ABSTRACTS

KEYNOTE LECTURES 1–5

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Veterinary virology in the post truth era

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The rise of populism worldwide is challenging science and its tenants and is fuelling the rise of anti-science movements. The latter attack scientific discoveries or science – based policy or scientific activity by amplifying wrong messages and generating and disseminating fake news. Inevitably, this will affect policy makers and funding agency's areas of interest and funding schemes. It is essential that we do not leave a void and that our perspective heard is clear and loud. As a call to responsibility, many scientists worldwide are ready to take a public stand in defense of the principles that we share as a community.

Based on my experience, taking a public stand can come with slanderous personal and institutional attacks as an attempt to shake institutional scientific credibility. The virology community as a whole is at risk of similar misrepresentation: we use verbiage in which we refer to "animal experiments", "cloning", "compulsory vaccination programs", "reverse genetics" and which include words and concepts that are now being questioned.

Defending the credibility of the veterinary services and of veterinary research in response to populist attacks should become one of our most urgent priorities. Leaders of the veterinary community worldwide should promote a reflection on this topic to improve vigilance and preparedness to limit the damage that such "viral" events can cause.

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Zoonotic arboviruses – Enhancing awareness

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Global mobility of goods and people as well as climate change effects which successively render central European ecotopes more suitable for reservoir species result in increased zoonotic arbovirus incursion risks.

Wild birds play an important role as reservoir hosts and vectors for zoonotic pathogens and their transmission. For several years we have therefore monitored migratory and resident birds by systematically setting up a German nation-wide wild bird surveillance network for zoonotic arthropod-borne virus infections like flaviviruses and alphaviruses. Special emphasis is laid on zoonotic flaviviruses, like West Nile virus (WNV) and Usutu virus (USUV). WNV is a mosquito-borne viral pathogen of global importance. Until July 2018, WNV was not detected in Germany, but an incursion is always possible. USUV is a close relative to WNV, which was introduced to Southern Europe approximately twenty years ago and has caused epizootics among wild and captive birds. USUV was first time detected in the Upper Rhine Valley in the year 2011 where it caused a massive die-off in Common blackbirds and is circulating since then in different regions of the country. In 2015/16 a dramatic increase in the number of USUV positive birds was detected with new cases showing up in the Berlin area, the Northern parts of North-Rhine Westphalia up to the Dutch border and in the region of Leipzig. This trend has continued so that we are seeing a massive bird die off almost all over Germany in 2018. Apart from USUV, Sindbis virus has repeatedly been detected by wild bird surveillance which proves that also vertebrates are clinically affected by this virus in central Europe.

In attempt to foster awareness and preparedness to emerging diseases and to build up diagnostic capacities at FLI and in African partner laboratories, we are collaborating with academic and state veterinary institutes in Mauritania, Sierra Leone, Guinea, Cameroon, Nigeria, Cameroon, DR Congo and Egypt. Approaches and surveillance results are presented and discussed.

Keynote Lecture 3

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Emerging lyssaviruses in bat hosts

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Background
Rabies, a fatal viral zoonotic disease, is one of the most serious viral zoonoses that is presently encountered worldwide and continues to pose a serious public health hazard. The Lyssavirus genus includes rabies virus and other recognised species, differentiated according to their genomic sequence. The lyssaviruses that infect bats include: European Bat lyssaviruses type-1 (EBLV-1) and -2 (EBLV-2), Bokeloh bat lyssavirus (BBLV), Aravan lyssavirus (ARAV), Khujand lyssavirus (KHUV), Irkut lyssavirus (IRKV), West Caucasian bat Virus (WCBV), Lleida bat Lyssavirus (LLEBV) and Gannoruwa bat lyssavirus (GBLV) are present in bat populations in Eurasia; Australian bat lyssavirus (ABLV) in Australian bat populations and Duvenhage lyssavirus (DUVV), Lagos Bat Virus (LBV) and Shimoni bat lyssavirus (SHIBV) in African bats and Taiwan bat lyssavirus (TBLV) in Asian bats. A further two African viruses, Ikoma lyssvirus (IKOV) and Mokola Virus (MOKV) have been described but never been detected in bats. A putative lyssavirus species that was detected in Finland, Kotalahti bat lyssavirus, awaits classification.

Methods
Phylogenetic analysis of complete genome sequences, together with geographic location and host species, provides strong evidence that new viral isolates can be classified as a viral species. As well as genetic relationships, lyssaviruses are grouped into phylogroups, based on both genetic and antigenic properties of the viruses. The grouping of viruses into phylogroups has important practical consequences or vaccine efficiency, since antigenic cross-reactivity is restricted to members of the same phylogroup.

Results
Novel lyssaviruses continue to be isolated from different bat species globally. Biologicals are available to pre-immunise individuals (vaccines) against rabies or treat potential exposures to the virus (vaccines and rabies immunoglobulin) as post-exposure treatments. Despite these tools, the continued annual mortality rate remains high, predominantly through a lack of availability of these preparations in areas where the virus is endemic. Whilst the human burden of the lyssaviruses remains unclear, human fatalities have been reported. It is remarkable that RABV has never been identified in Old World bats and, conversely, non-RABV lyssaviruses have never been detected in the New World.

Conclusion
Whilst vaccines are available to protect against all detected strains of rabies virus, their ability to protect individuals from the lyssaviruses varies notably. All these circumstances highlight the need for a universal pan-lyssavirus rabies vaccine, which could ultimately be given as a prophylactic childhood vaccine incorporated in the standard childhood vaccination schedule and able to prevent human rabies.

Keynote Lecture 4

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Next-generation sequencing – no limits (?)

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With the description of the first animal virus by Loeffler and Frosch more than 100 years ago, virology was born and the discovery and description of new viruses with conventional methods like virus isolation in cell cultures or embryonated eggs, electron microscopy, staining with specific antibodies or classical sequencing completed our knowledge. However, virus discovery slowed down over the years and molecular techniques including polymerase chain reaction or microarray detection mainly improved diagnostics within the known world of viruses. But with the availability of the so-called next-generation sequencing (NGS) technology, this novel and extremely powerful tool allowed completely new insights into the universe of viruses using a variety of techniques from whole genome sequencing or metagenomics. With the today available state-of-the-art platforms, NGS can be broadly used to identify e.g. also novel and unkown pathogens in different sample materials (even as point-of-care diagnostics) or characterize the complete genomes of all types of pathogens. The power of NGS-driven approaches to identify infectious agents is defined by the extreme amount of sequencing information that can now be obtained in a single sequencing run. By using these NGS-based pipelines, not only an enormous number of new viruses was described in the last years, but also new pathogens like the Schmallenberg virus, novel Bluetongue virus serotypes, new Astroviruses or the first zoonotic bornaviruses have been identified and genetically characterized. Nevertheless, these extreme number of sequence fragments resulting from NGS-analyses requires not only novel diagnostic pipelines including powerful software tools for big data analysis, but also a new dimension of knowledge and resources ("men, machines and money"). Here, the power and possibilities of modern NGS-based diagnostics in virology will be presented and discussed with the limitations of these developments e.g. concerning databases, quality control, ethics, taxonomy, funding and risk analysis.

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African swine fever: The biggest threat of the European swine industry. What can we do?

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Background

The current epidemiological situation of African Swine Fever (ASF) in the EU is a serious threat to the swine industry. ASF is not infectious to humans and does not directly affect public health. However, the ASF has a serious social and economic impact on the world trade of swine and swine products. The EU is the world second swine meat producer with 23.675 Tm in 2017, representing the 21,3% of the total swine meat production. The EU was also the main exporter of swine meat in 2017. This situation can be greatly affected by the ASF.

Methods

In this presentation, I will summarize my personal view of the current situation of ASF. A disease in which I have been working for the last 40 years in different epidemiological international scenarios. In general, ASF is usually underestimated.

ASF is a very complex disease whose ethology and immune response is still not well known, due to the complexity of the virus structure which encodes more than 165 genes. Even so, it's an epidemiological predictable disease (As an example: most of the epidemiological situations related with the virus spread and clinical forms of evolution, currently observed, were predicted and even published a few years ago). ASF is not a very infectious or contagious disease. However, ASF virus can go far away if we don't pay enough attention to its spreading mechanisms. The ASF virus animal infection is starting always in a new territory with naive animals, as an acute clinical form with relative high mortality. This situation, after some time, presents a clinical and immunological evolution to attenuated clinical forms even asymptomatic with the presence of carrier animals. That is the reason why, in these epidemiological situations, antibodies detection should always be include in a surveillance programme. This mentioned evolution is independent of the virus genotype.

Results

The main epidemiological factors and the disease evolution to the current situation will be analyzed. The attenuated and asymptomatic ASF isolates and its potential epidemiological role will be evaluated. Some specific and urgent epidemiological recommendations will be described as well as the role of a potential vaccine for wild boar and/or domestic pigs. A potential vaccination programme will be analyzed.

Conclusion

The ASF virus is doing a good job, we should work much better or the European wild boar will be endemically infected and sporadic and intermitten ASF outbreaks in domestic pigs will occur in the affected areas.

ABSTRACTS

ORAL PRESENTATIONS

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Surveillance of West Nile fever among equids and humans in the European Union and its potential impact on blood safety measures

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Background
West Nile fever is a viral zoonotic disease notifiable in humans and equids at the European Union (EU) level. To prevent human-to-human transmission of the virus via substances of human origin, blood establishments must defer donors for 28 days after leaving an affected area (where human cases were detected) unless an individual donation nucleic acid test is negative. The aim of the study was to define the impact of using equine cases as complementary trigger for blood safety measures (i.e. testing and deferral).

Methods
We analysed notifications of autochthonous human and equine cases from 2013 to 2016. Affected areas were defined at the third level of the Nomenclature of Territorial Units for Statistics (NUTS-3). We developed two alternative scenarios for estimating the impact of using equine data for triggering blood safety measures and calculated the impact for 2016. First, human cases or equine cases defined an affected area and the first of these cases triggered the start of the West Nile virus transmission season in the area. Second, equine cases, in addition to human cases, defined an affected area if at least one human case was detected in that area in one of the three previous years. For estimating the impact on blood safety measures under the alternative scenarios, we used the sum of the number of affected weeks multiplied by the population for affected areas for each scenario expressed as a percent increase compared to the baseline scenario, which is based on human cases only as trigger for blood safety measures.

Results
In 2016, 226 human cases and 188 equine cases were reported at the EU level. Fifty-nine areas (36.9 million inhabitants) were considered affected under the baseline scenario. Under the first modelled scenario, 78 areas would be considered affected (49.4 million inhabitants) representing a 35% increase in donation testing/deferral. Under the second scenario, 64 areas would be considered affected (39.8 million inhabitants), representing a 7% increase.

Conclusion
Considering equine cases as a complementary trigger for blood safety measures would increase donor screening sensitivity. Including any equine cases would have a major impact on testing requirements and deferral, without evidence, currently, of an association between equine and human cases. However, in areas where human cases have been reported in the past, using equine cases as a trigger would increase the timeliness of the blood donation measures with a limited impact on blood testing/deferral.

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Usutu virus infections in wild birds and humans between 2010 and 2017 in Austria and Hungary

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Prof. Dr. Herbert Weissenböck[1], Mr. Christof Jungbauer[4],
Mr. Stephan W. Aberle[5], Dr. Jolanta Kolodziejek[1],
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Background
Usutu virus (USUV, Flaviviridae) was first reported in Europe in Austria in 2001, where it caused wild bird (mainly blackbird) mortality until 2005. Since 2006 no further USUV cases were diagnosed in the country. However, the virus emerged in other European countries (Hungary, Italy, Switzerland, Spain, Germany and the Czech Republic) between 2005 and 2011. In 2016, widespread USUV-associated wild bird mortality was observed in Germany, France, Belgium and the Netherlands. In this study we report the results of passive monitoring for USUV in Austria and Hungary between 2010 and 2017.

Methods
Passive monitoring of wild birds was based on pathology and histopathology processing of dead birds found in nature. USUV RNA and antigens were detected by RT-PCR and immunohistochemistry. Blood donors were tested in 2017 in Austria using the nucleic acid test (NAT, Cobas) for West Nile virus (WNV) RNA. Positive samples were further tested by specific RT-PCRs. Viruses were identified by complete or partial genome sequence determinations. Genetic relationships were estimated by phylogenetic analyses.

Results
In Hungary, USUV caused sporadic cases of wild bird mortality between 2010 and 2015 (18 diagnosed cases), whereas in summer and autumn 2016 the number of cases considerably increased to twelve. In Austria, USUV was identified in two birds in 2016. Phylogenetic analyses of coding-complete genomes and partial regions of the NS5 protein gene revealed that USUVs from Hungary between 2010 and 2015 are closely related to the virus which emerged in Austria in 2001 and in Hungary in 2005, while one Hungarian sequence from 2015 and all sequences from 2016 clustered together with USUV sequences reported from Italy between 2009 and 2010. In 2017, USUV infection was determined in 22 wild birds in Austria and in five in Hungary. Five birds from Austria were infected with USUV belonging to the "Africa 2" genetic cluster. Additionally, seven of 12,047 blood donations from eastern Austria reacted positive to WNV in NAT. Confirmatory investigations revealed USUV RNA in six of these. Retrospective analyses of four blood donors diagnosed as WNV-infected in 2016 showed one USUV positive.

Conclusion
The results of the study indicate continuous USUV circulation in the region and exchange of USUV strains in Central Europe and Italy. Because USUV is a potential human pathogen, blood transfusion services and public health authorities in USUV-endemic areas should be aware of a possible increase of human USUV infections.

This study was founded by NKFIH K120118.

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Tick-borne virus detection in Caribbean ticks using NGS and high-throughput microfluidic real-time PCR (DOMOTICK Project)

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Background
Among hematophagous arthropods, ticks transmit the greater variety of pathogens of public health and veterinary importance whose (re)-emergence is recognized worldwide. Whereas the main human and animal tick-borne pathogens are well characterized in the Northern hemisphere, very few is known concerning the diversity of tick species and tick-borne pathogens circulating within the Neotropical zone of the Americas, especially concerning the Caribbean area.

Methods
We previously reported on the results of the DOMOTICK project concerning the detection of tick-borne pathogens in individual Caribbean ticks by using a combination of NGS and high-throughput microfluidic real-time PCRs using Taqman probes (BioMarkTM dynamic arrays, Fluidigm Corporation). Here, we focus on interesting viruses detected in these ticks. Total RNA extracted from 588 ticks collected in Guadeloupe and Martinique (Amblyomma variegatum, Rhipicephalus microplus) were sequenced by Illumina HIseq.

Results
Of 27,544 contigs generated, 1% matched with virus sequences available in GenBank of which 74% aligned with known tick-borne viruses. Out of these tick-borne viruses, four were of particular interest, related to viruses only described once in China in 2015. We detected one novel chuvirus, a circular RNA virus of 11,177 bases with 3 ORFs, with 55.6% of identity with the previously described Changping Tick virus. We also detected a new genotype of another chuvirus with 94.3% of identity with the Wuhan Tick virus, a circular RNA virus of 11,395 bases with 3 ORFs. The third interesting virus is a new genotype of the phlebovirus Lihan tick virus with 95 and 97% of identify on the S and L segments, respectively. The last virus detected is a four RNA-segmented new Jingmenvirus with 80% of identity with the original Jingmen tick virus and Mogiana tick virus. By using the BioMarkTM dynamic arrays, we were able to determine the prevalence of these four viruses in individual ticks from Guadeloupe and Martinique. Overall, the fours viruses were found in both tick species, but the new Changping-like virus was preferentially found in the Amblyomma ticks whereas the three others were preferentially found in the Rhipicephalus ticks. The prevalence of the viruses in Guadeloupe and Martinique did not show any bias in terms of geographical distribution.

Conclusion
The next steps of these studies will consist in assessing the veterinary and public health risks associated with these four new tick-borne viruses and trying to cultivate some of these viruses for better characterization.

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The characterisation of hepatitis E virus in UK pigs

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Background
Hepatitis E virus (HEV) infection is widespread in the global pig population. Although clinically inapparent in pigs, HEV infection is the cause of hepatitis E in humans and transmission via the food chain has been epidemiologically established. Studies are ongoing to understand how HEV enters the food chain with pigs/pork products being implicated as a source of infection. Following a 2013 study that investigated prevalence of HEV infection in UK slaughter-age pigs samples indicating the highest viral load (i.e. lowest real time PCR Ct value) were selected for further characterisation, the premise being that these strains were more likely to gain entry to the food chain.

Methods
This work was a continuation of an analysis of caecal content samples that had been collected as part of the 2013 Zoonoses in UK Pigs Abattoir Study, a cross sectional study of pigs being slaughtered at 14 high-throughput abattoirs (Grierson et al., 2015). At that time these samples (n=629) along with paired plasma samples had been tested for detection of HEV RNA and antibody. Five samples were subsequently selected for analysis by high throughput sequencing (HTS) based on original test data (Ct value). Four samples (006, 022, 493 and 557) were selected based on lowest Ct values and a fifth sample (090) was selected to enable an assessment of the sensitivity of methodology. These five samples had originated from different farms and from 4 different abattoirs in England.

Results
HTS was successfully used to obtain the complete coding sequence from five study samples. A natural in-frame insertion was observed within the HEV hypervariable region in two samples. To interrogate whether this mutation may be the cause of high-level viraemia and faecal shedding as observed in the sampled pigs virus isolation and culture was conducted.

Conclusion
Based on viral growth kinetics there was no evidence that these insertions affected replication efficiency in vitro, suggesting as yet undetermined host factors may affect the course of infection and consequently the risk of foodborne transmission.
Grierson et al., 2015 Emerg Infect Dis. 2015 Aug;21(8):1396–401.

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Prevalence and molecular characterization of hepatitis E virus in Lithuanian wild animal populations

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Background
Hepatitis E virus (HEV) is a sole member of Hepeviridae family that causes significant public health problems worldwide. Human and animal HEV cases in Europe had been attributed to HEV genotype 3. HEV can be transmitted to humans not only via uncooked meat, but through contaminated water and direct contact between animals and humans as well. HEV RNA presence in Lithuanian wild animal populations was determined using reverse transcription polymerase chain reaction (RT-PCR), and local isolate molecular characterization were carried out using phylogenetic analysis.

Methods
Wild boar (Sus scrofa) (n=505), roe deer (Capreolus capreolus) (n=93), red deer (Cervus elaphus) (n=15) and moose (Alces alces) (n=13) liver and serum samples were collected from 2014 to 2016. Viral RNA was extracted and RT-PCR analysis carried out using primers targeting ORF1 and ORF2 fragments. Prevalence and statistical difference analyses using Fisher's exact test were carried out and local isolates selected for genotyping using ORF2 fragment of HEV.

Results
25.94% [22.31–29.93] of wild boar, 22.58% [15.27–32.07] of roe deer, 6.67% [1.19–29.82] of red deer and 7.69% [1.37–33.31] of moose samples were positive for HEV RNA targeting ORF1 and 17.03% [14.00–20.55] of wild boar and 12.90% [7.54–21.21] of roe deer samples were positive for HEV RNA targeting ORF2. Phylogenetic analysis based on HEV ORF2 fragment confirmed that all tested HEV isolates in wild animal populations belong to genotype 3. Wild boar and roe deer isolates mainly clustered within 3i subtype of genotype 3 in addition to wild boar isolates that clustered within subtype 3h. HEV isolates from roe deer that cluster within subtype 3i had been described for the first time.

Conclusion
Direct evidence of HEV RNA prevalence in Lithuanian wild animal population has been obtained for the first time, confirming the possibility of natural HEV reservoir for human and domestic animal infections. Molecular characterization of local HEV isolates revealed a conservative genetic variation, consistent with results and isolates from neighboring and other European countries. Further studies are necessary to understand a complete epidemiological situation of HEV in both humans and animals on a national and international scale.

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Emergence of bluetongue virus serotype 4 in continental France in November 2017

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Background
In mainland France, bluetongue virus (BTV) emerged in 2006. After several vaccination campaigns of cattle, sheep and goats, no virus had been detected after 2010. France obtained an OIE/EU recognized BTV-free status in December 2012. In 2015, BTV-8 re-emerged in central France. In December 2016, BTV-4 was detected in South of Corsica. At the beginning of November 2017, a calf was tested RT-PCR BTV-4 positive in the context of pre-movement analysis. This presentation describes the detection of this case, the laboratory investigations that have been carried out to characterize the causative agent and the measures put in place to evaluate the BTV-4 spread and to control the disease.

Materials and methods
A 15-day-old calf which was born in Haute-Savoie moved to an assembly center in Loire prior to being exported to Spain. Blood sample was taken and rtRT-PCR analyses and isolation assays on KC, embryonated eggs and BSR cells were carried out. Next-generation sequencing was performed on RNA extracts from infected KC cells.
To evaluate the spread of BTV-4, blood cattle samples were collected in Haute-Savoie and neighboring departments in November and December 2017. Analyses were performed in Departmental Laboratories and BTV-4 positive samples sent to the ANSES National Reference Laboratory for confirmation.

Results
The calf was detected BTV positive with pan-BTV rtRT-PCR kit, negative with a BTV-8 rtRT-PCR and positive with a specific BTV-4 rtRT-PCR. After isolation of the BTV-4 strain, sequence analysis of the 10 double-stranded RNA segments showed a close relationship with the BTV-4 isolated in Hungary (2014) and in Corsica (2016). The nucleotide and amino-acid sequences identities were for all segments superior to 99.5 % with the homologous sequences published.
To date, 84 BTV-4 outbreaks were detected in Mainland France. Most of them (n =74) were in Haute-Savoie, the 10 others occurred in the departments of Ain, Haute-Saône, Saône-et-Loire, Jura, Maine-et-Loire and Yonne. Only one BTV-4 isolate has been obtained.

Conclusion
rtRT-PCR analyses from cattle samples collected during survey or investigations showed that BTV-4 was present throughout the Haute-Savoie department in November 2017, suggesting that BTV-4 was introduced several months before the first case detection. A compulsory vaccination campaign was decided in November 2017. However, the limited availability of vaccines led to a change in the BTV control strategy. On the first of January 2018, the whole of mainland France switched to BTV-4/BTV-8 restriction zone.

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Use of reverse genetics technique to study the early pathogenesis of Peste-des-petits ruminants virus

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Background

Across the developing world, Peste-des-petits ruminants virus (PPRV), places a huge disease burden on agriculture, in particular affecting small ruminant production and in turn increasing poverty in many developing countries. The current understanding of PPRV pathogenesis has been mainly derived from the closely related rinderpest virus (RPV). There are few studies that have focused on the late stages of pathogenesis of PPRV in the field and very little is known about the processes underlying the early stages of pathogenesis. It is believed that PPRV replicates mainly in the respiratory epithelium before disseminating throughout the host. We hypothesize that PPRV infects first the immune cells of the respiratory mucosa, but not respiratory epithelial cells and then migrates to the tonsil and local lymphoid organs for primary replication, after which virus enters the general circulation and secondary replication occurs in the epithelium of respiratory and gastro-intestinal tracts.

Methods

The application of reverse genetics techniques provides a tool to gain a better understanding of the molecular factors underlying virus host range and pathogenesis. Recently we have established reverse genetics system for PPRV and using this we have engineered a GFP tagged PPR virus (rMorocco 2008 GFP). Further, in the biosafety containment, we have infected targeted animals (goats) with this GFP tagged virus and following this virus in the body of infected goats in 6 hourly interval up to 96 hours.

Results

We could demonstrate that the virus primarily replicates inside the pharyngeal tonsil by 24 hours of post-infection and by 72–96 hours post-infection virus replicates in the epithelial cells of gastro-intestinal tract.

Conclusion

So PPR virus primarily replicates in lymphatic cells in tonsil and then spread to epithelium for secondary replication to cause the clinical disease. Work is ongoing to elucidate the cell types responsible for carrying the virus from the nasal mucosa to tonsil.

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Elucidating the role of T lymphocytes in pathogenesis during bluetongue virus infection in sheep

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Dr. Lyndsay Cooke[1], Dr. Marc Guimera Busquets[1], Dr. Amanda Corla[1], Dr. Martin Ashby[1], Dr. Mehnaz Qureshi[1], Dr. Christopher Sanders[1], Dr. Simon Carpenter[1], Dr. Katy Moffat[1], Dr. Karin Darpel[1]

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Background

Bluetongue is a non-contagious, haemorrhagic disease of ruminants caused by Bluetongue virus (BTV). BTV is an arthropod-borne Orbivirus (family: *Reoviridae*) transmitted to the mammalian host by infected *Culicoides* biting midges. The importance of specific T cell subsets in elucidating a protective immune response to BTV infection in respective ruminant hosts is still not fully understood, impeding future vaccine improvements. Furthermore, whilst certain T lymphocytes are known to become productively infected with BTV, the role of specific T lymphocyte subsets in BTV pathogenesis and transmission are yet to be fully elucidated.

Methods

Initially, 12-month old female sheep were administered intravenous injections of specific monoclonal antibodies (mAb) to deplete either CD8+ T lymphocytes (n=1) or WC1+ γδ T lymphocytes (n=1). Additional sheep (n=2) were injected with an isotype-matched depletion control mAb. Using a natural in vivo infection model, adult female *Culicoides sonorensis* were allowed to feed on blood containing BTV-4 MOR2009/07, a virus strain of moderate severity. After the extrinsic incubation period, *C. sonorensis* were allowed to blood-feed upon respective sheep. Further blood-feeding of uninfected *C. sonorensis* was carried out at peak viraemia and 21 days post-infection to assess onward transmission of BTV to the arthropod vector.

Results

The natural in vivo infection model resulted in successful transmission of BTV-4 to all sheep. T lymphocyte depletion and changes to cellular populations in whole blood during BTV infection were confirmed using a multi-colour flow cytometry panel. Viral dynamics were assessed throughout infection by qPCR and comparison to previous BTV infection studies suggest that *Culicoides* transmission of BTV (compared to needle inoculation) might shorten the initial dissemination phase of the virus in ruminants. Interestingly, whilst all sheep demonstrated the high fever, oedema and haemorrhage typical of BTV infection, the WC1+ γδ T lymphocyte-depleted sheep additionally demonstrated pronounced ulceration of the mucous membranes. Future study replicates will include an additional CD4+ T lymphocyte depletion group. Ongoing work is comparing BTV-specific antibody responses, T cell responses and cytokine profiles between sheep of different treatment groups and to samples stored from previous BTV infection studies.

Conclusion

In summary, blood-fed (rather than inoculated) *C. sonorensis* were successfully used here for the first time to infect sheep with BTV-4. Whilst only a single depletion replicate has been completed so far, additional replicates in coming months will further clarify these initial findings to elucidate the role of three specific T lymphocyte subsets (CD4+, CD8+ and WC1+) in BTV pathogenesis and immunity in sheep.

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Circulation of classical swine fever chronic and persistent forms refractory to vaccination and viral evolution under inefficient control programs

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Background
Classical swine fever (CSF) is a highly contagious viral disease in swine with high socioeconomic impact in endemic regions such as Asia, Central and South America, and in some Eastern European countries. CSF virus (CSFV) belongs to the Pestivirus genus of the Flaviviridae family. The fact that CSF has not been controlled in spite of the long-term vaccination programs with live attenuated C-strain vaccine conducted in endemic areas is a hindrance for CSF global control. Present study aimed to determine whether CSF subclinical forms may be present in a conventional farm in an endemic country with a history of vaccination failures, and to evaluate the vaccine efficacy under these types of infections in field conditions. Likewise, to characterize the circulating viral variants and to stablish their evolutionary relationship with previously reported CSFV circulating strains.

Methods
Six litters (n=62 piglets) born from vaccinated gilts were randomly chosen from a commercial Cuban farm. At 33 days of age, piglets were vaccinated with lapinized live attenuated vaccine, sera and rectal swabs were collected from sows and piglets and the sows were euthanized. After vaccination, sera and rectal swabs were collected at 21, 36, 66 and 96 dpv. Three litters were euthanized at 21 days post vaccination (dpv) and the remaining were maintained until 96 dpv and Tonsils were collected. CSFV RNA was detected by qRT-PCR and humoral response was evaluated. Virus isolation test was performed at 21 dpv. The E2 sequence from the tonsil of one animal from each litter was determined and positive selection pressure and functional divergence evaluation analyses were conducted.

Results
The presence of CSF chronic and persistent disease was demonstrated in an endemic country. The failure in the transmission of effective maternal immunity to the litters explained why the persistent infection turned out to be the predominant infection. Additionally we show the failure to respond to vaccination in animals with subclinical field infections. The E2 consensus sequence recovered from the six litters was the same. The residue 72 (R) on the E2 protein was identified under positive selection pressure in the CSFV circulating strain proving the relevance of this codon in the evolution of the CSFV 1.4 genotype.

Conclusion
Our results provide evidence about the repercussion of evolutionary changes of CSFV in the circulation of new strains with adaptive advantages that favour the generation of chronic and persistent forms with drastic implications for disease control in endemic countries under imperfect vaccination programs.

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Within-host diversity of low pathogenic avian influenza virus in various poultry species

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Background
Avian influenza virus (AIV) is an RNA virus characterized by high genetic diversity. In each replication cycle a swarm of genetically different variants is generated. The level of virus population complexity is highly relevant for virus adaptation, pathogenicity and transmissibility. However, little is known about the virus diversity generated during replication of low pathogenic avian influenza virus (LPAIV) in various species of poultry. To address this question, samples collected from experimentally infected turkeys, quail and ducks were subjected to deep sequencing with subsequent analysis of virus population diversity in individual birds.

Methods
Ten turkeys, ducks and quail were inoculated oculonasally with a dose of 10^6 EID50 of LPAIV H9N2 subtype. Oropharyngeal (OP) and cloacal (CL) swabs collected at 2 and 4 dpi, representing the beginning and peak of infection, were subjected to deep sequencing. The viral genome in each sample was amplified in RT-PCR. Prepared libraries were sequenced in MiSeq (Illumina). Reads were trimmed, mapped to a reference sequence and filtered. Variant calling was performed in VarScan and LoFreq. For each sample, the number of polymorphic positions (frequency >2%) and Shannon entropy were calculated. Additionally, genetic distances between pairs of samples were computed. Statistical analysis was performed with U-Mann-Whitney test.

Results
The highest diversity, expressed as the number of variant positions and Shannon entropy value, was observed in OP swabs from quail. Both metrics were significantly higher in quail than in turkeys. Due to the limited number of samples from ducks, the comparison with other species was not performed. However, noticeable differences in the virus diversity between OP and CL swabs were observed. Similarly, a higher heterogeneity in the virus pool in OP versus CL swabs was revealed in turkeys. In contrast to the genetic diversity at the level of individual birds, the genetic distance matrices showed higher intra-group diversity in turkeys than in quail. Considering each gene separately, the highest complexity was observed for HA gene, with significantly higher values in quail than in turkeys.

Conclusion
Significant differences in the virus diversity in various poultry species could help to further elucidate their role in the epidemiology of AIV. Additionally, the low virus complexity in cloacal swabs indicates that gastrointestinal tract is more selective environment than respiratory system, a feature that can be relevant for the fecal-oral route of transmission.

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Influenza viruses and paramyxoviruses enhance the adherence and invasion properties of streptococci by a sialic acid-dependent interaction with the capsular polysaccharide

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Background
Viral-bacterial co-infections, e.g. by influenza viruses and streptococci, may result in more severe diseases than the respective mono-infections. The underlying mechanisms may vary among different pathogens. We provide evidence that the hemagglutinins of some influenza viruses and paramyxoviruses are able to bind to the capsular polysaccharide of some streptococci by recognizing terminal sialic acid residues. In this way, virus-infected cells expressing viral hemagglutinins on the cell surface can increase the adherence of and invasion by streptococci.

Methods
Co-infections were performed with both immortalized cells (HEp-2, MDCK) and well-differentiated airway epithelial cells. The latter cells were obtained by generating precision-cut lung slices (PCLS) from porcine lungs. Cells were infected (i) by different influenza viruses that recognize either α2,3-linked or α2,6-linked sialic acid or (ii) by human parainfluenza virus 3 (HPIV3) that uses α2,3-linked sialic acid as a receptor determinant. The bacterial partners were either the porcine pathogen Streptococcus suis (S. suis) that contains α2,6-linked sialic acid in the capsular polysaccharide or the human pathogen group B streptococcus (GBS, S. agalactiae) that contains α2,3-linked sialic.

Results
Using porcine precision-cut lung slices (PCLS) we infected differentiated airway epithelial cells by swine influenza viruses. We found that the hemagglutinins expressed on the surface of virus-infected cells interacted with the capsular α2,6-linked sialic acid of S. suis resulting in enhanced bacterial adherence. In the late stage of infection, when the airway epithelium was destroyed, the porcine pathogen adhered to and invaded subepithelial cells independent of the capsular sialic acid. In the case of GBS that contains α2,3-linked sialic acid, adherence to and invasion of airway cells was greatly enhanced when the cells were pre-infected by avian influenza viruses. Co-infections by GBS and avian influenza viruses may be rare events. However, several human paramyxoviruses also use α2,3-linked sialic acid as a receptor determinant. We show that prior infection of cells by the human pathogen HPIV3 also enhanced the adherence and invasion properties of GBS.

Conclusion
Bacterial pathogens containing capsular sialic acid residues may use the interaction with virus-infected cells to enhance their adherence and invasion properties which my result in a more severe disease.

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Access to a main EHV1 receptor, located basolaterally in the respiratory epithelium is masked by intercellular junctions and revealed by pollen proteases

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Background
Equine herpesvirus type 1 (EHV1) outbreaks annually lead to respiratory, reproductive and neurological disorders in the horse population. Compared to other alphaherpesviruses, EHV1 has a unique strategy to limit its replication in the equine respiratory tract and infect leukocytes for reaching target organs. Since vaccines and antivirals are not fully efficient, a better understanding in EHV1 pathogenesis is needed, starting with its primary replication in the respiratory tract. Here, we determined (1) whether epithelial cell intercellular junctions (ICJ) are key boundaries in limiting EHV1 infection in the respiratory tract and (2) which factors affect these ICJ. EGTA and Lysomucil® alter extra- and/or intracellular calcium levels, enabling them to affect ICJ. Pollens exhibit proteolytic activities for efficient pollination, but when inhaled by humans or animals, these proteases can destruct ICJ.

Methods
Equine respiratory mucosal explants, originating from the slaughterhouse, were treated with various agents (EGTA, Lysomucil®, pollen proteases) to disrupt ICJ, prior to inoculation with EHV1. Destruction of ICJ was assessed by microscopic image analysis of hematoxylin-eosin stainings. EHV1 replication was visualised by immunofluorescence staining and confocal microscopy. To characterize attachment of EHV1, direct virus-binding studies were carried out with Dio-labelled EHV1-particles. Experiments in explants were corroborated in in vitro equine respiratory epithelial cells (EREC), cultivated at the air-liquid interface. EREC were inoculated at either apical or basolateral surfaces, following destruction of their ICJ, which was assessed by measuring the transepithelial electrical resistance.

Results
By using zymography, we confirmed that pollens of Kentucky bluegrass, white birch and hazelnut exhibit proteolytic activities. Together with these proteases, EGTA and Lysomucil® were able to alter ICJ in explants. Subsequent EHV1 binding to and infection of explants was greatly enhanced upon destruction of ICJ. In addition, EHV1 preferentially bound to and entered EREC at basolateral cell surfaces. Disruption of EREC ICJ with EGTA partially restored susceptibility to EHV1 at the apical surface, but had no impact on basolateral infection.

Conclusion
We demonstrated that integrity of the horse's respiratory epithelium is crucial in the defence against EHV1 infections. Veterinarians should be cautious when using Lysomucil® in the aerosol therapy of horses. In addition, it can be postulated that during pollen season, some horses might have a deficiency in epithelial integrity and therefore be more receptive for an EHV1 infection. Finally, by targeting a basolaterally located receptor in the respiratory epithelium, EHV1 has generated a strategy to efficiently escape from its host during reactivation from latency.

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Middle East Respiratory Syndrome coronavirus (MERS-CoV) vaccination efficacy in a direct-contact transmission model in llamas

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Background
Middle East Respiratory Syndrome (MERS) is caused by MERS-coronavirus (MERS-CoV), and dromedary camels are the main reservoir of the virus. MERS-CoV and related viruses are endemic in these animals in East Africa and Middle East. Thus, animal vaccination to prevent virus shedding might be a convenient way to prevent zoonotic transmission. However, since working with dromedaries under biocontainment environment is difficult, other camelid species may act as surrogates. In this study, the efficacy of a recombinant S1-protein vaccine against MERS-CoV in a direct-contact transmission model in llamas was explored.

Methods
The experiment was conducted in two separate boxes. In box 1, a group of llamas (n=3) were intranasal inoculated with 10^7 $TCID_{50}$ MERS-CoV Qatar (2015) strain. At 2 days post-inoculation (dpi) naïve llamas (n=5) were put in contact with challenged llamas. In box 2, one group of llamas (n=5) were primo vaccinated with 35 µg of a recombinant S1 protein, and with 50 µg (second immunization) 3-weeks later, both by intramuscular administration. Two weeks after the booster, vaccinated llamas were housed in the same box with MERS-CoV inoculated llamas (n=3), following the same schedule indicated for box 1. Virus nasal shedding was monitored by RT-qPCR during all the experiment, and sera was collected at immunizations, day of inoculation and weekly after challenge for seroneutralization assays and anti-MERS-CoV S1 protein antibody titres. Animals were euthanized 3-weeks after infection.

Results
Intranasal infected llamas from both boxes shed virus for a period of 2-weeks. In-contact naïve llamas from box 1 got infection as soon as 4-5 days after contact, with similar viral loads and periods of shedding observed in the inoculated animals. In box 2, inoculated llamas found the same infection pattern than those from box 1, but only one out of five vaccinated llamas got infection levels comparable to non-vaccinated in-contact animals. Sera from the S1-vaccinated llamas showed virus neutralization and antibodies against S1 capacity already at 3-weeks after the first immunization.

Conclusion
(i) The in-contact llama model was very efficient for transmission of MERS-CoV between animals, representing a more natural route of infection, (ii) the vaccine efficacy was proved by using the animal-to-animal MERS-CoV transmission model.

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Middle East respiratory syndrome coronavirus (MERS-CoV) early infection in alpacas

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Background
Middle East respiratory syndrome coronavirus (MERS-CoV) is a positive sense, ssRNA virus that causes fatal infections to humans. Although dromedaries are considered the main animal reservoir, alpacas are also reported as potential hosts for MERS-CoV infection. The present study aimed to investigate the early events of infection with MERS-CoV in alpacas.

Materials and Methods
Fifteen 6–8 month-old alpacas were used for this study. Three alpacas were euthanized prior to the start of the experiment, and served as negative controls. The remaining twelve alpacas were intranasal inoculated with 10^7 50% tissue culture infective dose of MERS-CoV Qatar 2015 strain. Nasal swabs were obtained on days 0,1,2,3 and 4 post-inoculation (p.i.) for virus detection by PCR analysis. Three alpacas were euthanized on days 1, 2, 3 and 4 p.i., respectively. At necropsy, respiratory tissues and cervical, mediastinal and mesenteric lymph-nodes were collected and fixed in formalin for immunohistochemical (IHC) examination with a primary antibody specific against the nucleocapsid protein.

Results
After challenge, all alpacas excreted virus, as evidenced by RT-qPCR of nasal swabs from 1 to 4 days p.i. No MERS-CoV was detected on day 0 p.i. either in nasal swab samples or in any tissue by IHC. On day 1 p.i., MERS-CoV was detected in epithelia of frontal/medial turbinate and medial/caudal trachea. On day 2 p.i., positive labelling was detected in most of the respiratory tract tissues, and cervical and mediastinal lymph-nodes. Very high viral loads were observed in frontal and medial turbinates. On day 3 p.i., MERS-CoV was mainly detected in turbinate samples and cervical lymph-nodes. On day 4 p.i., the viral antigen tissue distribution was similar to day 2 p.i., but with diminished viral loads and absence of virus in mediastinal lymph-nodes and tonsil.

Conclusion
Alpacas are highly susceptible to the MERS-CoV Qatar 2015 strain, as evidenced by high viral shedding and presence of virus in the upper and lower respiratory tract. Mediastinal and cervical lymph-nodes were shown to be infected. The peak of infection in different organs occurred on day 2 p.i. Viral loads in tissues declined from day 3 p.i. onwards, suggesting a potent innate immune response against MERS-CoV.

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Bats as reservoir hosts of novel and emerging viruses in Italy

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Background
The diversity of viral agents found in bats worldwide is still largely unknown, and it is thought that a great number of viruses are yet to be discovered and characterized. Herein we report the most recent virus detections obtained within a survey for emerging viruses associated with bats implemented in Italy.

Methods
The survey involved the analyses of dead animals collected from bat rehabilitation centers or from known roost sites between 2010 and 2017. Fecal samples collected from hospitalized individuals were also sampled. Fresh carcasses were fully necropsied, and tissue specimens from different organs were analyzed. Samples were examined through a diagnostic protocol broadly targeting viral agents, which was set up using both "classical" virologic methods (virus isolation and negative staining electron microscopy (nsEM)), and molecular techniques (PCRs and NGS sequencing) in order to enhance the possibility to detect newly-emerging viruses with a potential zoonotic importance or proving dangerous for bats.

Results
Almost 700 tissue and fecal samples from 12 different bat species (mostly pipistrelle bats) were collected and analyzed. The survey firstly provided evidence that insectivorous bats carry a wide variety of Mammalian orthoreoviruses (including new reassortant strains) and coronaviruses (COVs), both AlphaCoVs and BetaCoVs (MERS and SARS-like CoVs). Then, a novel and previously unknown Rhabdovirus, named Vaprio virus (VAPV), belonging to the genus Ledantevirus was isolated from organs of a Pipistrellus kuhlii spontaneously dead in a wild animal recovery centre. Preliminary screening for VAPV performed on 76 bats sampled in the same area revealed three more positivity with a prevalence of 5.2%. Moreover, an adenovirus type2, strongly related to a virus previously detected in bats from Germany [JN252129] in 2007, re-emerged in a new area in Northern Italy. More recently, a bat-borne poxvirus was also identified for the first time in bats in Europe. This virus was isolated from a pool of viscera of an adult male of Hypsugo savii with humerus fracture and osteomalacia. The preliminary genome sequence analysis performed on almost the whole-genome sequence (80%) revealed a significative aa similarity (84%) with the Eptesipoxvirus strain Washington [KY747497] isolated from the elbow joint of a big brown bat in US.

Conclusion
A quite large variety, including new and emerging viruses have been identified in bats in Italy. This provides new targets for the development of specific diagnostic and surveillance assays and opens new prospective for viral research in bats.

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Outbreaks of haemorrhagic fever with renal syndrome in the United Kingdom

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Background
Hantavirus species are the etiological agents of haemorrhagic fever with renal syndrome (HFRS) and responsible for thousands of human cases per year in Europe. The majority of HFRS cases in Europe are due to the bank vole associated hantavirus, Puumala Virus. In contrast, the European prevalence of the hantavirus associated with brown rats (Rattus norvegicus), Seoul Virus (SEOV), has historically been considered to be low. However, in recent years, SEOV has been linked to a growing number of moderately severe cases of HFRS in individuals known to be exposed to wild or pet rats in Europe.

Methods
Following a HFRS outbreak arising from contact with pet rats from a breeding colony in Cherwell, England, RNA from the kidney and lung tissue was screened using a pan-hantavirus RT-PCR (L gene). The kidneys from domesticated rats were similarly screened following a subsequent outbreak in South Wales.

Results
Seoul virus (SEOV) RNA was detected in 17 of 21 pet rats. In addition, SEOV RNA was detected in the heart, kidney, lung, salivary gland and spleen (but not the liver) of an individual imported rat from the Cherwell colony suspected of being the source of the SEOV infection. SEOV RNA was more reliably detected in kidney tissue than lung tissue in replicate testing. Partial hantavirus L segment sequence (333nt) for SEOV-positive pet rat strains were 100% identical to each other. The pet rat sequences shared 97% identity to the UK wild rat SEOV Humber strain.
Additionally, an outbreak of SEOV associated HFRS in South Wales was traced to a commercial facility breeding rats for sale as reptile food. Phylogenetic analysis confirmed that the SEOV strain responsible for the outbreak, whilst highly similar to the UK pet rat SEOV strain, was sufficiently different across the genome to indicate a separate cluster of infection within the commercial facility.

Conclusion
Our findings suggest that SEOV is likely to be more widely distributed in domestic and commercial rats than previously believed and may contribute to a greater number of the reported HFRS cases in Europe.

Oral Session 2.1
Emerging, zoonotic and vector-borne viruses 2

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Electron cryo tomography and determination of capsid structure of rabies virus

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Background
Rabies lyssavirus is an important zoonotic pathogen within the family Rhabdoviridae. Although efficient vaccines exist, the yearly human death toll is estimated to be 40,000–60,000 cases. No treatment of the disease is available to date, so once symptoms develop, rabies invariably leads to death. Capsid proteins are more and more being recognized as targets for the development of antivirals. This development is facilitated by the availability of structural data of the target. The structure of the closely related Vesicular stomatitis virus (VSV) N protein has been determined by crystallography, and its arrangement in intact VSV particles by single particle analysis. Data on rabies lyssavirus, however, are still lacking.

Methods
Electron cryo tomography and subvolume averaging were employed to analyse the morphology of rabies virus particles and its nucleocapsid.

Results
The rabies virus nucleocapsid is located in a regular helical arrangement beneath the virus membrane. Inside the nucleocapsid cone, no specific protein densities can be identified. The helical turn appears fishbone-like within the virus particle and are ±73 Å apart, with an angle of ±48° of the virus central axis.

Conclusion
The predicted N protein structure of rabies virus is not distinguishable from the respective crystal structure of VSV. Compared to VSV, our data show a different arrangement of the nucleocapsid within rabies virus particles, indicating the need for further analysis to exactly determine the arrangement of the protein-RNA complex.

Oral Session 2.2
Viral immunity; advances in vaccinology and antivirals

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Investigation of the duration of immunity induced by gene-deleted African swine fever viruses

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Background
Previously we showed that deletion of either the DP148R single gene or multiple copies of multigene family 360 and 505/530 genes results in attenuation of virulent African swine fever virus (ASFV) in pigs and induction of high levels of protection against challenge. These genes act to inhibit type I IFN.

Methods
To further evaluate these gene-deleted viruses as candidate vaccine strains we carried out additional experiments in pigs to test clinical signs and protection against challenge using different doses and routes of immunisation of these viruses. We also carried out an experiment to test the duration of immunity induced with a single dose of two different attenuated viruses.

Results
Immunisation with the Benin delta MGF resulted in a low transient fever for one or 2 days soon after immunisation but no further signs. This fever coincided with moderate levels of virus genome in blood which decreased gradually. The OURT88/3 isolate did not cause clinical signs post-immunisation and no viremia was detected. ASFV specific cellular responses were measured by IFN γ Elispot assays. These reached highest levels by day 24 post-immunisation and then decreased. ASFV specific antibody responses reached a plateau by about day 24 post-immunisation and were maintained during the experiment. None of the pigs survived challenge with virulent virus at day 130 post-immunisation. Those immunised with the Benin delta MGF virus survived for a longer time up to 10 days compared to 5 or 6. Levels of IL-10 and IFN γ both increased to varying amounts after challenge.

Conclusion
A single dose of 104 TCID50 of these attenuated ASFV strains does not induce longer term protection over a period of 130 days.

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Host factors involved in classical swine fever immunopathogenesis

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Background
Classical swine fever (CSF) is one of the most devastating diseases in swine. CSF virus (CSFV) belongs to the Pestivirus genus, Flaviviridae family. The capacity of CSFV to induce persistent infection is very well known. The present work aimed to elucidate some of the host factors that allow viral persistence in its natural host.

Methods
Three groups of pigs were used: Groups 1 and 2 were infected intranasally at 5 days after birth with a moderate virulence strain (Cat01) and a low virulence strain (Pinar del Rio), respectively, whereas group 3 were non-infected pigs. Serum and rectal swabs were collected weekly to evaluate viremia and viral excretion as well as to detect specific humoral immune response by ELISA and seroneutralization. At 6 weeks of age, pigs were euthanized and PBMCs and BMHCs were obtained for phenotypical analysis of lymphocytic and myelomonocytic surface markers by flow cytometry. Functional studies were performed to assess the IFN-γ and other cytokine production against specific (CSFV) and unspecific (PHA) antigens, using ELISPOT and ELISA assays, in co-cultures of PBMC and BHMC subsets obtained by immune-magnetic sorting.

Results
Pigs from group 1 showed permanent viremia in absence of specific humoral and cellular immune response, and also failed to respond to PHA, corresponding with persistently infected (PI) pigs. In group 2, pigs were immunocompetent (IMC) clearing the virus after infection and displaying specific humoral and cellular immune responses. Animals from groups 2 and 3 were able to mount an adequate IFN-γ response against PHA. Phenotypical analysis of BMHCs showed increased T-cell populations in PI animals. PBMCs from these animals also contained higher numbers of granulocyte precursors cells compared to those of IMC and naïve pigs. In functional studies, the response to PHA in PBMCs from naïve animals was diminished when co-cultured with a subset of BMHC from PI pigs. Likewise, co-culture of PBMCs from IMC animals with this subset of BMHCs from PI pigs drastically reduced the IFN-γ response against CSFV. These cells were further characterized by flow cytometry.

Conclusion
We have found, in PBMCs from CSFV PI pigs, cells that have a similar phenotype to immunosuppressive cell populations found in humans. Our findings illustrate the role of these cells for immunosuppression and virus dissemination in the host, favouring viral persistence.

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Abortigenic but not neurotropic equine herpesvirus 1 modulates the interferon antiviral defense

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Background
Equine herpesvirus 1 (EHV1) is considered as a major pathogen causing symptoms from mild respiratory disease to late-term abortion (mainly abortigenic strains) and neurological (mainly neurovirulent strains) disorders. The abortigenic strains replicate more efficiently in the respiratory epithelium (RE), since a higher amount of larger viral plaques and lower chemokine-expression was detected, compared to the neurovirulent strains. Based on the differences in the replication kinetics and chemokine-induction between both EHV1-phenotypes, we hypothesized that abortigenic strains may interfere with the type I interferon (IFN) secretion/signaling-pathway in the RE, while the neurovirulent phenotype might be less efficient. In this study, the effects of type I IFN were analyzed during abortigenic and neurovirulent EHV1-infection of primary respiratory epithelial cells (EREC) and nasotracheal mucosa explants (ME).

Methods
The nasal septum and trachea were collected from three horses in the slaughterhouse. EREC and ME were isolated, cultured at air-liquid interface and inoculated with either abortigenic or neurovirulent EHV1. At 10, 24, 48 and 72 hpi, supernatants of EHV1-inoculated EREC and ME were collected. ELISA and a CPE-reduction assay were used to quantify the IFNα-concentration and -bioactivity. The expression of IFNα was confirmed by immunofluorescence staining (IF). Next, EREC and ME were treated with recombinant equine IFNα (rEqIFNα) or Ruxolitinib (Rux), an IFN-signaling inhibitor, prior to and during EHV1-infection. At 48 hpi, ME, EREC and supernatants were collected, followed by IF staining and virus titration. The number of viral plaques, plaque latitudes and virus titers were deliberated in the presence or absence of exogenous and endogenous (rEq)IFNα, respectively.

Results
Similar levels of IFN (approx. 70 U/ml) were detected in explants inoculated with both types of EHV1 from 48 hpi to 72 hpi. Pre-treatment of ME and EREC with rEqIFNα decreased the replication of both EHV1 strains in an IFN concentration-dependent manner. However, in two out of three horses abortigenic strains induced higher virus titers and plaque sizes at a concentration of 100 U/ml compared to 10 U/ml, in contrast to the neurovirulent strains. Moreover, in the presence of Rux, neurovirulent viral plaques became wider compared to control, while the plaques of the abortigenic variants remained unaltered.

Conclusion
IFNα is induced by both EHV1-phenotypes in respiratory mucosae. We demonstrated that an effective IFN response inhibits to a moderate (EHV1 abortigenic) or high (EHV1 neurovirulent) extent the EHV1-replication in the respiratory tract. This knowledge is valuable to develop future intranasal vaccination strategies.

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Preliminary evaluation of a baculovirus-expressed subunit vaccine for the protection of white-tailed deer from clinical epizootic hemorrhagic disease virus serotype 2

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Background
Epizootic hemorrhagic disease virus (EHDV) is an arthropod-transmitted virus and the causative agent of epizootic hemorrhagic disease (EHD) in wild and domestic ruminants. EHD is an OIE notifiable disease and has been reported in the Americas, Australia, Asia, Africa, and the Middle East. In North America, cervid species are most affected by EHDV- particularly white-tailed deer (WTD) which experience severe clinical signs with high mortality. EHD also has had substantial impacts on farmed WTD populations resulting in significant animal losses and restrictions on animal movement causing hardship to the deer farming industry. No licensed vaccine is currently available in North America. The objective of this work was to evaluate the immunogenicity of recombinant subunit EHDV vaccines in mice, cattle, and WTD, and evaluate vaccine efficacy in WTD.

Methods
Recombinant structural proteins of EHDV serotypes 2 and 6 were constructed and expressed in a baculovirus system. Initially, immunogenicity studies using the purified proteins in vaccine formulations were performed in mice and cattle. Captive-bred WTD (n=3) were then immunized with EHDV serotype 2 (EHDV-2) antigen formulations followed by homologous challenge. Sham-vaccinees (n=3) served as controls. Animals were monitored for 14 days for clinical disease, and serological and viral parameters were measured. On D14 post-challenge, animals were euthanized and infection/protection was determined by pathology and RT-qPCR for viral RNA.

Results
Mice and cattle produced neutralizing antibodies to all formulations tested. One EHDV-2 antigen formulation produced robust neutralizing antibodies in vaccinated WTD. Following challenge, sham-vaccinated deer became febrile and developed hyperemia of the oral and ocular mucosa, facial edema and lymphopenia. Viral RNA was detected in all challenged, non-vaccinated WTD, with peak viremia occurring at approximately D6 (10^6-9 copies/ml whole blood). These deer had varying severity of gross and histological lesions consistent with EHDV and viral RNA was detected in a minimum of two of the four tissues tested (lung, spleen, liver, kidney). In contrast, clinical symptoms were not observed in vaccinees. Vaccinees also failed to develop PCR-detectable virus in the blood and tissues, and gross and histological lesions consistent with EHDV were absent.

Conclusion
An EHDV-2 antigen formulation produced robust neutralizing antibodies in all vaccinees and provided sterilizing immunity following a homologous challenge. These results suggest that a recombinant structural antigen subunit vaccine may be an effective tool in preventing clinical EHD and reducing virus transmission. Moreover, a single protein subunit vaccine has the benefit of being DIVA compatible.

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Dendritic cell based in-vitro assay to test immunogenicity of viral vaccine candidates

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Background
Currently available in-vitro vaccine testing assays do not accurately predict the immunity induced by viral or non-viral vaccine antigens in the host. The availability of such assays would help to reduce the number of in-vivo animal studies by screening several vaccine antigens at early stage. Dendritic cells are the most powerful antigen presenting cells, having the ability to activate naïve lymphocytes for the antigens that they present. We developed a bovine in-vitro monocyte derived dendritic cell (MoDC) based assay to measure vaccine immunogenicity and investigated the immunogenicity induced by of viral vaccine candidates.

Methods
Bovine MoDCs were generated from monocytes isolated from cattle peripheral blood using a commercially available cytokine cocktail. Immature MoDCs were pulsed with inactivated vaccine Rabies virus strain Pasteur (RIV) or inactivated Blue tongue virus BTV-4/SPA-1/2004 of serotype 4 (BTV) or CD40L or none. In later experiments, we used an irradiated (25KGy) RIV to pulse the MoDCs. Next, antigen pulsed MoDCs were cocultured with autologous naïve lymphocytes. After 7 days of co-culture, each treatment was repulsed with matching antigen pulsed MoDCs and 2 days later the activation of CD4 and CD8 cells were measured by expression of Ki-67 (proliferation marker) by flow cytometry. Expression of IFN-gamma (Real-time PCR and ELISA) and CD25 surface marker (flow cytometry) were also measured as additional activation markers.

Results
Both BTV and RIV pulsed MoDCs led to significantly increased activation and proliferation of CD4 and CD8 lymphocytes compared to control MoDCs (or CD40L in the case of BTV) as indicated by the increased expression of CD25 and Ki-67. Moreover, pulsing MoDCs with RIV caused significantly higher expression and production of IFN-gamma by CD4 and CD8 lymphocytes as compared to control MoDCs. The assay was also able to effectively measure the activation of both CD4 and CD8 lymphocytes following irradiation of the virus.

Conclusion
For the first time in any livestock species, a functional MoDC based assay that measures viral vaccine immunogenicity in-vitro is described by this work and is both effective and reproducible. The assay could also be used to measure vaccine immunogenicity of irradiated viruses and to design optimized irradiation protocols and other inactivation protocols that preserve immunogenicity.

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Nanopore sequencing in clinical veterinary virology: proof-of-concept studies and perspectives

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Background
Diagnostics in veterinary medicine is undergoing a revolution with the implementation of cutting-edge technologies. Next Generation Sequencing (NGS) is becoming more accessible in research laboratories for 10 years, but is still too expensive, hard to manage and non-adapted to clinical applications in veterinary medicine. Oxford Nanopore Technologies (ONT) is revolutionizing the field of NGS with its MinION, a portable NGS device for high throughput sequencing. This technology is based on the reading of DNA or RNA strand during its passage through a pore. Moreover, there is theoretically no read size limitation, allowing a full viral genome sequencing with just a few reads.

Methods
MinION sequencing was applied to the identification of two avian DNA viruses, belonging to poxviruses or adenoviruses. While several NGS studies are based on propagated viruses and/or PCR-amplification, we intentionally worked directly from tissues or swabs, to avoid amplification bias and facilitate further clinical studies.
Three cases of avian pox in laying hens in 2016 and 2017, and one case of viral pancreatitis in guinea fowl in 2017, putatively associated with an adenovirus, were included in the study. Total DNA was extracted using phenol:chloroform in order to limit DNA degradation. DNA was then submitted to library preparation using the ligation sequencing kit 1D before sequencing with R9.4 flow cells. Bioinformatics analysis consisted of sequence assembly using a reference and/or de novo assembly.

Results
We obtained whole genomes of avian poxviruses (n=3) and adenoviruse (n=1) from clinical material, without any isolation or PCR-based enrichment. The sequencing of the first APV sample produced 3 905 fowlpox virus sequences out of 163 824; the second and the third APV samples generated 3 265 and 2 234 fowlpox virus sequences out of 124 534 and 141 670, respectively. Concerning the guinea fowl pancreatitis sample, we obtained 4 781 adenovirus reads out of 764 001.

Conclusion
The MinION device from Oxford Nanopore Technologies is obviously suitable for the sequencing of veterinary viruses from clinical material. Despite a miniaturized system theoretically adapted to the field, several steps of our protocol, including bioinformatics, still remain lab-dependent. The error rate of the reads generated by the ONT technology is quite high regarding other technologies such as Illumina. However, for projects that focus on the identification of a virus based on its whole genome, the long-size reads generated by ONT remain essential.

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Nanopore sequencing as a revolutionary diagnostic tool for porcine viral enteric disease complexes identifies porcine kobuvirus as an important enteric virus

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Background
Diarrhea is a major problem for swine industry. Porcine epidemic diarrhea virus (PEDV) and rotavirus A (RVA) are known causes of enteric disease, but several novel viruses have been discovered in the feces of piglets using metagenomics sequencing. Most sequencing platforms require extensive sample preparations including amplification of viral nucleic acids and long analysis times. Here, the diagnostic capacities of MinION (Oxford Nanopore Technologies) were investigated.

Methods
In a first case, cell culture grown PEDV and RVA were pooled at high loads and sequenced for 24 hours using MinION. Next, a diarrheic sample of a one week old piglet on which no etiological diagnosis was made with traditional diagnostics, was analyzed for 3 h. In both cases, no amplification of viral nucleic acids was conducted. As a porcine kobuvirus was discovered for the first time in Belgium, a novel RT-qPCR was developed and a longitudinal field study, a retrospective and phylogenetic analysis were conducted.

Results
In case one, PEDV and RVA reads were detected rapidly (<30 seconds) after start of sequencing, resulting in high sequencing depths (19.2–103.5X) after 3h. De novo assembly resulted in recovery of the PEDV genome and RVA gene segments at identities between 95% and 99% compared to reference genes. In the second case, almost all (99%) of the viral reads belonged to bacteriophages. Contigs matching Bacteroides, Escherichia and Enterococcus phages were identified, which may have reshaped the piglet's gut microbiome. Three eukaryotic porcine viruses, mamastrovirus, enterovirus G and porcine kobuvirus were found. Suckling piglets shed kobuvirus from one week of age, but an association between peak of viral shedding (6.42–7.01 $\log_{10}$ copies/swab) and diarrheic signs was not observed in a longitudinal follow-up study on the case farm. Kobuviruses were widespread among Belgian diarrheic piglets (n=25, 56.8% pos) with loads up to 6.83 $\log_{10}$ copies/g. The 3D polymerase genes of Belgian kobuvirus isolates were moderately (90.1 to 97.2% nt identity) related to each other.

Conclusion
In conclusion, MinION enabled rapid detection of enteric viruses. New technologies will change the way how diagnostics will be performed in the near future. More extensive validations should be conducted to make sure that all members of the porcine enteric disease complex are accurately being diagnosed. Moreover, kobuvirus was found to be widely present in the Belgian swine population. Due to the lack of association between the peak of shedding and diarrhea, its true enteric pathogenicity should be questioned, while its subclinical importance cannot be excluded.

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Development of micro-amount of virion enrichment technique (MiVET) and application to transboundary animal diseases countermeasures

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Background
Transboundary Animal Diseases (TADs), such as African swine fever (ASF), foot-and-mouth-disease (FMD) etc cause vast economic losses throughout the world. Despite of the importance of identifying sources/propagation route of the spread, it is often confounded due to incomplete epidemiological evidence. Isolation/detection of the micro-amount of virion from environmental samples is rarely successful due to very low contamination level in the environmental samples. We have recently developed a "micro-amount of virion enrichment technique" (MiVET) in combination with the complex of polyclonal Ab and protein G-coated magnetic beads (pG-MB), and simple SDBS (sodium dodecyl benzenesulfonate) elusion. In this study, we have evaluated the performance of MiVET system.

Methods
Two avian influenza viruses (AIVs), consists of H5N2 and H7N7 subtypes, were artificially spiked into 1mL of 50% duck fecal supernatants and 50mL of PBS, respectively. IMB (immuno-magnetic beads, complex of commercially available anti-fluA polyclonal Ab and pG-MB) were added into the samples. Then, the mixture was incubated at 37 degree for 15 min. The tube was set onto a magnetic stand for 2 min, and then, supernatant was removed. After washing the IMB with PBS at 1 time, the supernatant was removed again. The IMB capturing virions were eluted 2 µL (for 1 mL of fecal supernatant) or 8 µL (for 50mL of PBS) of SDBS. The resulting all supernatant was used as template RNAs for real-time RT-PCR assay.

Results
The MiVET system successfully concentrated AIVs in fecal and PBS samples at least 10 and 100 times, respectively, in comparison to conventional column kit extraction. The MiVET system required less than 30 min from the beginning of incubation to the final RNA elution.

Conclusion
The MiVET system appears to be valuable in providing useful information to control programs for various animal diseases such as ASF, FMD etc, as well as identifying the source/propagation route of the spread.

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Widening the scope of PPR diagnostic: adaptation and development to target atypical host species and field situations

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Background
Peste-des-petits ruminants (PPR) is a highly contagious and devastating viral disease affecting sheep, goats, and a large number of species within the order Artiodactyla. Robust commercial serological and virological diagnostic kits are available to detect PPR infection, but they were mainly developed for domestic small ruminants (goat and sheep) and for high quality, invasive samples sometimes hard to obtain in the field. New tools are needed to detect PPR infection in atypical hosts (e.g. camels, wildlife) and in complex field situations. Here we present adaptation of existing methods and new diagnostic tools to resolve some of these issues. In some regions, farmers are reluctant to have their animals handled and tested for PPR infection. As well, in the case of wildlife survey, animal capture is very costly and demands complicated logistics. Detection of PPR virus in non-invasive samples (feces) can be extremely useful in such cases.

Methods and results
We adapted methods of RNA extraction, RT-PCR, RT-QPCR and antigen capture ELISA to detect PPR viral particles or genetic material from fecal samples. The methods were validated with samples collected during an infection experiment. Our protocol was then used with fecal samples obtained in the field in Tanzania in 2015, and compared to results obtained from ocular swab samples taken from the same animals. Another issue is that existing LFD tests used in the field do not allow for direct confirmation by PCR. Here we present a new rapid penside test, produced and distributed by IDvet (France), which can be performed without any lab equipment. Lastly, we also tackled the issue of PPR antibody detection from camelid sera, usually suboptimal because of their particular antibody structure.

Results
Our results show that virus particles can be detected in fecal samples from 4 days post infection (dpi) until at least 14 dpi. Sensitivity of RT-QPCR from fecal samples was similar to RT-QPCR and lateral flow device (LFD) on ocular swab samples. The penside test was as sensitive as the antigen capture ELISA test. Once dried, positive strips can be stored and used later on for confirmation by RT-QPCR or RT-PCR. A simple modification of the protocol of a commercially available competitive ELISA for PPR antibody detection (IDvet) increased the sensitivity of the test on camelid serum.

Conclusion
These tools will be extremely useful to unravel important questions that still remain about PPR epidemiology, notably the role of atypical host in PPR transmission dynamics.

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Surveillance for avian influenza in wild birds: an overview of strategies in Italy in 2017–18

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Background
Wild water birds are known to be natural hosts for avian influenza (AI) viruses, posing a continuing threat for direct and indirect introduction of these viruses into poultry holdings. Since the emergence of the Asian lineage H5N1 Highly Pathogenic Avian Influenza (HPAI) viruses, the main value of surveillance in wild birds in Europe changed. Programmes shifted from the detection on a voluntary basis of Low Pathogenic Avian Influenza (LPAI) viruses of H5 and H7 subtypes, to a compulsory risk-based surveillance targeting high risk bird species based on their migratory movements and likelihood of contact with poultry.

Methods
In Italy, according to Commission Decision 2010/367/EC, passive surveillance system by laboratory investigation of moribund or found dead wild birds was implemented in 2017. Besides, in Veneto region – one of the Italian highest densely populated poultry areas –, investigator-initiated activities on hunted birds and through environmental sampling was carried out in wetlands and marshlands placed along flyways of migratory birds, and hosting wintering sites for wild waterfowl populations. Samples positive for the presence of the M gene underwent testing to identify virus subtype and pathotype.

Results
Samples from 2028 wild birds found dead or symptomatic were collected on the whole Italian territory, from January to December 2017. AI viruses were detected in approximately 1% (n=19) of birds from five different orders: Anseriformes, Columbriformes, Accipitriformes, Falconiformes and Pelicaniformes. HPAI viruses were detected in sixteen birds (15 H5N8 and 1 H5N5), and a LPAI virus was identified in one bird (H6N2). Of the 815 cloacal and tracheal/oropharyngeal swabs collected from hunted birds in Veneto region, 1.5% (n=12) tested positive for AI viruses. Three of these were identified as LPAI H7 viruses in birds belonging to the Anseriformes order. From July 2017 to April 2018, 1095 environmental samples of fresh avian faeces were collected in the same region. Out of 17 samples (1.6%) testing positive for AI viruses, subtypes were determined only in 7 cases as H5, H7N3, and H9.

Conclusion
Passive surveillance is deemed the most suitable approach for early detecting HPAI viruses. However, complementary investigator-initiated strategies provide an effective way to study the genetic diversity of AI viruses circulating in wild bird populations, which carry the risk of spill-over to poultry and other species. Environmental sampling is simple and cost effective, and might be a valid alternative to sampling strategies based on the more-invasive and capture-dependent methods, allowing regulating sample sizes over time.

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One health challenge: detection of a swine H1N2 influenza virus in commercial turkeys

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Background
Three subtypes of influenza A viruses (IAVs), H1N1, H1N2 and H3N2 are commonly found in pigs in Italy. These were found to derive from mammalian or avian viruses or their reassortants. Since January 2017, 83 outbreaks of highly pathogenic (HP)IAV(H5N8) were reported in North Italy, mainly in industrial farms. Of these, 39 were reported in meat turkey farms in the same area, which is also characterized by the highest concentration of the national poultry and pig production. This study reports a natural infection of swine(sw)IAV H1N2 in a turkey farm located in North Italy in an area subjected to movement restrictions due to a previous H5N8HPAIV outbreak near the farm.

Methods
IAV and H5, H7 and other HAs genome detection was performed on pools of 10 swabs each by real time RT-PCRs. Full genome was performed using NGS approach. Serological analyses were performed by competitive ELISA and HI test using different antigens belonging to H5, H7, and other subtypes of avian IAVs and H1N1, H1N2 and H3N2 swIAVs.

Results
A flock of 15280 10-wk-old meat turkeys experienced anorexia, depression and respiratory signs in a farm located in Lombardia Region. 60 tracheal swabs (TS) were taken following clinical signs and 105 TS and 45 sera before slaughter. PCR for IAV on one pool, collected after clinical signs, showed positivity near to cut off, whereas six pools collected before slaughter resulted strongly positive. PCR for H5, H7 and other avian IAVs resulted negative. Full genome sequencing on positive samples taken at slaughter showed the presence of a swIAVH1N2. Genomic analysis evidenced an interesting gene constellation resulting from reassortment between H1 human-like (A/Sw/Italy/4675/2003-like) and human seasonal H3N2 of 1997–98. Twenty-five % of the samples resulted serologically positive towards swIAVH1N2 (HI titres from 16 to 32).

Conclusion
Interspecies transmission of SwIAVs to turkeys has been reported in several cases although mainly caused by H1N1 or H3N2 strains. This report provides evidence of a slow circulation of a swIAVH1N2 in a turkey farm and strengthens the arguments that IAVs can cross species complicating diagnosis and characterization of new isolates. The presence of both avian and human-type receptors on turkey tissues supports the finding that turkeys can be infected with IAVs containing avian or mammalian HA genes and can act as a potential intermediate host for interspecies transmission and spread of reassortment viruses between birds and humans.

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Characteristics of H5N8 highly pathogenic avian influenza epidemic waves in Italy in 2017

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Background
In 2017, the Italian poultry sector was involved in the H5N8 highly pathogenic avian influenza (HPAI) epidemic that spread across Europe in 2016/17. Eighty-three outbreaks were confirmed, affecting both rural and industrial sectors, and occurred in two distinct epidemic waves.

Methods
Epidemiological investigations were conducted in each infected premises. Information was collected on flock demographics, holding type and animal housing, including presence of wild waterfowl and proximity to wetlands, and biosecurity measures. Moreover, data on clinical signs and movements of live birds, eggs, personnel and vehicles on and off the farm were obtained. To generate hypotheses on the likely source of infection and potential further dispersal of the virus, at-risk contacts were investigated on a case-by-case basis. To assist outbreak investigation, samples tested positive for H5N8 virus were genetically characterized to reconstruct the evolution and transmission dynamics.

Results
The first epidemic wave lasted between January and May 2017, affecting a total of 16 farms located on the fringe of the densely populated poultry areas (DPPA) in northeast Italy. The large majority of affected farms (n=14, 87.5%) were close to wetlands where substantial populations of wild waterfowl were reported. Contact tracking activities did not identify links between cases, and sequenced viruses revealed considerable nucleotide differences.
The second epidemic wave started in July, 50 days after the previous case. Sixty-seven farms in the DPPA were affected, with sparse incursions into western and central Italy. Up to late September, epidemiological and genetic characteristics were comparable to the outbreaks reported in the first wave. Since October a larger number of secondary cases was observed. At-risk contacts were detected among related outbreaks, and phylogenetic analyses revealed 99–100% similarity between viruses isolated in those premises. Furthermore, a smaller fraction of outbreaks were reported occurring in proximity to wetlands (n=39, 58.21%).

Conclusion
Italy had been the only European country being largely affected by H5N8 HPAI viruses in summer/autumn 2017. Integration of epidemiological and genetic data allowed hypothesizing the origin of infection. Point source introductions from wild reservoirs more likely occurred in the first epidemic wave: viruses showed larger genetic distances between viruses, and scarce contacts occurred among infected farms. Conversely, lateral spread could be inferred when a potential inbound or outbound movement was found within the contagious period, and phylogenetic analysis revealed high similarity among viruses. The re-appearance of HPAI in July also suggested the potential role of residential wild birds in maintaining and spreading influenza viruses.

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Revision of critical point in last phases of eradication plan in Sardinia

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Background
African Swine fever (ASF) is a highly contagious infection in domestic pigs and wild boars. The infection, frequently characterized by high mortality, is caused by DNA virus (Asfarviridae family) and occurs in different clinical forms: peracute, acute, sub-acute and chronic without clinical signs. Animals which survived to acute or chronic disease form, still remain persistently infected, acting as virus reservoirs. ASF is endemic in Sardinia since 1978 and virus persistence in main territory is still due to the presence of illegal pigs in free range breeding. In this region, the main eradication disease problems are related to the disease persistence in domestic pigs and illegal free-ranging pigs, and the correctly hunting season management, aimed to control the infection in wild boar (WB) population. In February 2014, in accordance with the European Commission, the Sardinian Region Authority developed a new ASF Eradication Plan 2015–18. This plan provides measures to eliminate the free-ranging pigs and to incentivize good practices, with satisfactory results in term of reducing number of outbreaks and a decrease in ASF prevalence. Furthermore, some critical points still remain:
(1) not homogeneous sample during hunting season,
(2) WB habit change,
(3) difficulties in WB cases notification.

Methods
Following this evaluation, and in order to improve the eradication of ASF in WB, authors recommend a specific decision maker based on GIS-technology. Three fundamental points of intervention have been identified: change hunting season management and WB sampling procedure; re-classification of WB density and single population area; re-definition of "WB primary and secondary cases" with different approaches.

Results
The three critical points have been evaluated based on expert opinions and the actions to put in plan have been assessed on new area limits: hunter companies activities and veterinary controls have been plane on municipality level. The WB areal smoothing populations were redesigned using the WB density cluster and the "use of soil" layer. These new areas of reference have been used in order to evaluate different scenarios of epidemiological correlation in WB case or outbreaks.

Conclusion
The use of geo-reference will allow us to replaced previous WB macro-areal with new population density smoothing clusters and, consequently, these new areal will contribute in provide new WB primary and secondary cases definition.

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Attenuated and non-haem adsorbing African swine fever (ASF) genotype II European isolate induces subclinical to chronic infection

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Background
African Swine Fever (ASF) is a serious viral haemorrhagic disease of swine. Since its introduction into Georgia in 2007, ASFV has spread westward into Eastern Europe reaching the EU in 2014. The eastern European genotype II ASFVs are majority haem adsorbing (HAD) and induce acute clinical forms with mortalities close to 100%. However, the increase seroprevalence found in certain areas of the EU countries, and the recent description of genotype II ASFV of moderate virulence, suggest virus evolution towards less virulent forms. In this study we have assayed the virulence of HAD and non-HAD genotype II ASFVs isolated within the EU in 2016–2017. The ability of the non-HAD isolate to cross-protect was determined as a means of recreating potential reinfections in field conditions.

Methods and Results
One non-HAD genotype II ASFV was isolated from hunted wild boar in Latvia (2017). To assess its virulence, two domestic pigs were intramuscularly inoculated with the non-HAD ASFV and four pigs were kept as in-contact pigs. Two HAD genotype II ASFVs from Poland and Estonia were assayed in parallel. Infected pigs with both HAD viruses developed acute to subacute form of the disease, while pigs infected with the non-HAD ASFV developed variable clinical manifestations from subclinical to chronic infections. Animals developing chronic lesions had viremia which lasted for over a month, while subclinical pigs presented weak viremia peaks or even remained negative. In spite of negative viremia, the DNA was present in the oral swabs for a period of 22 days with sporadic shedding of infectious virus with titers over 10^2TCDI50/ml. The ASFV genome was occasionally detected in faecal samples although no infectious virus was recovered. All pigs developed antibodies from 7 to 14 days and persisted until the end (4 months). At 58 days after the infection, two non-HAD infected pigs were exposed to a heterologous virulent genotype II ASFV. Non-HAD infected pigs were fully protected without exhibiting any clinical signs of acute disease, although HAD virus was retrieved from the blood and tonsil of one pig, 36 and 68 days after the challenge.

Conclusion
This study demonstrates the evolution of the genotype II ASFV from virulent towards to attenuate strains that could have implications for the long-term persistence of virus in the field because of the cross-protection produced by the attenuated isolates. This may also explain the presence of seropositive animals where no clinical disease had been reported.

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Comparative analysis of the central variable region of ASF virus genome of Russian isolates between 2013 and 2017

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Background
In recent years, ASF has become widely spread in the Russian Federation, Ukraine, countries of European Union (Estonia, Latvia, Lithuania, Poland, Romania and the Czech Republic) and Moldova. Registration in 2017–2018 of ASFV cases in the Czech Republic, and Romania, new outbreaks in Moldova and central Poland are good examples of the vector of transmission and the high risk of infection in countries of Western Europe.
A modern approach to investigate the molecular epidemiology of ASF by sequencing the C-terminal region of the p72 gene (B646L), which, along with the newly discovered genotypes in Ethiopia and Mozambique, identified 24 genotypes (C. J. Quembo et al., 2017). Further differentiation in subgroups of closely related viruses is usually performed by analyzing the sequences of p54 (E183L) gene and the central variable region (CVR) in the B602L gene (Gallardo et al., 2009).

Methods
In the reference laboratory for ASF FGBI "ARRIAH" (Russia, Vladimir), a study was conducted on 54 isolates obtained from 23 Russian regions in the period 2013–2017 by sequencing the Central Variable Region (CVR) of the ASF virus genome.
By comparing obtained data, single nucleotide mutations were detected at 4 loci: T / C at position 994 of the B602L gene in 6 isolates (Sudogda-Vladimir 16-DP, Arkhangelsk 16-DP, Tambov 2016, Krasnodar 2016, Gorokhovets-Vladimir 17-WB / 325, Orel 17-WB / 337), T / A at position 1,136 in 6 other isolates (Anino-Moscow 13-WB, Kashino-Tver 13-WB, Karamzino-Tver 13-WB, Shihobalovo-Vladimir 13-WB, Sobinka-Vladimir 15-WB, Sobinka-Vladimir 16-WB) and T / C: 979 , G / A: 1,115 simultaneously in 14 isolates (Che07, Abk07, Arm 07, Az08B, Az08D, Oren08, Ing08, StPet09, Kalmykia09, Rostov 09, Dagestan09, Ukr12 / Zapo, Krasnodar 07/15, Crimea 01 \ 16 Martins).

Results
Analysis of the amino acid sequence of these isolates showed that tetramer-tandem repeats (CADT, NVDT, CASM) are compatible with Georgia 2007/1 isolate (8 tetramers), and only two amino acids have been altered thr206 → ala206 in 14 isolates and lys201 → glu201 in 6 isolates, two other nucleotide mutations were silent.

Conclusion
In the future, identified genetic markers can help in differentiating between circulating isolates, as well as tracking the spread of the ASF virus between territories both within the Russian Federation and with bordering countries.

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Detection of astroviruses and hepatitis E virus in wild boars

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Background
Wild boars are natural reservoirs of many viruses, which can be transmitted to domestic pigs significantly influencing pig farming industry. Other viruses, as for example astrovirus, sapovirus, and kobuvirus, were also detected in wild boars but their impact on health status of domestic pigs is not clear. Hepatitis E virus (HEV) found in pigs has zoonotic potential. We systematically identify viruses in pigs and wild boars in Slovakia. In this work we report detection and genetic typing of astroviruses and HEV in wild boars.

Methods
For astrovirus, all together 200 samples of tissue homogenates (the cocktail prepared from spleen, kidney, and lymph nodes) of juvenile and adult wild boars from different regions of Slovakia were used in this study. The total RNA was isolated by Trizol method, cDNA was prepared using random hexamers, and virus was detected by a semi-nested PCR using primers detecting wide spectrum of astroviruses. The amplicons were sequenced and then phylogenetic tree was constructed by MEGA 6.0 program.
For HEV, 30 liver samples of wild boars were collected in hunting regions of Eastern Slovakia. RNA was isolated by Trizol method, synthesis of cDNA by gene-specific PCR primer and virus was detected by a nested PCR with primers selected from ORF1 region. The phylogenetic analysis was carried out similar as for astroviruses.

Results
Of 200 samples analysed, 30 (15%) were positive for astroviruses. Genetic typing revealed that of 13 amplicons analysed, 8 were typed as porcine astrovirus lineage 2, and 2 as lineage 4. In addition, 2 identical sequences were grouped with chicken astrovirus which were different of astroviruses found in hens and pheasants from Slovakia. One nucleotide sequence was highly related to a sequence of a bat astrovirus analyzed in Germany. Of 30 liver samples, 15 (50%) were positive for HEV which originated from wild boars of five hunting regions of Eastern Slovakia. Phylogenetic analysis confirmed that viral isolates were typed as HEV-3 genotype which is genetically close to human HEV-3 isolates. No doubt that there is potential high risk for transmission of HEV from wild boars to human.

Conclusion
In this work porcine astrovirus was confirmed for the first time in parenchymatic organs of wild boars. In addition chicken astrovirus and bat astrovirus were detected as well. HEV-3 with zoonotic potential was often detected in liver of wild boars raising question on safe game meat.

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Identification of a new reassortant swine MRV3 during a PED outbreak in Italy

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Background
Mammalian Orthoreoviruses (MRV) are segmented dsRNA viruses belonging to the Reoviridae. MRVs can infect mammalian species, causing asymptomatic, respiratory, or gastro-enteric infections. MRV are dived into: MRV1, MRV2 and MRV3. Swine MVR3 was detected during gastro-enteric episodes in China and Korea, and recently in U.S.A. during Porcine Epidemic Diarrhea (PED) outbreaks. The first detection of swine MRV3 in Europe was in 2015 during severe PED outbreaks in North Italy: Lombardia region. Here we report the identification of a new variant of swine MRV3 in a neighbouring region (Veneto) during a PED outbreak.

Methods
In February 2016, a suspected case of PED in a swine farm was registered. Six faeces samples from six different animals were collected. All samples were tested by Real time RT PCR for PEDV and inoculated into confluent monolayers of VERO cells. Positive CPE cells were tested by RT-PCR targeting the L1 MRV. One CPE positive sample was processed on Illumina Miseq. Reads were qualitatively filtered and assembled independently with a de novo approach via IDBA-UD and GapFiller to produce the corresponding segment. Maximum Likelihood phylogenetic trees were generated using Iqtree v.1.6.1.

Results
The swine farm resulted positive for both PEDV and MRV3. The isolated strain (MRV3/IT/2016) showed high similarities with MRV3 detected in Lombardia in 2015 (MRV3/IT/2015), except for L2 and M2 segments. The L2 and M2 segments showed high similarity to swine MRV3 and MRV1 detected in U.S.A. in 2014 and China in 2011, respectively. The Italian MRV3/2016 possess the S1 gene highly correlated to bat MRV3 detected in Italy in 2012 and human MRV3 detected in Slovenia in 2013 and Switzerland in 2016. The recombination analysis confirmed that the American and Chinese strains might be the donors for the L2 and M2 genes, respectively. For all the other genes, the MRV3/IT/2016 showed a higher genetic similarity with other swine and human MRV strains. Additionally, the MRV3/It/2016 shows unique S1 aminoacid mutations compared to the swine MRV3s.

Conclusion
The novel variant of swine MRV3/IT/2016 most probably originated by a reassortant event involving: the MRV3/IT/2015, the American/2014 and the Chinese/2011 strain. Interestingly, so far swine MRV3 has been detected in Europe and USA during PED infections. The association of PED and MRV should be further investigated, given the importance of PED for the pig industry. The detection of swine MRV3 with high homology to human isolates warrants further studies on their potential zoonotic impact.

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Sequencing of the tandem repeat sequences (TRS) within the intergenic region between the multigene family 505 9R -10R genes: additional tool for subtyping genotype II African swine fever (ASF) isolates

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Background
Since the introduction of African swine fever virus (ASFV) in Poland in February 2014, 108 outbreaks in pigs and almost 2000 notifications in wild boar had been reported. The disease is creeping west bit by bit, arising concern for the large pig producers. Early detection and coordinated countermeasures are urgently needed. For these countermeasures, information on disease dynamics is mandatory and places the molecular epidemiology as an essential tool for epidemiological investigation. This study reports the genetic characterization of 372 Polish ASFV isolates belonging to genotype II by the analysis of an additional ASFV genome marker located at the left-end of the ASFV genome.

Methods and Results
372 Polish ASFV isolates (2014–2017) representatives of 322 wild boar cases and 50 domestic pig outbreaks were selected for genotyping. Sequences were compared with 32 genotype II European ASFVs including Russian and EU ASFV isolates (2007–2017). Initial approach was focused on the analysis of the tandem repeat sequences (TRS) located in the intergenic regions (IGR) between the I73R and I329L genes. With the exception of one wild boar ASFV isolate (case 237), all Polish viruses clustered within the IGR variant 2 (IGR2), according to the nomenclature of Gallardo et al 2014. The ASFV isolate Pol17/WB-CASE 237 collected 10 km far from the Belarussian border clustered within the IGR-1 variant, which never had been found in the EU, but is present in recent Russian ASFV isolates.
To gain more knowledge on the genetic evolution of ASFVs, the IGR located between the 9R and the 10R genes belonging to the multigene family (MGF) 505 was amplified. The results showed two different variants circulating within the wild boar population in Poland, at the Podlaskie province, close to the Belarusian border. One variant (MGF-1) was identical to the Georgia and Poland index cases, and the other variant, characterized by a TRS insertion of 17 nts (MGF-2), present 100% of homology with some ASFV isolates circulating in Russia since, at least, 2012. Identical TRS insertion was found from the ASFV isolated from the domestic pig outbreak nº 32.

Conclusion
This study reaffirms the suitability of the TRS for a higher resolution of ASFV molecular epidemiology. The two methods described can be applied to trace the variability of genotype II ASFV. With this additional information, a more confident clustering of ASFV isolates as well as molecular tracing of new outbreaks now seem to be possible.

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Successive (sub) clinical rotavirus infections in young suckling and nursery piglets on conventional and high health closed Belgian pig farms

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Background
Rotavirus A and C are commonly found in feces of young suckling and weaned piglets. The pathogenesis has been well studied in gnotobiotic pigs but their importance under field conditions has been poorly addressed. Here, occurrence and impact of rotavirus infections under real farm conditions were investigated.

Methods
For the first time, rotavirus shedding was quantitatively assessed during a longitudinal study in suckling and/or nursery pigs of seven conventional pig farms and one high health farm in Belgium. Five to ten animals per farm were selected and individual rectal swabs were collected every two to four days. RT-qPCR analysis for RVA and RVC was done to quantify the virus in feces and to make associations with growth and diarrhea. At the peak of viral shedding, the genotypes of genes coding for the outer capsid proteins VP7 and VP4 were determined. Escherichia coli was isolated on agar plates under aerobic conditions.

Results
In suckling piglets, clinical RVA infections were only observed in one litter of a conventional farm and were likely complicated by low environment temperature. A negative correlation (r^2=0.72, p-value 0.0079) between daily weight gain and RVC shedding in suckling piglets was observed on a high health farm, without signs of diarrhea. In nursery pigs, on all farms shedding of RVA started around weaning, leading to two or three successive replication waves with genetically heterogeneous strains. Similar successive RVC waves were observed on all farms after weaning, but mostly occurring in between or simultaneously with RVA shedding. Explosive diarrhea in weaned piglets was not observed, except on one farm with a coinfection of RVA, RVC and F18+ Escherichia coli. Impact of rotavirus replication on growth in weaned piglets varied between farms. Virus shedding after weaning was lower in pigs from the high health farm with strict cleaning and disinfection protocols. Applying strict cleaning and disinfection methods typically led to circulation of only two to three unique RVA VP7/VP4 combinations in the nursery barn, compared to five to seven unique VP7/VP4 combinations on farms using disinfection only.

Conclusion
In conclusion, successive subclinical rotavirus replication waves were occurring in all pigs on all Belgian pig farms studied. Severe rotavirus infections had an impact on growth in the suckling piglets, but only caused explosive diarrhea in weaned piglets during co-infections. Better hygienic measures led to an overall lower rotavirus infection pressure and caused less circulation of genetically different variants on the farms.

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The interplay between equine β-defensins and local viral pathogens in the horse's respiratory tract

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Background
The horse's respiratory tract needs to counter airborne pathogens on a daily base. Therefore, its epithelia are guarded with a repertoire of immune barriers, including antimicrobial peptides such as β-defensins. The three main equine β-defensins (eBD1, 2 and 3) have not been fully characterized in horses. Especially at the level of the respiratory tract, their distribution profile and their role as antimicrobial and immunomodulatory peptide remains obscure. Therefore, this study aimed at unravelling the interplay between local eBD and important equine respiratory viruses. Equine influenza virus (EIV), equine herpesvirus type 1 (EHV1) and equine arteritis virus (EAV) are three major viral causes of respiratory infections in horses.

Methods
First, eBD1, 2 and 3 were mapped in the horse's respiratory tract using RT-PCR and immunofluorescence staining. Concurrently, eBD were commercially synthetized, oxidized in-house and evaluated using MASS-spectrometry. In a synchronized assay, the temporal effect of the eBD on different steps in the infection of RK13 cells (by EAV and EHV1), MDCK cells (by EIV) or primary equine respiratory epithelial cells (EREC) (by EIV and EHV1) was studied. To characterize attachment of EHV1, direct virus-binding studies were carried out with Dio-labelled EHV1-particles. Next, the upregulation of eBD in EIV-, EHV1- and mock-inoculated EREC was examined using confocal microscopy and image analysis. Finally, chemotactic activity of the eBD on different equine blood leukocytes was investigated using a cell migration assay.

Results
Virus pre-treatment with eBD2 and 3, but not with eBD1, significantly decreased the formation of EIV and EAV plaques in MDCK and EREC or RK13 cells, respectively, compared to control. Remarkably, virus pre-treatment with the same eBD caused an increase of EHV1 infection in RK13 cells, but not in EREC. However, cell pre-treatment with eBD1, 2 and 3 did increase subsequent susceptibility of EREC to EHV1. Binding assays showed that these eBD enhanced EHV1-attachment by concentrating virus particles on the RK13 and EREC cell surfaces. Next, we demonstrated that EHV1-, and to a lesser extent EIV-infection, upregulates expression of eBD2 and 3 in EREC and that all eBD attract blood leukocytes.

Conclusion
While eBD act against EIV and EAV, they enhance EHV1 infectivity by concentrating virus particles on the cell surface. Moreover, EHV1 orchestrates EREC to synthetize eBD, which in turn attract blood leukocytes, essential for EHV1 spread within the host. Our results show that the ancient alphaherpesvirus EHV1 has generated eBD-exploiting strategies, while EIV and EAV remain susceptible to eBD.

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Equine herpesvirus 1 bridles T-lymphocytes to reach its target organs

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Background
Equine herpesvirus 1 (EHV1) replicates in the epithelium of the upper respiratory tract and disseminates through the body via a cell-associated viremia in leukocytes, despite the presence of neutralizing antibodies. 'Hijacked' leukocytes, previously identified as monocytic (CD172a+) cells, T- and B-lymphocytes, transmit the virus to endothelial cells of the endometrium or central nervous system, causing neonatal foal death, late-term abortion or neurological disorders. These clinical symptoms have extensive economic consequences in horse populations worldwide. Here, we verified the susceptibility of equine T-lymphocytes to EHV1, and aimed at unraveling the infection route of T-lymphocytes, either by cell-free virus or by cell-to-cell spread.

Methods
Primary blood- and lymph nodal-derived T-lymphocytes were cultivated in the presence of interleukin-2 (IL2) and inoculated with cell-free virus. At 1, 3, 6, 9, 12 and 24 hpi T-lymphocytes were collected, followed by immunofluorescence staining for immediate early (IE) and late viral proteins. Next, we investigated whether cell-to-cell spread might serve as an alternative route of T-cell infection. Activated T-lymphocytes were co-cultured with EHV1-inoculated fully permissive RK13 cells, primary respiratory epithelial cells (EREC) or autologous monocytic cells, followed by immunofluorescence staining. The production of progeny virus in T-lymphocytes was analyzed by determining the intra- and extracellular virus titers. Next, a viral plaque assay containing EHV1-inoculated T-lymphocytes and venous endothelial cells was carried out, to quantify transmittable infectious virus.

Results
Following exposure of primary T-lymphocytes to cell-free virus, IE proteins were detected in the cell nuclei at 1 hpi and replicative compartments were formed at 3 hpi. 'Leaky' late gB and late gC proteins were initially detected at 3 and 6 hpi, respectively. Furthermore, activation of T-lymphocytes with IL2 showed a significant increase in EHV1-positive T-lymphocytes, compared to quiescent T-lymphocytes. Once T-lymphocytes were co-cultured with EHV1-inoculated RK13 cells, EREC or monocytic cells, we detected EHV1 transmission and replication in T-lymphocytes, indicating that cell-cell spread is an alternative mode of viral transfer. Despite early expression of all viral proteins, the production of progeny virus particles remained restricted. However, upon cell-cell contact with target endothelial cells, infectious particles were formed in the T-lymphocytes and efficiently transmitted.

Conclusion
T-lymphocytes are susceptible for EHV1-infection and cell-cell contact transmits infectious virus to and from T-lymphocytes, which has significant implications for the understanding of the EHV1 pathogenesis and suggests that T-lymphocytes are important in the transport of EHV1 to the equine pregnant uterus or central nervous system.

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Typical morphological and structural alterations in equine canker point to a viral infection – are papilloma viruses really the missing link?

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Background

Recently described histological alterations in equine hoof canker, especially koilocytosis [1], in combination with bovine papillomaviruses 1 and 2 (BPV 1/2) reported to be involved in its etiology [2] led to the hypothesis that viral infection is a prerequisite for the development of clinical signs of canker. The aim of the present study was to investigate the degree of koilocytosis and the changes in the intracellular cytokeratin (CK) matrix in canker tissues in relation to the presence of BPV 1/2 infection.

Methods

Morphological and immunohistochemical analyses were performed on surgically removed canker tissues of 19 horses presented at our clinic for treatment of clinically obvious canker and on control tissues of seven hooves of unaffected horses. Light microscopy was used to grade for severity of koilocytosis, immunohistochemistry for CK filament distribution. All tissue samples were screened for BPV 1/2 DNA using PCR, emphasizing on the major transforming oncogene E5 and the late gene L1 [2].

Results

The presence of BPV 1/2 DNA in canker tissues was inconsistent (5/19), but there was consistent evidence of koilocytosis and changes in CK filament distribution patterns (19/19), both typical for viral infection. The degree of alterations was independent of the detection of BPV 1/2 DNA. No morphological or immunohistochemical alterations were seen in control tissues. In the canker affected population studied, the rate of BPV 1/2 infection was actually below the reported BPV 1/2 infection rate of healthy horses [3] and comparable to the rate of infection seen in our control group (3/7).

Conclusion

This study found changes typical for viral involvement, but no connection between BPV 1/2 infection and the presence or degree of koilocytosis or changes in CK filament distribution. Nevertheless, as these are typical pathomorphological and intracellular changes described in viral infection, the mystery of etiology of equine canker remains unsolved. Future studies will either identify a causative virus or the strong association between such cytological changes and viral infection will have to be questioned.

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The New Equine Virus (NEV): A lentivirus that has proteome identity with HIV-1

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Background

A New Equine Virus (NEV) was isolated from horses with anaemia and neurological signs that presented Equine Infectious Anemia virus (EIAV) – discordant results. These horses were positive in EIAVpv immunoblotting (gp90, gp45 and p26), but negative in EIAV official tests, AGID and commercial ELISAs.

Methods

In an attempt to propagate and isolate this virus, equine macrophage-like (EML) cells lines were established from three horses with EIAV-discordant results. The official EIAV tests used were the commercial AGID kit (IDVet) and two commercial ELISAs (VMRD, Eradikit). The EML cells were processed according to Fidalgo-Carvalho, et al (2009). Viral replication and propagation was made in newly established EML cells and equine dermal (ED) cells. Cytophatic effects were determined by plaque assays and PrestoBlue cell viability assays. To identify the EIAV genome, qPCR, RT-qPCR and conventional PCR were performed using several EIAV-specific primers. Purification of viral particles was obtained by ultracentrifugation in 20% sucrose gradient at 4ºC, 50.000g for 1 hour. Proteome analysis of purified viral particles was done by SDS PAGE, in-gel digestion and LC-MS/MS analysis using the AB Sciex® 5600 TripleTOF equipment and recommended procedure for peptide analysis. Protein identification was obtained using ProteinPilot™ software (v4.5, AB Sciex®). Viral ultrastructure was examined by positive and negative staining under Transmission Electron Microscopy (TEM) (JEOL JEM 1400 TEM).

Results

Cell syncytia formation and cytopathic effects (CPE) appeared after passaging and viral transfer between NEV-infected EML cells. Moreover, NEV can also replicate in Equine Dermal (ED) cells. But unlike EIAV, NEV is a highly cytophatic virus in ED cells with complete destruction of the cell monolayer after 7-12 days. After several attempts the EIAV genome failed to be amplified in viral RNA and/or proviral DNA obtained from equine PBMCs, infected EML cells and/or viral RNA obtained from gradient purified viral particles. Furthermore, proteome analysis showed distinct protein patterns between NEV and EIAV in SDS-PAGE. Moreover, mass spectrometry analysis of NEV viral particles purified and positive for Reverse transcriptase (RT) retrieved more than 76 short peptides (<30 aa). Of these 62 were blasted against public databases (UNIPROT KB or NCBI), and 14 blasted against Equigerminal database. Of the 62 peptides, 26 (42%) have sequence identity to part of the HIV-1 proteome, 45 (73%) have identity to retroviridae proteome, but none of these had identity to EIAV proteome. Purified NEV viral particles show a lentivirus core morphology by TEM negative staining. TEM positive staining of infected cells also showed that NEV viral budding occurs through the plasma membrane, such as other lentivirus.

Conclusion

Finally, the results obtained show that NEV is a lentivirus different from EIAV that has similarity with the HIV-1 proteome.

Oral Session 4.2
Viral infections of Equines

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Serological evidence of horses' infection with ZIKAV and DENV1 in French pacific islands

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Background
New Caledonia (NC) and French Polynesia (FP) are areas with high circulation of arboviruses. A large serosurvey among horses from NC and FP was carried out to investigate the seroprevalence of flaviviruses in the horse population.

Methods
293 equines were sampled and sera analyzed against flaviviruses using a competitive enzyme-linked immunosorbent assay (cELISA). The positive sera were then confirmed by flavivirus-specific microsphere immunoassay (MIA) and seroneutralization tests.

Results
This serosurvey highlighted that 16.6% (27/163) and 30.8% (40/130) of horses were positive with cELISA in NC and FP respectively while the MIA technique, targeting only flaviviruses responsible for neuroinvasive infections in horses (i.e. WNV, JEV, and TBEV) showed negative results for more than 83% (56/67) of ELISA-positive animals. Seroneutralization tests with the main flaviviruses circulating in the areas revealed that 6.1% (10/163; confidence interval [95% CI] 2.4%–9.8%) of sera in NC and 7.7% (10/130; 95% CI 4.8%–15.2%) in FP were positive for DENV1 while 4.3% (7/163; 95% CI 1.2%–7.4%) in NC and 15.3% (20/130; 95% CI 9.1%–21.5%) in FP were found positive for ZIKAV. Comparatively, seroprevalence of JEV and WNV flaviviruses were much lower (less than 2%) in the two areas.

Conclusion
This seroprevalence study in horses' population shows that horse population can be infected by DEN and ZIKA arboviruses and that these viruses induce seroconversions in horses. The consequences of these infections in horses and the role of horses as amplifier host of these viruses are some questions which deserve complementary investigation.

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Viral infections of Swine 2

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Porcine blood T-lymphoblasts support the replication of porcine circovirus type 2

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Background
Lymphoblasts are important target cells for porcine circovirus type 2 (PCV2) in pigs. Pigs suffering from PCV2-associated diseases (PCVAD) display lymphocyte depletion and monocyte infiltration in lymphoid tissues as a result of PCV2-replication in lymphoblasts. Little information is present on the kinetics of this replication. Therefore, this study aimed to generate a T-lymphoblast cell line as an in vitro model to study PCV2 replication.

Methods
To generate proliferating T-lymphoblasts, blood lymphocytes were isolated, stimulated with concanavalin A for 3 days and interleukin-2 continuously. Replication kinetics of PCV2 in T-lymphoblasts were investigated by a time-course study. PCV2 binding and entry were visualized with confocal microscopy. PCV2 entry and disassembly were examined by chemical inhibition of endocytic pathways and endosomal-lysosomal system acidification. To evaluate PCV2 infection, infected cells were immunostained and quantified by confocal microscopy, while the extracellular virus was quantified by titration.

Results
The development of a lymphoblast cell line was successful. After inoculation with PCV2 at a multiplicity of infection of 0.05, around 0.5% of T-lymphoblasts weakly expressed cap protein at 12 hpi. At 24 and 36 hpi, capsid was mainly found in the nucleus of 3.3% and 2.6% of the cells, respectively. Rep was always found in the nucleus of 2.7% of the cells at 24 hpi and 1.4% at 36 hpi. The titer of extracellular virus gradually increased from 10^3.6 $TCID_{50}$/mL at 1 hpi to 10^4.6 $TCID_{50}$/mL at 72 hpi. Around 26% of the T-lymphoblasts showed virus binding and internalisation. PCV2 infection was not affected by methyl-β-cyclodextrin. In contrast, it was decreased by (i) chlorpromazine (31±11% reduction), (ii) cytochalasin D (26±20% reduction), (iii) dynasore (34±18% reduction), and (iv) amiloride (16±6% reduction). In addition, PCV2 infection was significantly reduced by the lysosomotropic weak bases ammonium chloride (81±7% reduction) and chloroquine diphosphate (80±1% reduction).

Conclusion
The in vitro generated T-lymphoblasts support PCV2 replication. PCV2 uses clathrin-mediated endocytosis and macropinocytosis to enter T-lymphoblasts, with actin and dynamin being involved in the process. PCV2 needs an acid environment for virion disassembly.

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Vaccination with a recombinant PCV2-ORF2 vaccine does not interfere with the efficacy of a modified live vaccine against PRRSV

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Background

The vaccine against Porcine Circovirus 2 (PCV2) is one of the most efficient vaccines currently used. PCV2 is the primary agent of PCV-related diseases which include post-weaning multisystemic wasting syndrome (PMWS) and Porcine Dermatitis Nephropathy Syndrome (PDNS). Besides the reduction of clinical signs, vaccination with the PCV2-ORF2 vaccine is able to reduce viral and bacterial co-infections. For example, a reduction of the PRRSV loads during PRRSV infection was described. Nevertheless, vaccination against PRRSV is the most important strategy to combat PRRSV and available vaccines are mostly based on modified live viruses of the European or American species. With the knowledge of a reduced viremia after PRRSV infection by PCV2 vaccination one might postulate that a simultaneous vaccination with a PCV2-ORF2 vaccine has a negative influence on the efficacy of PRRSV-modified live vaccine.

Methods

24 four week-old PRRSV-negative piglets were divided into 5 groups, the 1st group (n=3) served as control group, the 2nd group (n=3) was immunized with a commercially available vaccine against PCV2 (CircoFLEX®, Boehringer Ingelheim), the 3rd group (n=6) was vaccinated with a MLV-PRRSV vaccine (PRRSFLEX®, Boehringer Ingelheim), the 4th group (n=6) was immunized with PRRSFLEX® + Carbopol, and the 5th group (n=6) was immunized with PRRSFLEX® together with CircoFLEX®. Blood was taken at different time points after vaccination (days 0, 7, 10, 14, 21, 28, 35) and analyzed for antibodies against PRRSV (IDEXX X3 Elisa), PRRSV neutralizing antibodies, the induction of PRRSV-specific T-cells in IFN-γ ELISPOT assays and flow cytometric analyses using intracellular cytokine staining for the detection of IFN-γ, IL-2 and TNF-α within T-cell subpopulations.

Results

Independent of a co-vaccination with CircoFLEX® or treatment with Carbopol all PRRSFLEX® vaccinated swine developed antibodies against PRRSV starting 10 days after vaccination. 14 days after vaccination most of the pigs showed neutralizing antibodies. Interestingly the neutralization titers of the double vaccinated group were more homogeneous. Most animals developed an antigen-specific IFN-γ response 14 days after vaccination. PRRSV-specific T cells showed both CD4 and CD8β phenotypes and especially in CD4+ T cells multifunctional PRRSV-specific cells producing IFN-γ, IL-2 and TNF-α could be detected.

Conclusion

PRRSFLEX® is able to induce PRRSV-specific antibodies within 10 days after vaccination, within 14 days neutralizing antibodies could be determined as well as PRRSV-specific IFN-γ producing T cells and multifunctional TH1 cells. A co-vaccination with CircoFLEX® had no influence on the efficacy of the modified live vaccine and the induction of a PRRSV-specific immune response.

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A recombinant strain from two genotype 1 PRRSV modified live vaccines shows increased replication and transmission in SPF pigs

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Background

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most costly disease for swine industry worldwide. This disease is characterized by reproductive failure in sows and respiratory disorders and growth retardation in growing pigs. The causing agent, PRRS virus (PRRSV) is a positive-sense single-stranded RNA virus belonging to the Arteriviridae family. In Europe, modified live vaccines of genotype 1 (MLV1) are commonly used to control PRRSV infection in swine herds.
In December 2014, following successive implementation of a PRRS vaccination program with Unistrain® (Uni) and Porcilis® (Porci) PRRS vaccines in a farm, a recombinant strain (Rec) from the 2 commercial attenuated strains was isolated. In order to assess the virulence of the recombinant strain, we set-up an in vivo study to compare clinical, virological and transmission parameters with the 2 parental MLV1 strains.

Methods

Three groups of 6 SPF piglets were respectively inoculated with one of the MLV1 (Porci and Uni groups) or the recombinant strain (Rec group). Twenty-four hours after inoculation, 6 contact piglets were added to each inoculated groups to evaluate PRRSV transmission. The fourth group was composed of 6 SPF piglets (negative control group). All animals were monitored daily. Blood and nasal swabs were collected twice a week after inoculation to monitor the viral genomic load. During necropsy, samples were collected for additional quantification of the viral genome in tissues.

Results

The vaccine and the recombinant strains did not induce clinical sign. PRRS viral load in inoculated piglets of Rec group was higher in serum, nasal swabs and tonsils in comparison with piglets from vaccine groups. The first viremic contact animal was detected as soon as 2 dpi in the Rec group compared to 10 dpi and 17 dpi for Porci and Uni groups respectively. Similarly to inoculated animals, the level of viremia of contact piglets from Rec group was 10 to 100 fold higher than for contact piglets from Porci and Uni groups. Estimation of transmission parameters by mathematical modeling showed a daily transmission rate (number of infected pigs by an infectious pig per day) of 0.57 for Rec group compared to 0.08 and 0.11 for MLV1 groups.

Conclusion

Our in vivo study showed that the recombinant strain from 2 MLV1 demonstrated higher replication and shedding and faster transmission compared to the parental vaccine strains. Considering these observations, measures should be implemented to avoid recombination phenomenon of PRRSV vaccine strains under field conditions.

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PRRSV-infection induces T cells with an early effector phenotype in the foetal placenta

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Background
Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important viruses affecting the global swine production. Infection of sows with PRRSV-1 and PRRSV-2 strains can cause reproductive problems like abortions, early farrowings, foetal death, and the birth of weak, congenitally infected piglets. So far, most research efforts focused on the respiratory form of PRRSV-infection but only little is known on PRRSV transmission, pathological mechanisms and the corresponding immune response in the uterus.

Methods
Three groups of gilts (n=4/group) were infected at day 84 of gestation with two different PRRSV-1 isolates, designated 720789 and AUT15-33, or kept non-infected. Around 21 days post infection sows were euthanized and foetuses analysed for preservation status. PRRSV loads were determined in various organs by qRT-PCR. From two foetuses per litter, lymphocytes and myeloid cells were isolated from uterine tissue following separation of the maternal endometrium and the foetal placenta. These cells were subjected to an immuno-phenotyping focusing on macrophages, NK cells, CD4, CD8 and γδ T cells alongside to molecules involved in the differentiation of these leukocyte subsets.

Results
Around 44% of foetuses (30 out of 68 foetuses) derived from sows infected with AUT15-33 were either autolysed, decomposed or meconium-stained. In contrast, only one foetus (out of 53) from 720789-infected sows was autolysed and another one meconium-stained, illustrating substantial differences in the virulence of the two strains. In utero PRRSV transmission to foetuses occurred in about 60% of foetuses from gilts infected with PRRSV AUT15-33, while less than 10% of foetuses from gilts infected with PRRSV isolate 720789 were virus positive in foetal thymus and/or foetal serum. Immuno-phenotyping results showed an increase of both CD4 and CD8 T cells with an early effector phenotype (CD8α+CD27+ and CD27dim, respectively) in lymphocytes isolated from the foetal placenta of AUT15-33-infected sows in comparison to foetuses from 720789-infected sows and non-infected control sows. In lymphocytes isolated from the maternal endometrium a rise in early effector CD8 T cells was found in both infected groups in comparison to non-infected sows.

Conclusion
Our results indicate an involvement of T cells located in the foetal placenta, in the uterine immune response against PRRSV. Whether these activated T cells are of maternal or foetal origin and the potential role of these cells in a hitherto uncharacterized immunopathogenesis of PRRSV infections requires further investigation.

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Correlation of serological response after vaccination against FMDV and protection against challenge in pigs

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Background
Vaccination is one of the most effective ways to protect animals against infection with FMDV. For cattle, the correlation between antibody levels and protection against challenge has been studied extensively. For other species, these data are scarce. The objective of this study was to estimate whether the VNT titre after vaccination in pigs could be correlated with protection. For this, we used data of previously performed experiments in which vaccinated pigs were challenged with FMDV.

Methods
We used data of previously performed experiments. Data for each pig (n=63) consisted of: the vaccine strain (O Taiwan or O Manisa), the challenge strain (O Taiwan or O Netherlands), the VNT titres (log10) against the vaccine strain, challenge method (intradermal inoculation, contact with (non-)vaccinated infected animals), type of challenge (homologous or heterologous to vaccine) and protection status. The relationship between antibody titre and the probability of protection against either infection (virus excretion) or disease (clinical signs) was evaluated by fitting a binomial regression model where protection was the response variable and antibody titre was the explanatory variable. Additionally the effect of the type of challenge and the used challenge method on the probability of protection was evaluated by adding these variables in the model. The titre at which 50% protection of the vaccinated pigs is expected (VNT50) was calculated.

Results
The VNT titre elicited by vaccination had a significant association with protection. Neither the type of challenge nor the challenge method had a significant effect in the models. The estimated VNT50 against infection was 1.8 (1.4–2.2). To protect against disease, a lower VNT50 was needed, 1.4 (1.0–1.7).

Conclusion
Two models were developed where the expected probability of protection for pigs against challenge with FMDV could be quantified. The antibody thresholds (VNT50) that were estimated could be used as correlates of protection to assess vaccine efficacy and or vaccination effectiveness.

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The novel pestivirus species "LINDA virus"

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Background
A so far unknown pestivirus species was discovered in 2015 in an Austrian piglet producing farm during virological examinations of severe congenital tremor cases. Affected piglets suffered from pronounced hypomyelination of the white substance in cerebellum and spinal cord and exhibited high mortality rates.

Methods
"Linda" virus (Lateral shaking Inducing Neuro-Degenerative Agent") could readily be isolated and propagated in cell culture providing a basis for the experimental study of this agent. Cell culture growth was assessed on different cell lines, immunological reagents and a qRT-PCR assay against Linda virus were established. Positive sera were generated by virus infection using a small scale animal experiment. For this purpose, 21 post-weaning piglets (12 weeks old) were housed in a biological safety unit (BSL2) and divided in three different groups. One group was infected intra-nasally and one group intra-muscularly with 1 x 10^7 TCID50 each. A mock infected group served as a separated control, while 3 sentinel animals were included in the infection groups at the day post infection.

Results
Linda virus was of low pathogenicity for the post-weaners. All infected animals and one sentinel animal showed a sero-conversion measurable by high titers of virus neutralizing antibodies. Linda virus was detectable in the sera of infected animals and shedding via oral, nasal and faecal routes was substantiated by qRT-PCR analysis. Furthermore, all infected animals displayed Linda virus RNA presence in the tissue of their tonsils at necropsy four weeks after infection. A sentinel animal was infected proving that Linda virus was shed after the experimental infection.

Conclusion
The severe neurological symptoms observed in the initial outbreak of Linda virus were not reproduced in the experiment and hence, the neonatal neuronal symptoms were most probable a consequence of persisting intra-uterine infections. Further experiments are needed to study the pathogenicity of Linda virus after intra-uterine infections.

Oral Session 5.2
Viral infections of Carnivores

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Canine respiratory viruses in New Zealand

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Background
Infectious canine tracheobronchitis (ICT), often referred to as "kennel cough", is an acute, highly contagious respiratory disease of dogs. It usually affects young dogs in a multiple-dog environment. Typically, affected dogs present with acute onset of paroxysmal dry hacking cough and nasal/ocular discharge. The aetiology of ICT is complex, and the expression of disease is likely to be affected by a number of host-, environment- and pathogen-related factors. Traditionally, canine para-influenza virus (CPiV), canine adenovirus 2 (CAdV-2), and Bordetella bronchiseptica were regarded as most commonly involved in ICT, and are incorporated into available vaccines. However, other pathogens have also been detected from diseased dogs including canine herpesvirus 1 (CHV-1), canine reoviruses, canine distemper virus as well as several novel pathogens such as canine influenza viruses, canine respiratory coronavirus (CRCoV), canine pneumovirus (CnPnV), canine bocavirus, canine hepacivirus, and canine picornavirus (CanPV). The objective of the present study was to identify what viruses circulate among selected New Zealand dog populations and to determine which of these are likely to be aetiologically involved in ICT.

Methods
Shotgun sequencing was used to determine what viruses were present in pooled samples of oropharyngeal swabs collected from dogs with ICT (n=50) and from healthy dogs (n=50). Subsequently, real-time PCR assays were used to assess the frequency of detection of canine respiratory viruses (CPiV, CAdV-2, CHV-1, CRCoV and CnPnV) in individual oropharyngeal swab samples. In order to gain some insight into the epidemiology of CRCoV in New Zealand, canine sera (n=100) were randomly selected from diagnostic submissions on a monthly basis from March to December 2014, and analyzed for the presence of CRCoV antibodies.

Results
Sequences of CHV-1, CRCoV, CnPnV, CanPV and influenza C virus were identified in the pooled sample from dogs with ICT, but not from healthy dogs. Individual samples from ICT-affected dogs were most commonly positive for CnPnV (26.8%) followed by CAdV-2 (8.9%), CPiV (3.7 %), CHV-1 (3.6%), and CRCoV (1.8%). Only CAdV-2 (5.0%) and CnPnV (18.3%) were identified in individual samples from healthy dogs. Overall, 52.8% of 1015 sera tested were positive for CRCoV antibody. There was an association between CRCoV seropositivity and age of dogs, as well as presence of respiratory disease.

Conclusion
This is the first identification of CnPnV, CanPV and influenza C virus among New Zealand dogs. Possible involvement of "newer" pathogens in ICT may provide one explanation for occasional development of ICT in fully vaccinated dogs.

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Mapping mutations related to cross species infection in canine distemper virus isolates from the Serengeti National Park

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Background
Canine distemper virus (CDV) is a member of the Paramyxoviridae family, genus morbillivirus, order Mononegavirales. CDV infects a wide range of carnivores, both domestic and feral dogs as well as wild animals. CDV gave rise to the closely related phocine distemper virus and has spread to non-canine species. In 1993/94 over 35% of the lion population as well as other species in the Serengeti Park died due CDV infection and a further outbreak occurred in 2001. Of major concern with regard to possible future zoonosis is CDV infections in non-human primates in 1989, 2006 and 2008. To understand how CDV adapts to new species it is crucial to determine the associated mutations. Changes in the virus haemagglutinin (H) protein (which allows use of cell entry receptors) and in other genes which may give rise to increased virulence need to be determined. Signalling lymphocyte activation molecule (SLAM) is a universal receptor for morbilliviruses required for initial infection but differs between species requiring virus adaption. We have isolated viruses from a lion, 2 hyena's, a bat eared fox and a domestic dog from the 1993/94 Serengeti outbreak and carried out extensive sequencing analysis of the hemagglutin (H), phosphoprotein (P) and fusion (F) genes from the 1993/1994 outbreak. Sequences were compared to other CDV strains including the monkey isolates.

Methods
Viruses were isolated in VeroDogSLAMtag cells, sequenced and nucleotide alignments made using the NCBI BLAST programme. Unrooted neighbour joining phylogenetic and fast minimum evolution trees were constructed using DNASTAR software Megalign package with the CLUSTAL W method.

Results
Changes in the H gene including the SLAM receptor binding site were identified which correlate with adaptation to non-canine hosts in the Park. These mutations differ to those observed for primate adaption. Differences in the P gene sequences between disease and non-disease causing CDV strains were identified which may relate to pathogenicity in domestic dogs and wildlife including lions.

Conclusion
The Serengeti CDV strains sequenced in this study were more closely related to one another and to more recent isolates from wild dogs around the Park than to other published isolates. Mutations observed in the virus P gene sequence may relate to increased virulence. Mutations in the H gene within the SLAM receptor binding site and other sites in this protein were identified in 3 of the strains which correlate with adaption to non-canine hosts and differ to the changes for adaption to primates.

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Detection of a gE-deleted PRV strain in an Italian red fox

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Background
Aujeszky disease (AD) is a notifiable disease that results from infection by Pseudorabies virus (PRV) that causes substantial economic losses to the swine industry. Suids are the natural reservoir of PRV whereas the disease is self-limiting in other species. AD in pigs is controlled by using live and inactivated gE-deleted vaccines. Although PRV has been eliminated from domestic pigs in many European countries, AD is continuously being reported in wild boars and in related hunting dogs. Clinical cases have rarely been detected in other wildlife such as foxes, Iberian lynxs, panthers and skunks. This case reports AD caused by a gE deleted PRV strain in a red fox in Italy.

Methods
A fox displaying atypical behaviour was found in an urban area in Central Italy and submitted to a Veterinary Hospital. Death occurred within 48 hours. The presence of PRV DNA in the brain was determined by specific gE/gB genes real-time PCRs. Rabies, canine distemper, Parvovirus, Hepatitis E and Leptospirosis were excluded by direct immunofluorescence and PCRs. Partial sequencing of the gC gene was also performed on clinical specimens. Virus isolation was attempted through inoculation in PK15 cells. The virus isolate was tested for PRV gE and gB antigen detection by two virological ELISAs using MAbs specific to gB and gE proteins. Full sequencing on the virus isolate was attempted by NGS approach.

Results
Clinical signs included ataxia, poor coordination, unsteady walk, tendency to stumble and diarrhoea without skin lesions. Only PRV gB was detected by real time and traditional PCRs. Virological ELISAs performed on the virus isolate resulted positive for gB antigen detection and negative for gE. Phylogenetic analysis of gC gene showed that the fox PRV sequence belonged to a different clade from those circulating in Italy but was closely related to the Nia strain. Full genome sequencing is in progress.

Conclusion
This case reported the isolation of a PRV strain in a fox suffering neurological signs found in an urban area. These results support the idea that the virus is a gE deleted mutant closely related to a vaccine strain and implicates domestic pigs as the primary source of infection for the fox. However, the epidemiologic link between a PRV vaccine strain and the infection in a fox remains unclear. These findings underscore the importance of biosecurity measures on pig farms and the relevance of virological surveillance.

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Inhibition of feline coronavirus replication by different antiviral molecules

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Background
Coronaviruses are responsible for serious diseases such as the Severe Acute Respiratory Syndrome (SARS) or the Middle East Respiratory Syndrome (MERS) in humans and a fatal disease called Feline Infectious Peritonitis (FIP) in cats. There is currently no effective vaccine or specific treatment against these viruses. In this study, two antiviral molecules, Ribavirin and Cyclosporine A, were tested in vitro against the feline infectious peritonitis virus (FIPV). Ribavirin (RBV) is a nucleoside analogue. Cyclosporin A (CsA) is an inhibitor of cyclophilins, cellular proteins often used by viruses as cofactors.

Methods
FIPV was cultivated in feline kidney cells (CrFK). The viral RNA in cell supernatants was quantified by qRT-PCR. Cytotoxic effect of the drugs was determined by using the "CellTiter-Glo Luminescent Cell Viability Assay" (Promega) according to the manufacturer instructions. Increasing doses of drugs were used to determine both the cytotoxic and viral inhibithory concentrations.

Results
Inhibitory concentrations IC50 and IC90 were estimated at 1 µM and 2 µM respectively for Cyclosporine A and 2 µg/ml and 10 µg/ml for Ribavirin. However, at this concentration Ribavirin is cytotoxic which prevents its use in vivo. In order to understand the mode of action of Cyclosporine A, kinetic treatments were conducted with a concentration of 2 µM of CsA. The drug was administered in the culture medium of FIPV-infected cells at either 2 h, 4 h, 6 h or 18 h post-infection (p.i) and was left throughout the infection. A significant decrease of viral production was observed with the addition of CsA at a concentration of 2 µM at 2 h p.i and 4 h p.i. By adding the treatment at 6 h p.i, inhibition was also observed but not stastically significant. Treatment at 18 h p.i did not induce a significant decrease of viral production.

Conclusion
Our data demonstrate that RBV has a modest antiviral effect whereas CsA at 2 µM inhibits viral replication by up to 90%. This molecule inhibits the early stages of the viral cycle but at a post-entry step. All of these studies will bring new knowledge on the specific mode of action of these antiviral molecules in the purpose to develop therapeutic treatments against Feline Coronaviruses.

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Humoral immune responses to non-structural proteins of feline coronavirus

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Background
Feline infectious peritonitis (FIP) is a fatal disease of cats caused by a virulent variant of feline coronavirus (FCoV), referred to as FIP virus (FIPV). FIPV is believed to emerge de novo via mutations of a relatively non-pathogenic variant of FCoV, designated feline enteric coronavirus (FECV), within individual hosts. Infection with FECV is common among cats, while FIP is relatively rare. Factors that determine the switch from a largely subclinical and localized enterocyte infection to a systemic monocyte/macrophage infection with a fatal outcome are currently poorly understood. Similar to other nidoviruses, non-structural proteins (nsps) of FCoV are one of the first viral proteins abundantly produced within the infected cells. However, there are currently no data related to immune responses to FCoV nsps. Hence, the aim of this study was to determine whether cats infected with FCoV develop humoral immune responses to selected nsps of the virus, and if so, whether the targets for such immune responses differ between cats with different disease outcomes.

Methods
Custom peptide chips were commercially synthesised. Each included 28,426 12-mer sequences covering all available variants of the entire non-structural polyprotein 1ab of FCoV. Each chip was then hybridised with the following samples:
1. Chip1: control serum from a cat negative for FCoV antibody.
2. Chip2: Pooled sera (n=5) from FCoV antibody positive healthy cats.
3. Chip3: Pooled sera (n=5) from FCoV antibody positive FIP cats.

Selected peptides (n=11) were then used as antigens in ELISA-based format and tested with sera or effusion samples from individual cats. A total of 92 samples from 72 cats were tested, including non-FIP (n=42) and FIP-affected (n=30) cats.

Results
There was minimal binding of the negative control serum to Chip1, with clear binding detected to Chip2 and Chip3. All 11 peptides tested showed some level of binding to FCoV Ab positive sera (variable between cats) and no biding to the control FCoV Ab negative serum in ELISA. Although none of the peptides tested reacted exclusively with sera from FIP cats, one peptide appeared to be preferentially recognized by FIP-affected cats, with the mean corrected OD450 of sera from FIP cats significantly higher than OD450 of sera from either FCoV-antibody positive non-FIP cats or FCoV-antibody negative cats.

Conclusion
Cats develop immune responses to nsps of FCoV. The targets for such responses differ between cats. It may be possible to utilise this knowledge for improvements in the diagnosis of FIP.

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Viral infections of Carnivores

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Clinical importance and molecular epidemiology of canine minute virus infections in dogs

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Background
Canine minute virus (CMV) is a parvovirus that affects dogs belonging to genus Bocaparvovirus. Even though it has been described more than fifty years ago its clinical importance and epidemiology is still unknown. In experimental infections CMV was responsible for reproductive disorders and diarrhoea in puppies while in field conditions diarrhoea and respiratory illness were recorded. CMV was also isolated from asymptomatic animals. The purpose of this study was to determine clinical importance of CMV infection as well as to give insights in the epidemiology of CMV infection.

Methods
In period from 2015 till 2017 a total of the 274 rectal swabs from dogs presented with signs of the acute gastroenteritis were collected at veterinary clinics in Zagreb. Additional rectal, nasal and vaginal swabs from 120 breeding bitches, with history of reproductive disorders, were sampled from 24 different breeding kennels in Croatia. As a control, rectal swabs from 75 healthy dogs were included. For all animals included in this study data, regarding signalment, medical history, course and outcome of the disease were collected as well. All collected samples were tested for presence of the CMV, using polymerase chain reaction (PCR) targeting VP2 capsid gene. For positive samples in additional PCR reactions were used to amplify most of non-structural genes. All PCR products were sequenced. Statistic description and analysis were made using Dell Statistica version 12. For phylogenetic analysis MEGA7 software was used.

Results
CMV infection, with prevalence of 10.22%, was significantly more prevalent ($p<0.0001$) in dogs with signs of gastroenteritis, than in dogs with reproduction disorders (2.5%) and in healthy population (2.67%). Statistics analysis showed significant ($\chi 2$, $p=0.0013$) age disposition with no evidence of infection in animals under two months of age. Interestingly there was no more overt signs of respiratory infections in CMV infected dogs (Fisher, $p=1$). Sequencing and phylogenetic analysis showed circulation of two distinct groups of strains with one group only infecting dogs between two and four months of age. There were a number of amino acid substitutions in non-structural viral proteins.

Conclusion
CMV seems to be an important pathogen of gastrointestinal systems of dogs in field conditions with age disposition different than the previously described. Sequencing and phylogenetic analysis showed that epidemiology of this disease is more complex than it was thought, with possible unique model of the evolution on the molecular level, which could serve as evolution model of bocavirus infections in other species.

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Bluetongue virus serotype 8: infection kinetics of two European strains in sheep

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Background
Bluetongue is a haemorrhagic disease of ruminants caused by bluetongue virus (BTV: genus Orbivirus, family Reoviridae), a diverse virus that is transmitted by Culicoides biting midges. A European outbreak of BTV serotype 8 (BTV-8) during 2006-2008 caused significant morbidity and mortality in the naive ruminant population and represented the first incursion of BTV into northern latitudes. Following a successful vaccination campaign, no further cases of BTV-8 occurred in Europe until November 2015 when BTV-8 caused outbreaks in France. This BTV-8 strain has caused largely sub-clinical infections despite sharing 99% nucleotide similarity to the 2006-2008 strain. We investigated the infection kinetics of this new BTV-8 alongside the 2007 BTV-8 UKG strain in British Mule sheep to determine the differences in clinical severity.

Methods
Two groups of 8, BTV-naïve sheep were subcutaneously and intradermal inoculated with 1.5 ml of 5.75 Log10 TCID50 ml-1 of UKG2007 BTV-8 or FRA2017 BTV-8 (hereafter UKG or FRA, respectively). A single uninoculated sheep was included per group as a contact transmission control. Over a 22 days post infection (dpi), EDTA blood and serum samples were taken and subsequently analysed for the presence of BTV-specific antibodies (competitive ELISA and serum neutralisation test) and nucleic acid (RT-qPCR). Virus isolation on KC cells (C. sonorensis) was also performed. Animals were monitored throughout for clinical signs.

Results
BTV RNA was detected by 2 dpi and peak viraemia occurred between 5–8 dpi. A mean viraemia of 7.05 and 5.75 log10 genome copies ml-1 was detected for the UKG and FRA strains, respectively. Virus was isolated from 2 dpi to 9 dpi for UKG and from 5 to 7 dpi for FRA. One animal infected with FRA did not develop viraemia but had seroconverted by 9 dpi as did all other infected animals. Neutralising antibodies were detected by 7 and 9 dpi for UKG and FRA, respectively. Bluetongue clinical signs (pyrexia, depression, facial oedema, reddening of mucosal membranes and coronary bands) were seen more frequently in UKG infected sheep. A single animal from each group was euthanized on humane grounds at 9 dpi (UKG) and 17 dpi (FRA).

Conclusion
The newly emerged BTV-8 induced mostly mild clinical disease compared to UKG. However, one sheep infected with FRA developed severe lameness under these experimental conditions. Morbidity rates in field conditions are likely to be higher and coupled with animal movement restrictions would still have an economic impact on the farming industry.

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Vector competence: The role of viral proteins in transmission of bluetongue virus by midges

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Background
Transmission of vector-borne virus by insects is a complex mechanism consisting of many different processes, such as viremia in the host, uptake, infection, replication and dissemination in the vector, and delivery of virus to susceptible hosts leading to viremia. All these processes must be sufficient to maintain virus circulation. In addition, transmission efficiency of virus is strongly influenced by many factors such as temperature and the environmental condition. Bluetongue virus (BTV) is the prototype vector-borne orbivirus. Conventionally, BTV serotypes 1–24 are spread competent biting Culicoides midges (typical BTVs). New serotypes 25–29 were discovered in goats, and belong to a group of atypical BTVs with specific characteristics, including lack of virus growth on Culicoides (KC) cells (Batten et al., 2012; Breard et al., 2017; Chaignat et al., 2010). Extensive studies have shown that most reassortants of BTV1 and 26 replicated well in both mammalian (BSR) and Culicoides (KC) cells, whereas some reassortants did not replicate in KC cells (Pullinger et al., 2016). Apparently, RNA dependent RNA polymerase VP1, subcore protein VP3, and outer shell proteins VP2 and VP5 are involved in 'differential virus replication'. Previously, we demonstrated uptake by, and replication of, BTV11 in competent *Culicoides* sonorensis midges, and preliminary studies showed the role of several viral proteins in virus propagation in midges (Feenstra et al., 2015).

Methods
We generated 'synthetic' BTV11 aiming to rescue BTV11-based mutants. Regarding vector competence, we investigated virus replication in midges after blood feeding, including uptake, replication, and dissemination to the head.

Results
The minimal virus dose resulting in 50% infected midges (Midge Alimentary Infective Dose [$MAID_{50}$]) was appr. 15 $TCID_{50}$ of BTV11 in competent C. sonorensis midges. This corresponded to appr. 5x10*5 $TCID_{50}$/ml BTV11 1:1 diluted in blood, whereas a 20x higher virus titre in fed blood resulted in close to 100% BTV11 infected midges. As expected, replication of BTV11 expressing VP126 was strongly inhibited in KC cells, whereas BTV11 variants expressing different 11/26 chimeric VP1 proteins replicated well in both cell types. Some BTVs infect competent midges only after intrathoracic inoculation (Feenstra et al., 2015), indicating that these viruses cannot escape the midgut barrier following ingestion.

Conclusion
The midge feeding model is useful to study the role of viral proteins in virus replication *in vivo*. Current findings indicate that several viral genes are involved in differential virus replication *in vitro* and *in vivo*. Future research will focus on viral factors associated with vector competence.

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Widespread circulation of influenza D virus in Luxembourg

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Background
Influenza D virus, a new member of the Orthomyxoviridae family, has been recently discovered in pigs with respiratory symptoms in the USA. Influenza D virus is implicated in bovine respiratory disease complex and cattle are now considered as the main host, while several livestock species seem susceptible. Although few studies have shown that the virus geographic range is large, deeper epidemiological data are still scarce outside North America.

Methods
Sera from 450 cattle (44 herds, 2016) and 545 pigs (n=258, 27 farms, 2012; n= 287, 29 farms, 2014–2015) from Luxembourg were screened by hemagglutination inhibition assay to detect anti-influenza D antibodies. Nasal swabs from 659 (n=232, 56 farms, 2009; n=427, 36 farms, 2014–2015) asymptomatic pigs and few lung samples from calves with respiratory disease were screened by real-time RT-PCR.

Results
An overall seroprevalence of 80.2% (361/450) was found in cattle and 97.7% (43/44) of the herds had at least one seropositive animal. The average within-farm seroprevalence was 83.0% (range 20–100%). Seroprevalence rates significantly increased with the age of the animals and no difference between production orientation (milk or meat) was observed. Most of the cattle investigated were born in Luxembourg (90%, 405/450), demonstrating that our results cannot be explained by importation of seropositve animals alone and that IDV transmission takes also place in the country. In contrast to cattle, the IDV seroprevalence in swine was low but increased during recent years (0% in 2012; 5.9%, 17/287 in 2014–2015). Seropositive animals were detected in 20.7% (6/29) swine herds.
So far, influenza D RNA was detected in a calf with respiratory symptoms, coinfected with bovine respiratory syncytial virus (genome sequencing ongoing). In pigs, the low virus prevalence in nasal swabs (0%, 2009; 0.7%, 2014–2015) and the low viral RNA concentration (9.7 to 94.5 copies/µl) were not conducive to amplification of genetic material for sequencing.

Conclusion
The results of this study investigating the epidemiology of the newly discovered influenza D virus in Luxembourg showed that the virus is enzootic in cattle, similarly to what was reported in Italy. Considering trade and cross-border grazing, the regions beyond Luxembourg's borders are likely similarly affected by the virus, and influenza D was indeed found in France, Italy and Ireland. The virus affects swine to a lesser extent but increasing seroprevalence over the years in Luxembourg, as was found also in Italy, suggest the importance of monitoring influenza D in swine herds in the future.

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Efficacy of a MAY 97 foot-and-mouth disease virus vaccine against heterologous challenge with a field virus from the emerging A/ASIA/G-VII lineage in cattle

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Background
In 2015, outbreaks of foot-and-mouth disease (FMD) in the Middle East were discovered to be caused by a new emerging viral lineage, A/ASIA/G-VII. In vitro vaccine matching data generated by the World Reference Laboratory (WRLFMD) indicated that this virus poorly matched (low r1-values) with vaccines that were being used in the region. However, previous studies have shown that regardless of a poor antigenic match, high-potency vaccines can protect against heterologous challenge. A collaboration was started in which was investigated whether vaccines that were present in national (The Netherlands, Australia) or the EU (UK) emergency vaccine banks could protect against challenge with FMDV A/ASIA/G-VII. Because A/MAY/97 gave the most promising results in pilot studies, this vaccine was tested in a PD-50 experiment.

Methods
A PD-50 experiment was performed in which 5 cattle were vaccinated with a full dose, 5 cattle with a 1/3 dose and 5 cattle with a 1/9 dose of vaccine. Vaccines were prepared similarly as would be done during an emergency vaccination. All vaccinated cattle and 3 control cattle were challenged intra-dermolingually at 21 days post vaccination with FMDV A/ASIA/G-VII. Cattle were monitored for clinical signs of FMD between 1 and 7 days post challenge. Cattle with lesions on their feet were considered as non-protected (similarly as prescribed by the European Pharmacopeia). The PD-50 of the vaccine was calculated with the Spearman Karber method.
Further, nose swabs, saliva samples and blood samples were collected and tested for presence of virus (PCR, VI) and serum samples were tested for antibodies (VNTs, NS-ELISA) against FMDV.

Results
All cattle from the full vaccine dose, 4 cattle from the 1/3 vaccine dose and 2 cattle from the 1/9 vaccine dose were clinically protected against challenge with FMDV A/ASIA/G-VII. The corresponding PD-50 was calculated to be 6.47. As a result of the tongue challenge, all nose and saliva swabs tested positive. Also all cattle tested positive by NS-ELISA. However, no viraemia was detected in the protected cattle. The VN-titre of the full dose group was 2.22 (log10) against FMDV A/MAY/97, and 1.38 against FMDV A/ASIA/G-VII.

Conclusion
A high potency vaccine can protect against heterologous challenge, even if in vitro results predict a poor antigenic match. Probably, this is highly dependent on the quality / vaccine potency of the vaccine.

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Molecular-genetic and biological characterization of lumpy skin disease virus isolated in Saratov region of Russia in 2017

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Background
The first LSD outbreak in the Russian Federation was reported in July 2015 in the Republic of Dagestan. Later the disease was registered in other regions. The first case of LSD in Saratov region occurred in June 2017. The preliminary diagnosis was confirmed by detection of capripoxviral genome in the biopsy samples collected from affected cattle. The analysis was done by real-time PCR method, described by T.R. Bowden et al. (2009). Surprisingly, these samples of animals with clinical signs of LSD were negative for the presence of genome of field strains of LSD virus in the methods, based on amplification of EEV gene. Therefore we decided to investigate the molecular-genetic and biological characteristics of a new isolate of LSD virus, named Dergachevskyi.

Methods
For molecular-genetic characterization of isolate Dergachevskyi GPCR, P32 and EEV (LSDV127) genes were amplified. The obtained nucleotide sequences are deposited in the GenBank under the numbers MH029290, MH029291, and MH077561, respectively.

Results
Phylogenetic analysis conducted on the basis of the concatenated sequences of three genes showed that isolate Dergachevskyi belongs to the cluster of vaccine strains of LSD virus: Neethling-LSD vaccine-OBP, Neethling-Herbivac vaccine, SIS-Lumpyvax vaccine, Neethling vaccine LW 1959. GPCR gene of isolate Dergachevskyi contains the insertion (12 bp) that is present in this gene of the vaccine strains of LSD virus. The GPCR gene of isolate Dergachevskyi has 99% identity with Neethling-like vaccine strains and 98% with strain KSGP-0240. EEV gene of isolate Dergachevskyi has 100 % identity with the Neethling-like vaccine strains. At the same time, this gene of isolate Dergachevskyi has a lower level of nucleotide identity with the strain KSGP-0240 (95%). It's explained by the presence of the deletion (24 bp) in EEV gene of Neethling-like vaccine strains and the absence of this deletion in EEV gene of strain KSGP-0240. For estimation of biological features of isolated virus, 4 calves were experimentally infected with the suspension of biopsy samples from sick animals. These calves showed typical clinical signs of LSD including the appearance of skin nodules, purulent discharge from the nose and eyes, and lacrimation.

Conclusion
Our results have shown that isolated LSD virus that caused outbreak in Saratov region in 2017 has a genotype of vaccine strains, while experimentally infected with this isolate animals demonstrated clinical signs which are typical for field isolates. To explain this phenomenon the further experiments are needed.

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Pathological and molecular characterization of an unusual outbreak of Peste-des-petits ruminants (PPR) in Nubian ibex (*Capra nubiana*) in Israel

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Background
Peste-des-petits ruminants virus (PPRV, Paramyxoviridae: Morbillivirus) causes a devastating disease, with severe consequences when infecting flocks of domesticated small ruminants. In Africa and the Middle-East, wild animals are usually not severely affected upon exposure to the virus, while PPR circulating in Asia was reported to be lethal to wild ruminants. PPR was first diagnosed in Israel in 1993, and occasional acute PPR cases were diagnosed in recent years in domesticated animals. Here we describe the first recorded acute outbreak of PPR in Nubian ibex (*Capra nubiana*) in Israel, resulting in the death of 70% of the infected flock.

Methods
Clinical inspection of the infected animals raised the suspicious of PPR infection. Deceased animals were subjected to post-mortem analysis and histological examination. Tissues and body fluids were used for RNA extraction and PCR analysis. Initial molecular analysis included PPR-specific RT-qPCR test, and endpoint PCR test. Positive samples were sequenced. Finally, the complete viral genome was obtained, using Illumina Miseq sequencing.

Results
Infected animals exhibited lethargy and watery to bloody diarrhea, and death usually occurred within 48 h. The most consistent gross pathologic findings were hemorrhagic abomasitis and enteritis. Oral lesions and pulmonary lesions were rare, contrary to what is usually observed in domesticated animals. Histological analysis revealed necrohemorrhagic enteritis and abomasitis, with viral inclusion bodies. RT-qPRC and end-point RT-PCR confirmed the presence of PPRV in numerous tissues. Finally, the entire genome of the virus was sequenced, and its analysis showed that it belongs to lineage IV. Comparison of the entire Fusion (F) protein and Hemagglutinin (H) protein genes obtained from African and Asian samples suggests that the examined virus is closer to the lineage IV PPR viruses circulating in Asia, than to the African ones.

Conclusion
The reported case is considered unusual for two major reasons: although serological studies showed that wild animals in Africa, including Nubian ibex, are often exposed to PPRV, they are not usually developing acute illness. Second, the pathological manifestation of the infection, as evident from the post-mortem analysis, is different from what is usually observed in domesticated small ruminants. As Asian PPR is reported to be virulent to wild ruminants, this may explain the increased virulence of this virus towards Nubian ibex. This outbreak therefore highlights the importance of PPRV surveillance in wild ruminants, and the importance of research aimed to understand the factors affecting the virulence of PPRV towards wild animals.

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Viral infections of Birds

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Primary chicken bursal cells: an in vitro alternative to in vivo infection that sheds new light on infectious bursal disease research

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Background
Infectious bursal disease virus (IBDV) of chickens induces an immunosuppressive and sometimes lethal disease of economic importance worldwide. In the host, IBDV mainly replicates in developing B cells contained in the cloacal bursa (also known as bursa of Fabricius). Current in vitro cell culture systems for propagating the virus are not ideal as the majority of field IBDV isolates require mutations in the capsid protein to allow replication. Furthermore these mutations very often induce a loss of virulence in vivo, altering the viral phenotype. Therefore bursal B cells, the main targets of IBDV in vivo, represent an attractive model to study this virus. Unfortunately, these cells quickly undergo apoptosis following their isolation from the cloacal bursa. This study aimed at maintaining bursal B cells ex vivo to use them as a relevant model for IBDV infection.

Methods
Primary chicken bursal B cells were isolated from specific pathogen-free chickens by density centrifugation. The impact of various stimuli on cell viability and on viral yield upon infection was measured. Optimally stimulated cells were then infected by vaccine and field IBDV strains to follow viral replication dynamics by titration and flow cytometry.

Results
Stimulation of primary bursal B cells with phorbol 12-myristate 13-acetate (PMA) resulted in high viability and high viral yield upon infection. PMA-stimulated bursal B cells were successful in (i) isolating viruses from field samples even in the case of co-infected bursae, (ii) producing high-titer viral stocks without inducing nucleotide mutations in the capsid protein responsible for adaptation to cell culture, (iii) producing viral antigen used for diagnostic. An IBDV titration assay based on PMA-stimulated B cells was developed and showed a similar to increased sensitivity compared to the reference in ovo method. Proof-of-concept cross-neutralization data were obtained by viral neutralization assays performed on stimulated B cells. Kinetic studies of viral replication revealed striking differences, up to 2 log10 (TCID50/mL) in viral titers between some vaccine strains and some field isolates. Mathematical modeling of replication is ongoing in order to correlate in vitro data such as viral yield and cell death induction to in vivo immunosuppressive properties of various strains.

Conclusion
PMA-stimulated primary B cells are a promising alternative to in vivo experiments for IBDV studies that paves the way to (i) improved classical virology tools and diagnostic reagents, (ii) better understanding of molecular basis of immunosuppression and pathogenesis, (iii) cross-neutralization studies to quantify vaccine-induced protection and viral antigenic escape.

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Metagenomic identification of the virome associated with runting stunting syndrome in broiler chickens

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Background
Runting-stunting syndrome (RSS) of commercial broiler chickens causes arrested development, severe stunting, poor feed conversion, lesions within intestinal tracts and frequently diarrhoea. Generally, birds are younger than 3 weeks when affected and flock culls can number thousands of birds. Affected chicks may be more than 50% lighter than unaffected house mates of the same age. RSS has an unknown and complex aetiology involving enteric viruses that may also impact upon the bacterial repertoire of the gut. Experimental studies of individual viruses have failed to identify a single aetiological agent although challenge infections by several enteric viruses have each resulted in a lesser degree of stunting giving rise to a hypothesis that a community of viruses, termed the virome, may be responsible for clinical cases of RSS. Furthermore, gut homogenates from clinical RSS cases, filtered to remove bacteria and larger pathogens, have been sufficient to reproduce RSS in specific pathogen free birds, indicating that the cause is viral.

Methods
Gut contents from two to seven broilers of between 13 and 21 days of age from each of five RSS-affected and two unaffected commercial flocks were pooled to make seven flock samples, which were processed by filtration and ultracentrifugation to enrich for the virome. Viral nucleic acids were extracted, amplified via whole genome or whole transcriptome amplification and prepared into separate DNA and cDNA libraries that were multiplexed using molecular barcodes. Whole genome metagenomic studies of the gut virome were performed using a next-generation sequencing (NGS) shotgun approach and bioinformatics analysis.

Results
Sequencing results produced 2,036,415 high quality reads which were assembled into >64k contiguous sequences (contigs), the majority of which were between 250 & 500 bp. Twenty DNA and RNA viral families were identified comprising 31 recognised genera and 7 unclassified genera.

Discussion
The presence of diverse viral families, species and strains was observed with greater diversity in RSS-affected flock samples. These included chicken astrovirus, avian nephritis serotypes 1 & 2, chicken megrivirus, chicken parvovirus, and sicinivirus 1, a novel picornavirus. Many of the same viruses were detected in both RSS affected and normal birds although differences were seen in the number and types of strains present and in the proportions of viruses. Additionally, a high proportion of bacteriophage were identified in three of the affected flock samples and in one of the unaffected flock samples suggesting that bacteriophage naturally regulate the microbiome in both healthy and sick birds.

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Intergenomic comparison of an in vitro-attenuated FAdV-4 strain and its virulent counterpart provides substantial evidence for virulence modulation in fowl aviadenoviruses via adaptation of right-terminal genome contents

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Background
Fowl aviadenovirus species FAdV-C, in particular strains typed as FAdV-4, cause hepatitis-hydropericardium syndrome (HHS) in chickens, which has seen severe episodes of emergence in some countries, most recently documented from China.
So far, typing by extensive sequencing of the hexon neutralization epitope has created a bias in the available data sets, indicating a uniform array of FAdV-4 strains with no apparent link to the strains' individual pathogenicity, promoting interest in the genomic composition beyond the established single-gene approaches for FAdVs.

Methods
Based on highly virulent FAdV-4 AG234, isolated from an HHS-outbreak, and its descendant INT4, generated by passaging on an atypical cell line (QT35), this is the first study addressing intergenomic plasticity in an in vitro-attenuated FAdV pair with experimentally confirmed pathogenicity differences. Complete genomes were obtained by Illumina-sequencing, de novo-assembly, and manual editing combined with annotation against reference strains. Screening for diversity in multiple-sequence alignments was implemented by different software-assisted analyses.

Results
The AG234/INT4-counterparts shared 99.9% identity and, unlike other FAdV-4s with reported deletions, contained all ORFs with high coding potential. Left-end and core-genomic ORFs remained stable during the process of attenuation, with sporadic mutations assumed as random due to their unique or non-discriminatory distribution across all analyzed FAdV-4 strains. Structural proteins were relatively conserved between INT4 and its progenitor, consistent with the growing awareness that FAdV epitopes cannot be reliably correlated to pathogenicity. Contradicting the long-sustained hypothesis that virulence resides in the receptor-binding FAdV fiber, the distal domain was even the most conserved in both FAdV-4 fibers. The majority of differences between AG234 and INT4 mapped to the 3'-genome terminus. Stereotypical variation occurred in non-coding, repetitive regions, particularly flanking a sequence stretch comprising ORF16 and ORF17. Longer patterns and copy numbers in the tandemly repeated TR-E of apathogenic INT4 could be well aligned with the separation between other reported pathogenic and apathogenic strains. Furthermore, INT4 had acquired mutations in ORF16 which were discriminatory for a cluster of strains considered as apathogenic.

Conclusion
Whilst outruling some earlier postulated regions, our results pinpoint candidate virulence markers in the FAdV right-terminal genomic region. Its potential to encode host-modulatory functions is supported by our finding of evolutionary independence between FAdV core-genomic and terminal contents. Tandem repeat-variation may present another adaptation of FAdVs, as indicated by preliminary results from amplification of repeats of different lengths across various passages.

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Detection of a novel recombinant (QX-793/B) variant of infectious bronchitis virus, related to the Xindadi (XDN) type, following vaccination with a 793/B vaccine and field infection with IBV QX

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Background
Avian infectious bronchitis virus (IBV) belongs to the family Coronaviridae and is the aetiological agent of highly contagious disease in chickens, causing economic losses in the poultry industry worldwide. The virus exists in a wide range of genetically and antigenically distinct types due to mutation and recombination, complicating the prevention and control.

Methods
Sampling of a farm which consisted of three flocks (A, B and C) was performed in a longitudinal scheme: before onset of lay (age: 17 weeks), at peak of lay (age: 40 weeks) and at end of lay (age: 72 weeks). At each sampling point 30 tracheal and 30 cloacal swabs were taken from individual birds and five birds per flock were necropsied to record gross pathological lesions. Swabs and organs from necropsied birds were processed for IBV detection by RT-PCR combined with partial sequencing of the S1 gene and application of a specially developed RT-PCR to screen for recombinants. Recombination analysis was performed with RDP software. To determine the distribution of IBV variants and to reconstruct the emergence of the recombinant IBV, deep amplicon sequencing of partial S1 gene was undertaken.

Results
Throughout sampling IBV type 793/B (GI-13) was detected in all flocks and Massachusetts type (GI-1) at peak of lay. Besides anticipated IBV vaccine types (IB 793/B and IB Massachusetts strains), IBV QX type (GI-19) was detected from flocks B and C at peak of lay, when birds showed typical pathomorphological lesions for IBV. Even though typical IBV lesions were not observed at the end of lay, the IBV QX type could be still detected. Further to this, partial S1 gene sequence analysis of samples obtained from flock C at the end of lay revealed an IBV type related to the Xindadi (XDN) variant, so far reported from Spain without information on applied vaccines. Recombination analysis revealed that the detected XDN variant originated from a recombination between QX and 793/B types, identifying samples from the rearing farm (type 793/B) and the peak of lay (QX type) as pertinent parent strains.

Conclusion
This is the first study reporting the detection of a new recombinant IBV variant in a well monitored layer flock with detailed information on the vaccination programme. Complexity of the study which includes vaccination data, longitudinal sampling, post-mortem investigations together with advanced molecular typing of IBV, enabled the reconstruction of the emergence of the recombinant IBV variant based upon vaccine and field virus.

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The microbiota modulates viral replication in ducks infected with a H5N9 highly pathogenic avian influenza virus

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Background
In contrast to chickens, which are very susceptible hosts for avian influenza virus, ducks often show little or no clinical signs, even following infection with a highly pathogenic avian Influenza virus. The aim of this study was to test the hypothesis that the microbiota could contribute to the control of highly pathogenic avian Influenza virus replication in ducks.

Methods
We analysed virus replication following infection with a highly pathogenic avian influenza H5N9 virus by comparing groups of ducks in which the gut microbiota was significantly depleted by a large spectrum antibiotic treatment to undepleted ducks, which received no antibiotic treatment.

Results
Bacterial culture and 16S ribosomal RNA gene qPCR showed a strong depletion of the gut microbiota after antibiotic treatment. Virus shedding analysis showed that antibiotic-treated H5N9 virus infected ducks showed significantly higher cloacal shedding at day 3 and 5 post-infection, but no difference in oropharyngeal shedding at all-time points.

Conclusion
Our results provide evidence that the microbiota contributes to the control of virus replication in ducks. Ongoing analyses will determine how depletion of the gut microbiota modulates the innate response to highly pathogenic avian influenza virus infection in ducks.

Oral Session 6.2
Viral infections of Birds

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Pathology and tropism of clade 2.3.4.4. H5 HPAI infection in ducks, France, 2016–2017: field and experimental data

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Background
Clade 2.3.4.4. H5N8 Highly Pathogenicity Avian Influenza viruses (HPAIVs) emerged in Europe in 2016. HPAIVs display a tissular pantropism, which implies that they could spread in feather pulp. We investigated the clinical and pathological features of 2.3.4.4. H5N8 HPAIV in ducks, in both field and experimental infections. We specifically investigated viral tropism in tissues and feather pulp.

Methods
Five field cases of H5N8 HPAI were investigated in ducks in South-West France from December 2016 to March 2017. On each case, a complete clinical analysis was performed on farm. Up to 5 clinically affected birds were selected and necropsied. On each bird, gross lesions were recorded and tissues were sampled for histopathology, immunohistochemistry and viral load titration in tissues. Tracheal, cloacal swabs and feather pulp samples were sampled on 10 to 15 birds.
An experimental H5N8 infection was performed on 10-week-old mule ducks: 5 birds were infected, 5 were in direct contact in the same isolator and 5 placed in another isolator, in aerosol-contact with the first one. All birds were monitored and sampled for 10 days post-infection.

Results
In spontaneous cases, nervous signs were often noticed, with great variations in incidence and intensity. Gross lesions were observed in a majority of affected birds, with a marked myocarditis and less frequently, splenitis and pancreatitis. The main microscopic lesions consisted in severe subacute lesions of necrotizing myocarditis, non suppurative meningoencephalitis, splenitis and uveitis. Acute necrotizing hepatitis and pancreatitis were also observed in some ducks.
Tissular viral loads were much higher in ducks compared with the "European" H5 HPAI viruses emerged in France in 2015, suggesting a particularly high virulence, associated with a much more severe clinical and pathological picture. RNA loads detected in feather pulp were at least equivalent and in most cases up to 103 higher than those detected in either tracheal or cloacal swabs. In contrast, experimental infection resulted in a totally asymptomatic infection in all birds, despite PCR detection starting before day1 post-infection and Ct PCR values as low as 16 in some birds at peak of infection. Viral loads were higher in tracheal than cloacal swabs and very significant in feather pulp.

Conclusion
These pathological data confirm strikingly variable pathological profiles of clade 2.3.4.4. H5N8 infection in ducks. Viral excretion was consistently higher in trachea than in cloaca and viral detection was often higher in feather pulp than in swabs.

Oral Session 7.1
Viral infections of Ruminants 2

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Small ruminant morbillivirus V and C proteins inhibit the activity of the interferon-stimulated response element

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Background
Small ruminant morbillivirus (SRMV) is the causative agent of the disease Peste-des-petits ruminants (PPR). SRMV is present in four different genotypes (lineage I–IV). These genotypes are considered to not differ in pathogenicity, but rather describe the geographic distribution. Morbilliviruses have six genes encoding eight proteins, where the non-structural V and C proteins are encoded within the P gene via editing (V) and translation initiation from the second AUG codon (C) (1). V and C proteins of morbilliviruses have previously been demonstrated to effectively regulate the host's type I interferon response (IFN-α/β) (2). One critical step in this pathway is activation of the interferon-stimulated response element (ISRE), which leads to transcription of IFN stimulated genes. Here we study the immunomodulatory effect of the SRMV V and C proteins from representatives of the four different lineages on the activity of ISRE.

Methods
V and C proteins from the four different lineages were synthesized and cloned to a vector backbone (GenBank acc.no.: EU267273, KY628761, KJ867543, and KJ867541). All isolates originate from the African continent. In HEK 293T cells we utilized a pISRE-Luc reporter plasmid system. Luciferase activity was measured and normalized to renilla readings for each well as relative light units (RLUs). Activation of ISRE was calculated by comparing stimulated cells to not stimulated cells.

Results
The results show a clear inhibition by the SRMV V protein and also by the SRMV C protein. The V proteins of the different lineages inhibit the luciferase activity of pISRE-luc with 80.5–93.3 % compared to the cells transfected with an empty vector. Additionally the C protein inhibits the activity, however to a lesser degree (17.4–43.3 %).

Conclusion
In conclusion, the SRMV V and C proteins share the ability to suppress the host's immune response with its relatives in the genus morbillivirus. Of the two, V is the main immunomodulatory protein, showing a clear inhibition of pISRE-luc. This will be studied further utilizing other reporters for different steps in the interferon induction pathway to more precisely elucidate the immunomodulatory properties of SRMV.

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Identification of Peste-des-petits ruminants, Georgia

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Background
The Peste-des-petits ruminants virus (PPRV) is the cause of a highly infectious transboundary animal disease that primarily affects sheep, goats and small wild ruminants. It is presently being targeted by international organizations for global eradication by 2030. Between January and March 2016, outbreaks of PPR were reported in three farms located near Tbilisi, the capital of Georgia. Of 3,740 susceptible sheep 415 (11%) showed symptoms of PPR.

Methods
Organ and swab (nasal and ocular) samples were collected and tested in the Laboratory of the Ministry of Agriculture, Tbilisi using a PPR Antigen Capture ELISA (ID.Vet, France). Six positive samples were individually adsorbed onto the matrix of a ViveSTTM transport tube (ViveBio, USA) and were shipped to the Institute for Veterinary Disease Control, Austria, for further characterization. Upon arrival in Austria, the samples were eluted from the ViveSTTM with 1 ml of Dulbecco's Modified Eagle's Medium High Glucose medium and stored at –80°C. Total RNA was extracted from 200 µl aliquots using an RNeasy kit (Qiagen, Germany). The extracted RNA samples were analysed by RT-PCR to amplify a fragment of both the PPRV Nucleoprotein (N) and Fusion protein (F) genes. Three of the six samples tested were positive by RT-PCR. Amplicons were purified and sent for sequencing using standard Sanger methods at LGC genomics (Berlin, Germany). A phylogenetic tree of N and F gene segments from a representative selection of PPRV sequences available in GenBank was estimated using the maximum likelihood method available in MEGA6 employing the Kimura-2 parameter model of nucleotide substitution and 1000 bootstrap replications.

Results
The phylogenetic analysis revealed that the PPRVs present in the three Georgian samples were identical and belonged to lineage IV. Notably, the N gene fragment sequences were more related to those of viruses from, Egypt, Eritrea, Ethiopia, and Sudan while the F gene fragment sequences clustered with viruses from Egypt, Ethiopia and Sudan. Unexpectedly, the N and F gene fragment sequences for viruses isolated from countries close to Georgia (e.g. Turkey, Iran and Iraq) were less similar to the Georgian viruses.

Conclusion
This is the first report of PPR in Georgia. Since there is no obvious connection between Georgia and Egypt, Eritrea, Ethiopia or Sudan through the trade or import of small ruminants, further work is required to fully understand PPRV circulation at a regional level.

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Imaging of BVDV host cell entry

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Background
Bovine viral diarrhea virus is an economically important pathogen of cattle within the family Flaviviridae, genus Pestivirus. Its enveloped virus particles integrate three viral glycoproteins, Erns, E1 and E2. E2 is interacting with the cellular receptor CD46 to mediate virus entry via clathrin-coated pits. Fusion occurs in the endosome and results in the release of the viral nucleocapsid complex into the cytoplasm.

Methods
E2 protein of the cytopathogenic BVDV strain C86 was tagged with a fluorophore and resulting virus particles were purified. Bovine CD46 was tagged with a fluorophore and inducibly expressed in porcine SK6 cells. The system was fixed at different time points after virus addition and imaged on a Zeiss AiryScan microscope.

Results
Association of virus particles could readily be observed at 5, 8, 10 and 12 min after virus addition.

Conclusion
We have established a system for the monitoring of BVDV host cell entry which will be employed to monitor virus entry using life cell imaging and to correlate these data with electron cryo microscopy to further elucidate morphological changes during virus entry.

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A novel ovine picornavirus in lambs with polioencephalomyelitis

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Background
Over the last years, several cases of 2–3 week old lambs presenting with progressive neurological signs related to lesions of polioencephalomyelitis and ganglionitis were reported. There was additional evidence of insufficient colostrum intake in some lambs. While the findings were typical of neurotropic viral lesions, all known differential diagnoses including Louping ill virus could be excluded and the cause of the disease remained unclear.

Methods
As a generic approach, a metagenomic analysis via next-generation sequencing was performed using extracted nucleic acids of the central nervous system of affected lambs. Sequencing data was classified using the software pipeline RIEMS and all sequences of viral origin were assembled. The detected complete viral genome sequence served as basis for the development of a specific RT-qPCR. The sample materials were also inoculated on two different baby hamster kidney cells (BHK), African green monkey kidney cells (Vero), sheep fetal thymus cells (SFT-R), murine neuroblastoma cells (Neuro-2a), and a fetal goat tongue cell line (ZZ-R).

Results
A novel ovine picornavirus could be detected and genetically characterized. The full-genome sequence has only 56% pairwise sequence identity to the nearest known relative, a bovine picornavirus. Phylogenetic analysis showed that the novel virus clusters with several unclassified picornaviruses between the genera Enterovirus and Sapelovirus. Viral RNA was only detected in affected lambs with encephalitis. The presence of viral RNA is strongly correlated with the site of the severe lesions, e.g. very high viral loads were detected in the spinal cords and cerebella. The diagnostic metagenomic analysis did not reveal any other pathogen potentially responsible for the observed clinical signs. Attempts at virus isolation in cell culture were not successful.

Conclusion
The complete genome sequence of a novel ovine picornavirus causing severe polioencephalomyelitis and ganglionitis in pre-weaned lambs was determined. Due to the similarity of the pathogenesis in comparison to Louping ill virus and particularly ovine astrovirus, ovine picornavirus should be considered in the differential diagnosis of progressive neurological signs in lambs. Further studies are ongoing to assess the distribution of the novel picornavirus in sheep populations.

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Antigenic relationships between the bluetongue virus outer coat protein VP2 from multiple serotypes, expressed in *Nicotiana benthamiana*

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Background
Bluetongue is a disease that affects wild and domesticated ruminants, caused by the bluetongue virus (BTV), which is transmitted primarily by arthropod-vectors belonging to certain *Culicoides* species. The larger BTV outer-coat protein, VP2 (encoded by genome segment-2 (Seg-2)) elicits VP2 binding antibodies (bAbs) in infected animals, a subset of which are protective neutralising antibodies (nAbs). VP2 is the least conserved of the BTV proteins and these nAbs can be used to differentiate distinct BTV 'serotypes' in neutralisation-assays. To-date 27 BTV serotypes have been recognised, which can also be differentiated/identified by Seg-2 sequencing, and grouped into 'nucleotypes' based on the closer phylogenetic relationships between some serotypes. Some antigenic similarities do also exist between the different BTV serotypes that are revealed by low-level, or one-way cross-neutralisation reactions.

Methods
Recombinant VP2 proteins of 17 BTV serotypes/topotypes, were expressed using a transient, plant-based system, in *Nicotiana benthamiana*. The expressed proteins were purified then used to immunise rabbits to obtain polyclonal antisera. Sheep polyclonal reference antisera, raised against reference strains of the different BTV serotypes were also obtained from the Orbivirus Reference Collection at The Pirbright Institute. These antisera were all tested for cross-reactivity against the BTV reference-strains in serum neutralisation tests (SNT), and by Indirect ELISA (I-ELISA) using the plant-expressed VP2 proteins. The antigenic relationships observed were compared and quantified using antigenic cartography.

Results
A broad range of antigenic relationships were detected, with greater cross-reactivities between the different serotypes by I-ELISA than by SNT. The plant-expressed VP2 proteins induced strong neutralising antibody responses in rabbits that were more cross-reactive between certain BTV serotypes, than the post-BTV-infection sheep antisera. Using the rabbit antisera, the recently described 'novel' BTV serotypes (BTV-25, BTV-26 and BTV-27) exhibited strong, two-way cross-reactions between BTV-26 and BTV-27 but only very weak relationships between BTV-25 and BTV-27.

Conclusion
The strong immunological responses to VP2 detected by both I-ELISA and SNT, confirm the antigenic relevance and suggest a native structure for the VP2 proteins expressed in plants. However, the antigenic cross-reactivities detected between BTV serotypes did not correlate exactly with the phylogenetic relationships of Seg-2. The antigenic differences/relationships of VP2 have important implications for the potential to develop serological serotype-specific diagnostic tests for disease surveillance and for development of multivalent/cross-reactive vaccines.

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Bovine hepacivirus field infections: immune response, course of infection, and host tropism

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Background

Bovine hepacivirus (BovHepV) is a recently added member to the growing genus Hepacivirus within the family Flaviviridae. Animal hepaciviruses are rarely characterized so far. Apart from Norway rat hepacivirus which represents a promising hepatitis C virus surrogate model, only equine hepaciviruses have been studied to some extent. BovHepV has been initially identified in bovine samples and was shown to establish persistent infections in cattle. However, consequences of those chronic infections, humoral immune responses and the possibility of an extended host spectrum have not been explored so far.

Methods

We here analyzed the dissemination of anti-NS3-antibodies in cattle herds in Germany by luciferase immunoprecipitation system (LIPS) and the presence of viral RNA by qRT-PCR. Moreover, for twenty-five selected cattle of a BovHepV positive herd the presence of viral RNA was monitored over one year in two to three months intervals in order to discriminate acute versus persistent infection. Finally, the host tropism including zoonotic potential of bovine hepaciviruses was investigated.

Results

It could be shown that 19.9% of investigated serum samples from individual cattle had antibodies against BovHepV. In 8.2% of investigated samples, viral RNA was detected by qRT-PCR and subsequent phylogenetic analysis revealed a novel genetic cluster of BovHepV variants. However, in persistently infected dairy cattle, no serum antibodies were found, and biochemical analyses did not hint at any liver injury in those animals, although presence of viral RNA was confirmed by fluorescent in-situ hybridization. Apart from a single sample of a pig providing a positive reaction in the antibody test, neither BovHepV-specific antibodies nor viral RNA were detected in porcine, equine or human samples.

Conclusion

BovHepV infections are widely distributed in cattle herds in Germany but seem to be restricted to the bovine host. Potential mechanisms involved in suppression of humoral immune responses will be revealed in future studies.

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Characterization and genetic diversity of lagoviruses collected in apparently healthy European hares (*Lepus europaeus*) and genetically distinct from European brown hare syndrome viruses (EBHSV)

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Background

European brown hare syndrome virus (EBHSV) is a calicivirus of the Lagovirus genus, GII.1 genotype. It was first described in 1980 and causes high mortalities in *Lepus europaeus* and *Lepus timidus*. The genogroup GI corresponds to Rabbit haemorrhagic disease virus (RHDV)-related viruses. One hypothesis to explain the origin of pathogenic lagoviruses in leporids is the evolution of non-pathogenic viruses towards pathogenic forms. Presence of non-pathogenic lagoviruses in hares was previously suspected after identification of EBHSV seropositive hares from countries without description of EBHS. The first non-pathogenic lagovirus, named Hare Calicivirus (HaCV), was characterized in Italy in 2014 from healthy hares born and reared in a farm. The genetic relationship based on capsid protein (VP60) gene sequences showed that they formed a new genetic group. This study aimed at characterizing lagoviruses collected in apparently healthy French hares to improve knowledge on these viruses.

Methods

We screened HaCV in 199 duodenum samples from hunted hares in 2014–2015. Extracted RNAs were amplified by RT-PCR with primers designed in conserved VP60 gene regions, sequenced (Sanger) and genotyped (BLASTn). Entire VP60 genes were amplified and sequenced for phylogenetic analyses (Maximum likehood, tMRCA). Structure of the VP60 from HaCV/E15-219 was predicted by homology modeling (Jpred, Prof, Psi-pred) using X-ray crystallographic structures of related viruses as templates. For one HaCV, the complete genome was amplified twice by overlapping PCRs. The extremities were acquired using RACE methods.

Results

Twenty HaCV strains were genotyped. Eleven VP60 genes and one complete genome were sequenced. Phylogenetic analysis confirmed that the French and Italian HaCVs form a separate genetic group to EBHSV/GII.1. Nucleotide sequence analyses showed high genetic diversity within the HaCV (73.1% to 94.1% identity) and 75% identity with the nearest lagovirus, an EBHSV. Modeling of HaCV/E15-219 VP60 secondary structure revealed differences, despite overall homology, with EBHSV/O282/Sweden/1984. The carbohydrate-binding site described for RHDV was conserved in HaCV and EBHSV. HaCV also showed the same genome organization as other lagoviruses. Nucleotide identity was 88% with EBHSV with one codon insertion in the p16 gene. A preliminary tMRCA estimate around 200 years prior to EBHSV tMRCA was found, although more HaCV sequences will be required to validate this result.

Conclusion

Phylogenetic distances and the low percentage of sequence identity between HaCVs and EBHSVs are not consistent with a direct origin of the pathogenic forms from non-pathogenic ones. In addition, pathogenicity does not correlate with the presence of the carbohydrate-binding site.

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In vivo infection dynamics of deformed wing virus (DWV) in honeybees

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Background

A marked increase in honeybee colony mortality and the consequent loss of pollinators is a major issue of food security worldwide. Varroa mite infestation and accompanying deformed wing virus (DWV) infections are major factors in the decline of bee populations. The mite acts as a vector for DWV, which is injected into the hemolymph of bee larvae by the mites feeding behaviour. This leads to the occurrence of typical clinical symptoms of DWV, including wing deformities, a shortened abdomen and ultimately causes colony mortality. Although the clinical signs of DWV infections could be reproduced by artificial injection into bee pupae, the exact mechanisms leading to pathological wing deformities are unknown. We aim at elucidating the pathogenic mechanisms leading to development of the eponymous wing deformities.

Methods

To unravel the mechanisms of DWV pathogenesis, we generated a recombinant DWV carrying a monomeric GFP (acGFP) reporter gene (DWV-GFP) at the 5'-end of the ORF using standard cloning techniques. To ensure the release of the GFP protein from the polypeptide we inserted a gene encoding the 2A peptide sequence of porcine teschovirus between GFP and the L-protein. Transcription of the DWV-GFP cDNA into infectious RNA followed by transfection into bee pupae resulted in viral replication. Furthermore, a bee cell culture system was established in order to study cellular infections.

Results

DWV-GFP was stable over several passages and led to clinical symptoms similar to wild-type DWV, expression of viral proteins and fluorescence of bees. The reporter gene allowed tracking the virus spread in cell culture and life bees in real time. GFP positive cells primarily occurred in the epidermis and the wing tissue of infected pupae indicating active replication in these tissues. Expression of viral proteins and the 27kDa GFP molecule were confirmed by Western Blot. Cryo-EM structure analysis of DWV-GFP showed that its capsid has identical structure to wild-type DWV. We performed immunohistochemistry analyses to compare infection patterns where we observed a specific IHC staining in neuronal, glandular and connective tissue cells.

Conclusion

In contrast to other non-enveloped RNA viruses, it was shown that the enlargement of the DWV genome is possible without structural alterations. The wild-type like phenotype and the stability of DWV-GFP make it an ideal tool for monitoring the dynamics of DWV infections, analysis of antiviral agents and immune modulators. Our data suggests that the epidermis is a significant region of DWV replication causing the particular pathology of this virus in honeybees.

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First discovery of the Moku virus in Asian hornets and honeybees in Europe

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Background

The European honeybee (Apis mellifera) is the most commonly managed bee in the world and is a key contributor to the pollination of food crops and wild plants. Honeybee mortality is due to multiple stressors including predators. The Asian yellow-legged hornet (Vespa velutina nigrithorax) (AH), a natural predator of honeybees, has a native range spanning from India through China and as far as Indonesia. It is a particularly efficient invader because of its distinctive biology and behaviour. It was accidentally introduced from China into France in 2004 and spread to neighbouring countries, including Belgium since 2011. In invaded areas, hornets' feeding sites are primarily apiaries, because they present an attractive, abundant and defenceless prey source. V. velutina nigrithorax do not only contribute to the loss of honeybee colonies by hunting, it also interacts with them and can act as viral reservoirs infecting them via spillover events.

Methods

To explore this possibility in Belgium, we performed a viral metagenomic analysis of AH collected in 2016 (pool of 10 specimens). To confirm the discovery and possible spread of the Moku virus (Iflaviridae family) in 2017, two specific RT-PCR were designed (targeting conserved regions of the genome) and performed on 6 AH coming from 3 nests and on 6 pools of honeybees collected in 2017 coming from 3 apiaries attacked by AH.

Results

a) Viral metagenomic analysis: BlastN alignment showed a positive match to Moku virus. Template-based assembly using Moku virus genome (GenBank accession number KU645789) and primer walking PCR with Sanger sequencing permitted a full-genome reconstruction. The full viral genome sequence is 10,032 nucleotides in length (GenBank accession number MF346349). It has a mean nucleotide identity of 96.0% to the Hawaiian Moku virus strain KU645789, both viruses showing an open reading frame of the same length (9,153 nucleotides) sharing an amino acid identity of 99.0%.
b) PCR analysis: the presence of Moku virus was identified both in AH (4 among 6 tested) and in honeybees (1 pool among 6 tested).

Conclusion

For the first time in Europe, we report the detection of Moku virus in both invasive AH and honeybee in Belgium. This constitutes an unexpected report of this Iflavirus outside of Hawaii, where it was recently described in social wasps. Although its virulence is currently unknown, its potential spread raises major concern for European honeybee populations.

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First detection of Lake Sinai virus in honeybees and bumblebees in Slovenia

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Background
Lake Sinai virus (LSV) is a new unclassified RNA virus which infects honeybees and was discovered with next generation sequencing (NGS) in US in 2008. Since the discovery, several different lineages of LSV were found in US, Spain, Belgium and Turkey in multiple bee species. No data regarding LSV infections was available for our country until this study.

Methods
From one archive sample of honeybees, the complete LSV genome was sequenced by the Ion Torrent NGS technology. From October 2016 till January 2018, 56 honeybee samples (pool of 10 bees per sample) from 32 different locations and 41 bumblebee samples (one bumblebee per sample) from 5 different locations throughout Slovenia were collected. Samples were tested by specific RT-PCR method which amplifies a 603 base pair product of LSV RdRp genome region.

Results
The first positive sample of LSV in Slovenia (M92/2010) was discovered from an archive honeybee sample collected in 2010. The complete genome sequence of the LSV M92/2010 strain consists of 5.926 nucleotides and is the first determined complete genome sequence of LSV3 lineage, with 86% nucleotide identity to the most closely related strain NT-LSV3 from Australia (KY465717). 75.92% of tested honeybee samples and 17.07% of bumblebee samples were found positive for LSV by the RT-PCR method.

Conclusion
This is the first evidence of LSV infections in honeybees and bumblebees in Slovenia. The complete genome sequence of the LSV M92/2010 strain is the first determined complete genome sequence of LSV3 lineage. The detected prevalence of the LSV in honeybees was very high, while in bumblebees was lower. The knowledge about LSV transmission or pathogenicity in honeybees and bumblebees is still limited and further studies of this new honeybee virus will be followed.

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The study of rotavirus phylogenetic diversity, re-assortment and Interspecies transmission and associated virome in Northern Irish livestock and wildlife

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Background
Rotavirus (RV) is a highly infectious pathogen of livestock causing diarrhoea and dehydration and substantial economic loss. RV is an RNA virus with a segmented genome that has evolved significantly creating diverse strains. RV is believed to be endemic within livestock and the genotyping of positive isolates will allow the diversity of the rotavirus to be phylogenetically mapped for evidence of re-assortment and interspecies transmission. Diarrhoea in livestock is often caused by a combination of infectious agents such as viruses, bacteria and protozoa. Rotavirus positive samples and rotavirus negative samples were examined using a metagenomics approach through next generation sequencing (NGS) to study the virome of symptomatic animals.

Methods
RV in symptomatic livestock faeces samples (n=326) was screened by PCR for the RV VP6 gene including bovine, porcine, equine and caprine faeces samples from Northern Ireland. A small screening of foxes and other animals was also carried out. Positive RV samples were genotyped for the RV capsid genes VP4 and VP7 by PCR and sequenced by Sanger sequencing. RV positive samples were then selected for screening by de novo NGS using a metagenomics approach. 20% faeces homogenates were nuclease treated and extracted. The viral nucleic acid RNA and DNA were amplified by Repli G WTA and WGA. Libraries were multiplexed and prepared using Nextera-XT library kits. 133 libraries were run on the MiSeq reagent V3 600 cycle as pairend reads. Data was quality checked by Fast QC, assembled in SPAdes, processed through NCBI blast (n) and then MEGAN to display the taxonomical content.

Results
The prevalence of RV VP6 gene was n=108 (33%). Initial Sanger sequencing results showed porcine G3, G4 and G5 and bovine P7 and P13 strains. Preliminary metagenomic results indicates re-assortment and interspecies transmission with a positive bovine sample showing RV acquired re-assortment from human and equine RV strains. Other novel and highly divergent viruses were detected in samples such as gemycircularvirus, smacovirus and odonata-associated virus.

Conclusion
The preliminary data shows there is a large viral community present in both RV positive and RV negative animals. The metagenomic profiling through NGS data set will give a better understanding of the symptomatic virome in livestock.

ABSTRACTS
POSTER PRESENTATIONS

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Molecular characterisation of a previously undetected bluetongue virus serotype 8 strain in Cyprus

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Background
Bluetongue is a haemorrhagic disease of ruminants, transmitted by Culicoides biting midges, caused by the bluetongue virus (BTV). The BTV genome contains 10 double-stranded RNA segments which can be independently re-assorted between strains. There are currently 27 recognised serotypes, with multiple strains per serotype.
Since 1998, frequent incursions of BTV into Europe have led to significant disease outbreaks within naïve ruminant populations and several BTV strains circulate in central and southern Europe. In 2006, for the first time, BTV was detected in Northern Europe. Clinical disease was observed in sheep and cattle due to infection with BTV-8. Following a vaccination campaign across Europe BTV-8 was controlled. However, it re-emerged in France in 2015 and continues to circulate. In Cyprus 2016, sheep and goats showing clinical symptoms of bluetongue were confirmed to be infected with BTV-8, previously undetected in this country. Here we discuss the possible origins of this strain.

Method
BTV was propagated on KC (C. sonorensis) cells from which BTV RNA was extracted using TRIZOL Reagent. Libraries were prepared using the Nextera XT DNA kit and run on a Miseq instrument. Sequence reads were aligned to reference genomes using the BWA-MEM tool. Phylogenetic analysis was performed using MEGA7 and the reliability of the generated trees was estimated by bootstrap analysis of 1000 replicates of the sequence alignment, using the neighbour-joining method.

Results
Based on segment 2 phylogenic analysis, the Cyprus isolate was identified as BTV-8 and is most closely related to a BTV-8 strain from Israel (99.6% identity ISR2008/13). This BTV-8 strain also shared 99.4% identity with two BTV-8 isolates from France (2008, 2015). Further analysis revealed that segment 4 and segment 9 of CYP2016 are more closely related to BTV-4 (ISR2006/12) and BTV-15 (ISR2006/11) stains, respectively, rather than to BTV-8 (FRA2008, 2015 or ISR2008).

Conclusion
A previously undetected BTV-8 strain was introduced to Cyprus in 2016 causing overt clinical symptoms in infected animals. This suggests that the re-assortment of segment 4 and 9 in BTV-8 CYP2016 has led to increased virulence as current BTV-8 infections in France are mainly asymptomatic.

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Diversity and pathogenicity of chicken astrovirus

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Background
Chicken astrovirus (CAstV) is the most recently identified virus of the family, Astroviridae, and like other family members, is a leading cause of enteritis and diarrhoea in the young. CAstV is a separate species to the other main astrovirus of chickens, avian nephritis virus, but shares similar virion morphology; short, single stranded RNA genome (~7kb) and minimalistic gene organisation comprising three open reading frames coding for a protease, a polymerase and a capsid protein. The hypervariable capsid gene has proved useful for classification of the many, diverse strains in circulation which arise due to the rapid evolution of CAstV genomes by antigenic drift. Phylogenetic analysis of the capsid protein sequence of multiple strains revealed there are 2 major groups (A & B) that are antigenically separate sharing low levels of amino acid genetic identity (38–40%; Smyth et al, 2012). Since this initial A/B classification of CAstV strains, specific strains have been identified in association with kidney disease of young chicks, runting stunting syndrome and the White Chicks hatchery disease.

Methods
The CAstV capsid gene RT-PCR test (Smyth et al., 2010) was applied to diagnostic and surveillance samples from broiler flocks. The capsid gene sequences were determined by either Sanger sequencing or next generation sequencing and international sequences were also obtained from the online repositories. The amino acid sequences from multiple CAstV capsid genes were phylogenetically compared using the Geneious bioinformatics programme.

Results and Discussion
It was found that the majority of the strains clustered into groups according to either geographical location and/or pathogenicity. Two additional B group subgroups were formed which included strains associated with high mortality and kidney disease in young broilers (Biii) and with the white chicks hatchery disease (Biv). Better understanding of CAstV diversity in relation to pathogenesis and disease should help improve clinical diagnoses in cases involving this common enteric virus.
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Characterisation of novel inhibitors of the IRF3 and NF-kappa B pathways encoded by African swine fever virus

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Background
The African swine fever virus genome contains 150 to 167 open reading frames. The encoded proteins include many that inhibit the host's innate defence systems thus enabling rapid virus replication and disease progression.

Methods
To expand knowledge of ASFV encoded proteins that can inhibit innate responses we screened a library of 60 plasmids, each expressing a single ASFV protein, for those that could inhibit induction of an IRF3 dependent luciferase reporter gene utilising a type I IFN promoter containing DNA binding motifs for IRF3. A similar screen was employed to identify inhibitors of a NF-κB dependent luciferase reporter gene.

Results
We identified 6 novel ASFV proteins that inhibited induction of the IRF3 reporter by greater than 75% using Sendai virus as the inducer. The point of action of inhibitors was further investigated by overexpression of individual proteins from the IFN induction pathways. This approach indicated that most of these proteins acted at or below the activation of the TBK1 kinase. Interactions of these novel inhibitors with host proteins and their localisation within cells is under investigation. NF-κB activation is required for transcription of several different genes including type I IFN and those involved in the inflammatory response. We identified 9 novel ASFV proteins that inhibited induction of NF-κB by greater than 50% using TNF-α or IL-1β as the inducers.

Conclusion
These novel inhibitors of host innate immunity represent targets for deletion to produce rationally attenuated ASFV vaccine candidates.

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T-Cell epitopes in serotype specific proteins of African swine fever virus

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Background
African swine fever virus (ASFV) causes high mortality in domestic pigs and wild boars approaching 100%. There is no effective and safe universal vaccine available against ASFV. Immune correlates and mechanism of protection against African swine fever virus (ASFV) remain undiscovered and hamper ASFV vaccine development. Well known, that both arms of immunity are important to develop protection against ASFV. We have shown earlier that serotype-specific proteins of ASFV are able to modulate protection. Here, we investigated further the role of ASFV serotype specific proteins in T-cell mediated immunity.

Methods
Peptide library (132 peptides) mimicking C-type lectin and CD2v ASFV proteins was designed. Animal experiments using live attenuated virus (LAV) (Congo-a) and homologous challenge model (Congo-v) were carried out to induce solid immunity as described previously (Titov et al. 2017). PBMC were isolated from recovered animals and used for ELISPOT IFN-y assay (BD, USA). The individual peptides were used to stimulate IFN-y secretion. The number of spots was counted by Immunospot S6 (CLC, USA).

Results
Animal experiments based on homologous ASFV model demonstrated 100% protection. PBMC from all recovered animals (n=6) showed IFN-y positive recall to peptides mapped on C-terminus of C-type lectin. Several minor functional T-cell epitopes were also discovered in C-terminus of CD2v protein of ASFV.

Conclusion
The results indicate that serotype-specific proteins are important antigens for ASFV specific immunity and contain T-cell epitopes, which appeared to be a non-essential component of an efficacious vaccine against ASF.

Acknowledgments
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Molecular characterisation of Polish African swine fever virus strains

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Background
After more than 10 years since African swine fever introduction to Europe, the disease continues to pose a serious threat not only for European, but also worldwide trade of swine. Up to date, a number of 108 outbreaks in pigs and over 2000 cases in wild boars were confirmed in Poland; however the vast majority of them occurred in the past year. In spite of taken preventive measures, the number of positive cases is growing rapidly, as well as affected area, which is consistently extending westward. Genotyping based on the sequencing of B646L and E183L genes, grouped Polish isolates within genotype II. Analyses of the IGR region (I73R-I329L ORFs), revealed that Polish viruses had a TRS insertion identical to that found in Ukrainian, Belarusian and Lithuanian isolates, but differing from reference Georgia2007/1 sequence, indicating common origin of this strains. Another study, concerning molecular evolution of EP402R and MGF505-2R genes involving 67 Polish strains, revealed minor genetic diversity within analysed genes, indicating slow but consistent molecular evolution of these regions.

Methods
The next generation sequencing (NGS) of 7 Polish isolates on MiSeq (Illumina) was performed; subsequently the obtained viral reads were mapped against the reference sequence (Georgia 2007/1) using CLC Genomic Workbench (Qiagen). Final sequences were analysed using Geneious R9 software (Biomatters).

Results
The length of all obtained sequences ranged from 189,393 to 189,405 nt. Global alignment revealed over 99.9% identity in comparison to the reference Georgia 2007/1 strain. The majority of all mutations were represented by point deletions or insertions which were observed mainly within non-coding regions. Regarding variations within ORFs, a number of 25 point mutations/deletions (within 21 genes) was found in all Polish sequences; nevertheless further investigations are needed to elucidate their impact on predicted protein function.

Conclusion
Currently, a growing accessibility of next generation sequencing techniques will probably result in obtaining whole sequences of other European ASFV strains. The sequenced Polish isolates proved slow but consistent molecular evolution of viruses circulating in Europe for the past 10 years and provide a baseline for further comparative analyses of related strains.

Acknowledgements
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Rabies virus belonging to phylogroup C has spread to Poland – complete genome analysis

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Background
Rabies is an acute viral infection of the central nervous system caused by viruses of the genus Lyssavirus, the family Rhabdoviridae and the order Mononegavirales. It is a global disease that occurs on all continents with the exception of several islands and archipelagos. Rabies virus (RV) is mainly transmitted with saliva during biting by rabid animals. Depending on the continent different animal species are RV reservoirs. The genetic diversity of the rabies virus throughout the world has a strong geographic pattern, which results from the virus spread. Four phylogenetic groups of RV were distinguished in Europe since the last decade of the XX century whereas six additional lineages of RV primarily originated from Eurasia were detected through Southern and Eastern Europe. The main goal of the study was the phylogenetic analysis of Polish rabies virus isolates collected for the last decade to investigate the spatial and temporal distribution of the recent RV isolates.

Methods
For this purpose 925 RV isolates collected in the frame of rabies surveillance between 2008–2017 tested rabies positive with FAT (Fluorescent Antibody Test) were involved in the study. Phylogenetic analysis conducted on 570 bp fragment of N gene coding nucleoprotein of Polish RV isolates together with representative GenBank sequences was performed using Mega 5.0 software and Bayesian coalescent approach. For a new Polish RV representative of phylogroup C genome sequencing applying Illumina technology was done.

Results
The study revealed the circulation of two major rabies virus phylogroups in the territory of Poland between 2008–2017 with a prevalence of North Eastern European (NEE) isolates over Central European (CE). In 2008 RV isolate representing phylogroup C previously detected in Eurasia and Eastern Europe was identified for the first time in 2 rabid foxes collected from Eastern area of Poland (Podlaskie voievodeship) nearly Polish-Belarusian border. Full length sequence analysis revealed the highest genetic homology with Russian strains collected at relevant time in the territory of Russia.

Conclusion
The study demonstrates the phylogroup C of RV is further distributed than has been previously reported.
The study was funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015).

Key words
rabies, Poland, phylogenetic

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Determination of exosomes infectivity in cattle infected with bovine leukemia virus (BLV)

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Background
Bovine leukemia virus (BLV) is a member of the Retriviridae family, genus Deltaretrovirus. BLV is a causative agent of bovine leukemia. The BLV genome has two identical RNA positive single strains. In molecular structure, is composed of three principal genes: env, gag and pol which encode the viral envelope, structural proteins and the polymerase, respectively. It has two other genes of a smaller size, rex and associated with viral regulation-tax. Exosomes (Evs) are small membranous microvesicles (40–100 nm in diameter). In humans they are present in various physiological fluids. Evs contain miRNA, mRNA and membrane and intracellular proteins. It has been suggested, that they play a role in intracellular (cell-to-cell) communication through either direct contact of exosomal surface antigens or via the transfer of RNAs and proteins.

Aim
The aim of the study was to isolate exosomes and determine the membrane and intracellular proteins, the presence of BLV proteins gp51 and p24 and infectivity of exosomes in samples taken from BLV infected cattle.

Materials and Methods
Exosomes were isolated from the blood sera and supernatant of dendritic cells (DCs) from BLV infected cell cultures and control cows. Immunological status of animals was determined by ELISA and qPCR. Exosomes were isolated by differential centrifugation. Filtered supernatant was ultracentrifuged at 120,000 x g for 3 h, at 4°C. The pellet of EVs was resuspended in PBS and stored at –80°C for further analysis. Purified protein from each sample was used for Western Blot analysis. Proteins separated on SDS-PAGE were transferred to an Immobilon-P PVDF membrane. BLV proteins were detected using monoclonal antibodies specific to a D-D9 epitope on BLV gp51 and a BLV p24. Lysosomal markers: CD63, CD9 and flotillin-1 were determined. The visualization was performed with chemiluminescence method.

Results
The presence of viral proteins gp51 and protein p24 was detected in exosomes isolated from the sera and supernatants of DCs culture infected with BLV, while these proteins were absent in exosomes isolated from healthy cattle samples. Cellular markers: CD63, CD9 and flotillin-1 were present in both BLV infected and negative samples.

Conclusion
BLV markers: gp51 and p24 were present in exosomes isolated from the sera and supernatants of the BLV infected dendritic cell culture. These findings suggest that BLV proteins are released into exosomes in sera and supernatant, and could be transferred into recipient cells as an alternative route not requiring virus infection.

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Phylogenetic analysis showing new insights of epidemiology, evolution and taxonomy of HEV in Brazil

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Background
Hepatitis E is an emerging infectious disease worldwide distributed. Its causative agent, Hepatitis E virus (HEV), has been found in different animal species such as rabbit, deer, wild boar and domestic swine. Natural HEV-3 infection in swine as well as human autochthonous cases were reported in Brazil, however, only partial genomic fragments have been obtained. Over the last two decades, the amount of sequences increased and, naturally as a mechanism of evolution, it can be observed a high genetic variability and divergences between new HEV genetic groups and subgroups. Subtyping has led to inconsistences among studies and in 2016, Smith et al., standardized HEV subtypes prototypes. Nevertheless, due to the lack of genomic data, Brazilian and South American prototypes were not available, consequently, limiting the knowledge regarding molecular epidemiology and evolution of this virus in the continent.

Methods
Sequences were obtained from positive samples described in previous studies from different regions in Brazil (Northeast, Southeast and South) with more than 3000 Km distant from each other. RNA was isolated from both feces and sera samples using TRIzolTM Reagent (Invitrogen) following manufacturer's instructions. One set of primers were designed to amplify the capsid region on an alignment using 156 partial genomic Brazilian and HEV-3 subtypes references sequences. Another sample was sequenced using primer-walking methodology. RT-PCR was performed using SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen) as per manufacturer's instructions. Sequences were compared to Genbank entries including HEV-3 reference for each subtype. Then, evolutionary study was conducted through distance-based (Neighbor-Joining – NJ) and Maximum Likelihood (ML) inferences methods with Kimura-2-parameter evolutionary substitution model. Bootstrap confidence value of 1000 replicates was used to check the reliability of phylogenetic tree branches.

Results
We obtained the first HEV whole-genomic and five complete capsid sequences from Brazilian strains. Preliminary evolutionary analysis showed that Brazilian sequences clustered into four distinct clades. Two isolates clustered together with reference subtypes 3i and 3f. Four other sequences clustered as outgroups into a-b-j and a-c-h clades and could not be attributed to any HEV-3 subtype.

Conclusion
Data on evolutionary analysis are preliminary so far; however, our analyses suggest that Brazilian sequences should be grouped in two new proposed subtypes. We are currently performing further studies involving Bayesian coalescent-based methods to better understand the evolutionary relationship among Brazilian and other South American strains alongside its impact on the evolution of HEV strains distributed worldwide.

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Importance of the ciliary activity of the airway epithelium in preventing influenza virus infection

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Background
To prevent the detrimental attack by foreign substances, e.g. viruses, bacteria, or physical particles, the respiratory tract is equipped with specialized cells such as ciliated cells and mucus-producing cells. The latter cells generate a protective blanket of mucins that is transported by the ciliary beating out of the airways. We analyzed whether the ciliary activity itself also counteracts virus infection. We chose porcine precision-cut lung slices (PCLS) as a culture system of differentiated airway epithelial cells to analyze the effectiveness of the ciliary activity in preventing influenza virus infection.

Methods
In order to cause reversible ciliostasis, porcine precision-cut lung slices (PCLS) were treated with sodium chloride at different concentrations for different time periods. The effect of NaCl was evaluated by determining the ciliary activity. PCLS were infected with an H3N2 isolate of swine influenza virus in the presence or absence of ciliary activity. The effect of the ciliostatic treatment was evaluated by determining the amount of infectious virus released into the supernatant.

Results
The lowest concentration of sodium chloride that induced complete ciliostasis was 2%; this ciliostatic effect was completely reversible. When the PCLS were treated for up to 30 min, the ciliary activity was fully recovered. Longer treatments resulted in partial recovery. Treatment of swine influenza viruses (H3N2 subtype) with 2% NaCl for 30 min had no effect on the infectivity of the virus as determined by plaque assay on MDCK cells. PCLS infected for 30 min under ciliostatic conditions released two times (at 24hpi) and three times (at 48hpi) more infectious influenza virus into the supernatant than did PCLS infected in the presence of ciliary activity.

Conclusion
The ciliary activity of the airway epithelium reduces the efficiency of influenza virus infection. This approach is applicable also to other viral and bacterial pathogens.

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Co-infection of a natural pair of low and highly pathogenic H7N7 avian influenza viruses in chickens and embryonating chicken eggs

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Background
Avian influenza virus (AIV) of the subtypes H5 and H7 are known to spontaneously mutate to highly pathogenic variants (HP) from precursor viruses of low pathogenicity (LP). Due to HPAIV outbreaks, vast economic losses in poultry production and zoonotic propensities (of both LP and HPAIV) associated with these viruses justify stringent control measures. A better understanding of the processes initiating the molecular changes involved in the emergence of HPAIV and its escape from the index bird which is essentially infected with the antigenically identical precursor virus are required.

Methods
A recently detected pair of LP/HPAIV H7N7 viruses from two spatio-temporally linked outbreaks caused by LP and subsequently HP AIV in two neighbouring layer farms in Germany in 2015 was used for co-infection studies in embryonating chicken eggs and in 6-week old chickens. Co-infection was by mixtures of antigenically identical LP and HPAIV H7N7 at varying titres of the HP variant and constant titres (10^6 EID50 per 0.5 mL) of the LP virus. Furthermore, in ovo infection of the same co-infection mixtures was carried out in specific pathogen free 10- and 14-day-old embryonating chickens eggs to test for a simple method to clarify pathogenesis where a minor population of HPAIV is present among a majority LPAIV population.

Results and Conclusion
Co-infection in chickens with 10^6 EID50 of LPAIV requires at least 10^4 EID50 of antigenically identical HPAIV to produce overt clinical signs and death and spread to uninfected sentinel chickens, while in contrast, an HPAIV mono-infection started with an inoculum of 10^3 EID50 is already sufficient to induce high mortality. Regarding the in ovo study, ECE of older incubation age embryos (14-day-old) already mounted some resistance against both LP and HP AIV infection as compared to 10-day-old embryos. In ovo as well as in vivo, HPAIV strains display systemic tissue tropism (including brain and heart), while the spread of H7N7 LPAIV is limited to the allantoic membrane (ECE), and the oral respiratory and only occasionally the digestive tract. Further evaluations are on-going. Our results will cast new light on how HPAI viruses might escape their primary host after de novo emergence and compete with co-circulating LP AIV precursors. In addition to this, the in ovo model evaluated in this study shows potential for determining pathogenicity of mixtures of different AIV pathotypes and could provide an alternative to using hatched chickens.

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Porcine bocavirus NP1 interferes with the IFN α/β signaling pathway

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Background
Porcine bocavirus (PBoV) is a non-enveloped single stranded DNA virus in the family Parvoviridae, subfamily Parvovirinae, genus Bocavirus. PBoVs was first isolated and identified in 2009 in Sweden, where the virus was found in the lymph nodes of pigs with post-weaning multisystemic wasting syndrome (PMWS) (1). The full-length genome sequence of PBoV exceeds 5 kb and contains three open reading frames (ORFs) that encodes non-structural proteins (NS1, NP1) and structural proteins (VP1, VP2). Recent studies have demonstrated that PBoV might be an immunosuppressive pathogen, as the NP1 has been shown to suppress interferon (IFN) production (2,3). The aim of this study is to analyse whether PBoV from different subgroups interact with the host immune system in a similar manor and to the same extend, starting with investigating PBoV ZJD.

Methods
To begin to investigate whether PBoV ZJD interferes with type I interferon, HEK 293T cells were co-transfected with pISRE-Luc and PBoV ZJD proteins (NS1, NP1, VP1 and VP2). At 24-h post transfection, the cells were treated with poly (I: C) as an activator of the IFN signalling pathway. Luciferase was measured using a dual luciferase reporter assay.

Results
The activation of the ISRE promoter was induced by poly(I:C) stimulation, while this induction was strongly reduced by NP1. The NP1 protein of the PBoV ZJD inhibits the luciferase activity of pISRE-luc about 80% compared to the control.

Conclusion
In conclusion, we demonstrated that PBoV ZJD NP1 protein shows a clear inhibition of pISRE-luc and functions as an antagonist of the IFN signalling pathway. We are currently investigating the mechanism behind this inhibition of the IFN signalling pathway.

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Comparison of the porcine epidemic diarrhea virus (PEDV) shedding in semen from infected specific pathogen-free boars (SPF) with a French S-InDel PEDV strain or a US S-non-InDel PEDV strain

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Background
In 2013, a severe PED epidemic struck the United States of America with two types of virus circulating: S-InDel and S-non-InDel strains (Insertion/deletion in S gene) having different virulence characteristics. PEDV is mainly transmitted by the oro-fecal route but shedding in semen although already suspected has never been studied. This study aimed at comparing, in experimental conditions, the detection of PEDV in semen from specific pathogen free boars inoculated either with a PEDV US S-non-InDel or a PEDV French S-InDel strain.

Methods
The study has been carried out in Anses' animal facilities according to the regulations on animal experimentation. Two rooms with 2 boars housed in two separate pens were used (one room for each strain). The boars were inoculated orally with 5 ml of an inoculum titrating 10^8 PEDV genome/ml (each strain). The experimental trials lasted 51 days post inoculation (DPI). Regular sampling of semen (daily the first week post-inoculation and twice a week after) and of feces (twice a day the first week post-inoculation and then three times a week) were performed to quantify PEDV genome by RT-qPCR. Seroconversion was assessed by PEDV ELISA.

Results
All the boars had diarrhea after inoculation. Transient presence of the PEDV genome for the two boars infected with the S-non-InDel strain was detected by RT-qPCR in the seminal (5.06 × 10^2 to 2.44 × 10^3 genomic copies/mL) at 2 and 35DPI for one of the boar and at 0.5 and 28DPI for the second one and in the sperm-rich fractions of semen (5.64 × 10^2 to 3.40 × 10^4 genomic copies/mL) for three distinct periods comprised between 3 and 16 days. PEDV genome was also detected by RT-qPCR in the sperm-rich fraction of semen (6.94× 10^3 and 4.73× 10^3 genomic copies/mL) for the two boars infected with the S-InDel strain but only once at 7 DPI.

Conclusion
The results of this study evidenced the possible detection of PEDV in semen, intermittently for the S-non-InDel PEDV strain and only once at 7DPI for the S-Indel strain. The shedding of PEDV could be observed in the sperm-rich and seminal fractions for the S-non-InDel strain while the shedding of PEDV for the S-InDel strain could only be observed in the sperm-rich fraction. The infectious nature of the PEDV detected in semen should be confirmed but it raises new questions in term of disease spread within the pig population with the use of potentially contaminated semen.

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Intradermal DNA vaccination harbouring a combination of conserved HA-peptides reduces viral shedding in two different experimental swine influenza virus infections

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Background
Swine influenza A viruses (swIAVs), though not causing abundant mortality, need to be under control since swine can be acting as mixing vessels favoring genome reassorment of diverse Influenza viruses. For example, the pandemic H1N1 virus that arose in 2009, which owned a combination of gene segments from avian, pig and human lineages and which rapidly reached pandemic proportions. In order to confront and prevent these possible emergences as well as antigenic drift phenomena, vaccination strategies are of vital importance. Our research group is mainly focused on the use of potential immunogenic peptides which can be acting as multivalent vaccines and has reported the use of one conserved hemagglutin peptide (HA-peptide) that recognizes and neutralizes different influenza virus subtypes.

Methods
In the present work, a combination of different conserved HA-peptides expressed along with flagellin as an adjuvant interfering in the innate immune response was selected and reverse-translated to a pCDNA3.1(+) plasmid. Two studies in conventional farm pigs were performed in order to test the vaccine efficacy. In the first study, we immunized influenza virus seronegative animals with 600 µg of pCDNA3.1(+) plasmid (applied twice in three-weeks of interval) and challenged them intranasal with pH1N1 virus. In the second study we use influenza virus seropositive animals immunized and challenged with SwH3N2. In both studies we made use of a needle-free IDAL (Intra Dermal Liquid Application) vaccination. Sera, nasal swabs, BALF and PBMCs were collected for analysis during the course of the experiment.

Results
Both experiments demonstrated a complete elimination or significant reduction of the viral shedding within the first week after challenge suggesting vaccine efficacy against both the subtypes (H1 and H3) and, that maternally derived antibodies (MDA) were not an obstacle for the vaccine approach tested herein. Antibodies against both H1 and H3 subtypes also increased after first vaccination and pre-challenge time-points in the vaccinated group. Cellular and mucosal (IgA) immune responses need to be further evaluated.

Conclusion
HA-peptides could potentially be used as a multivalent vaccine against Influenza viruses.

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Influence of cell type and cell culture media on the propagation of foot-and-mouth disease virus with regard to vaccine quality

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Background
Suspension culture of BHK cells allows large-scale virus propagation and cost-efficient vaccine production, while the shift to animal-component-free cell culture media without serum is beneficial for the quality and downstream processing of the product. Foot-and-mouth disease virus is still endemic in many parts of the world and high-quality vaccines are essential for the eradication of this highly contagious and economically devastating disease.

Methods
Changes to the viral genome sequence during passaging in an adherent and a suspension cell culture system were compared and the impact of amino acid substitutions on receptor tropism, antigenicity and particle stability was examined. Virus production in suspension cells in animal-component-free media and in serum-containing media as well as in adherent cells in serum-containing media was compared. Infection kinetics were determined and the yield of intact viral particles was estimated in all systems using sucrose density gradient centrifugation.

Results
Capsid protein sequence alterations were serotype-specific, but varied between cell lines. The A24-2P virus variant had expanded its receptor tropism, but virus neutralization tests found no changes in the antigenic profile in comparison to the original viruses. There were no differences in viral titer between a suspension and an adherent cell culture system, independent of the type of media used. Also, the usage of a serum-free suspension culture system promoted viral growth and allowed an earlier harvest. For serotype O isolates, no differences were seen in the yield of 146S particles. Serotype A preparations revealed a decreased yield of 146S particles in suspension cells independent of the culture media.

Conclusion
The selective pressure of the available surface receptors in different cell culture systems may be responsible for alterations in the capsid coding sequence of culture-grown virus. Important vaccine potency characteristics such as viral titer and the neutralization profile were unaffected, but the 146S particle yield differed for one of the tested serotypes.

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Bluetongue disease – from suspicious field sample to serotype identification in 2 hours

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Background
Bluetongue virus (BTV; genus Orbivirus, family Reoviridae) is a double-stranded (ds) RNA virus with 10 genome segments. VP2 is the primary target for neutralizing antibodies and defines the serotype. Today, more than 27 serotypes are known and new serotypes are under investigation. Beside the group-specific BTV-genome detection further serotype characterization is of importance to understand the epidemiology of the disease and for efficient outbreak control.

Methods
A low-density RT-qPCR array representing a panel of group- and serotype specific assays was combined with an internal control system. For BTV serotype detection both published (Maan et al., 2016) and newly developed in-house PCR-systems were combined. The different primer-probe-mixes were placed in 48 wells of a 96 well plate stored at –20 degree until use. The RT-qPCR array can be started in less than 15 minutes. First, the template RNA will be added to the prepared primer-probe-mixes in the PCR plate and heat denatured at 95°C for 3 min. After cooling, the RT-qPCR mastermix will be added in each well and a PCR run of around 90 min can be started. Beside the fast identification of the BTV-serotype in clinical cases, the developed low-density RT-qPCR-array can be easily extended with novel BTV-serotype assays or assays for differential diagnosis like EHDV or FMDV.

Results
So far, we analyzed the analytical sensitivity of the array system with 24 typical BTV serotypes using log 10 dilution series. Loss of analytical sensitivity around 1 to 2 Cq-values compared to the group-specific BTV assay was observed, like BTV 1, 4, 13, 19, 23 and 24. For serotypes 2, 15, 16, 17, 20 and 21 similar sensitivity like in the pan BTV-assay could be observed. In addition, the serotype specific assays for serotypes 6, 8, 9, 10, 11, 12, 14, 18 and 22 were even more sensitive than group-specific BTV assay.

Conclusion
A panel of serotype assays could be successfully established, and to our experience, it is feasible to obtain fast and reliable results for serotype identification using the multi-well BTV low-density PCR-array. However, assay parameters for several serotypes need to be changed for further improving the sensitivity of selected serotype-specific PCRs within the BTV-typing array. The adaption of the primer/-probe concentration as well as the split of published and in-house assays in independent wells on the array are possible solutions. For BTV serotype 23 newly designed primers might be necessary.

Key words
BTV, serotypes, qRT-PCR, sensitivity

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Inactivation of classical swine fever virus in serum samples

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Background
Classical swine fever (CSF) is one of the most important diseases of pigs, which – in case of an outbreak – is associated with enormous economic losses in agriculture. Shipping of serum samples is frequently requested with the objective of serological analyses and is of major interest with regard to biological safety in exchange of sera between laboratories. In order to minimize the risk of virus contamination at the consignee's premises and to comply with legal shipping regulations, this study aimed to establish a pragmatic approach for reliable CSF virus (CSFV) inactivation in sera. Considering the fact that complement inactivation through heating is routinely applied on serum samples prior to shipment, the basic idea was to combine heat treatment with the dilution of a serum sample in a detergent containing buffer in order to facilitate the inactivation process.

Methods
First experiments targeted the assessment of CSFV inactivating detergent concentrations along with heat treatment at 56°C for 30 min. For this purpose, cell culture supernatants containing viable CSFV as well as infectious serum samples collected from animals experimentally infected with CSFV were applied. After adjustment of the detergent concentration the protocol for inactivation was validated using a panel consisting of 33 serum samples with different virological and serological properties. After detergent-heat treatment the samples were analyzed in two CSFV antibody ELISAs, in one CSFV antigen ELISA, in VNT and in virus isolation over two passages.

Results and Conclusion
The combination of heat treatment and dilution of the sample in a detergent containing buffer resulted in a complete virus inactivation without impairing the serological properties with regard to antibody detection in CSFV antibody ELISAs and VNT.

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Immunization against African swine fever virus using potential T-cell antigens delivered via adenovirus and vaccinia vectors

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Background
African swine fever virus (ASFV) ccauses an important disease of domestic swine and wild boar for which there is no vaccine. Protective immunity mechanisms against ASFV are poorly understood due to the complexity of this virus, which encodes over 165 genes, several able to control immune responses. The cellular arm of the immune response is a determinant for effective protection from virulent ASFV following immunization with related attenuated isolates. However, viral epitopes eliciting T-cell responses to ASFV are still largely unknown.

Methods
In the first systematic screen for ASFV T-cell antigens, pools of viral peptides representing 132 predicted proteins encoded by OUT88/3 were incubated with lymphocytes from ASFV immunized pigs. Responses were assessed through IFN-gamma ELISPOT assay. Eighteen viral proteins were identified and the viral genes were cloned in replication deficient adenovirus and modified vaccinia Ankara (MVA) as expression vectors and tested in immunization experiments. In a first experiment, inbred pigs were primed and boosted with a pool of adenoviruses expressing twelve of the T-cell stimulating genes, or adenovirus expressing GFP as control. A second experiment used outbred animals and heterologous prime and boost. Groups of six pigs were immunized with the same pool of adenoviruses expressing twelve ASFV genes, or with a smaller pool expressing nine genes, six of which different from the previous. Control animals were immunized with adenovirus expressing influenza NP. Boost was performed with MVAs expressing the same pools of ASFV genes or control NP. IFN-gamma producing T-cells and antibody production in the immunized animals, in response to ASFV or specific to the different viral proteins, were also assessed.

Results
In the first experiment, after challenge with virulent ASFV, two animals in a group of four immunized with the pool of adenoviruses were partially protected, exhibiting delayed and reduced clinical signs and lower viral loads in blood and tissue in comparison to the controls.
In the second experiment, three animals from the six immunized with the twelve vectored ASFV genes were partially protected after challenge with virulent ASFV, exhibiting reduced clinical signs, while all the animals in the group immunized with nine ASFV genes developed acute disease similarly to the control animals.

Conclusion
Both immunization regimens with the vectored ASFV antigens induced specific cellular and humoral responses, although no clear correlation between such responses and the partial protection in some of the animals could be established at this point. These models of immunization seem promising for inducing protection using different combinations of ASFV antigens.

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Suppression of influenza A virus replication in chicken embryonic fibroblasts lacking CD209L and ANP32A by the CRSPR/Cas9 system

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Background
Limited protection of current vaccines against avian influenza virus (AIV) infection underscores the urgent need for developing novel antiviral strategies including genetic modification in poultry. Genome editing such as by the CRISPR/Cas9 system has become an available genetic technology to generate disease-resistant animals in a gene-specific manner. As an anti-AIV gene target, we paid attention to the functions of two proteins, CD209L which is an authentic endocytic receptor for AIV entry and ANP32A which is an essential host partner co-opted to support AIV replication. To investigate antiviral effects in chicken embryonic fibroblasts (CEF) lacking CD209L or ANP32A in vitro, we established stable CEF lines lacking each gene.

Methods
CEFs were transfected with pX459 expressing Cas9 and sgRNA for each gene and selected with puromycin. DNA was extracted from established CEF lines and confirm each gene sequence. For the investigation of viral one-step growth in CEFs lacking CD209L or ANP32A, the cells were seeded in 35 mm diameter dishes (1 × 106 cells per dish). Strain A/Puerto Rico/8/1934 (PR8) at a multiplicity of infection (m.o.i.) of 0.01 was absorbed onto the cells at 37°C for 1 hour, and the cells were then washed to remove non-adherent virus and cultured in 3 ml DMEM. Each of five hundred microliters of supernatant was harvested from the infected cells at 16 hours post-infection, and plaque formation assay was performed using MDCK cells cultured into DMEM containing 0.8% SeaPlaque Agarose (Lonza) and 2 µg/ml of TPCK-treated trypsin.

Results
In both of CEF line lacking each gene, the virus production was significantly suppressed, as compared with that in normal CEFs. Interestingly, suppression of AIV replication was much stronger in CEF lines lacking ANP32A than them lacking CD209L. These results indicated that lacking of each gene, especially ANP32A, led to suppress AIV replication.

Conclusion
Our findings in the present study suggested that ANP32A is a candidate of anti-AIV gene targets in developing influenza-resistant poultry.

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Development of a multiplex real-time RT-PCR assay for the differential detection of bluetongue virus serotypes

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Background
Bluetongue is an economically-important disease affecting mainly sheep, cattle and goats. Substantial financial costs are directly attributed to reduced animal production and restrictions on trade. Over the past 20 years, there have continued to be widespread incursions of different bluetongue virus (BTV) serotypes across Europe. The detection of multiple serotypes co-circulating in the same country is also observed, including the recent detection of BTV-4 and BTV-8 in France. As part of improved disease surveillance and control of bluetongue, particularly in neighbouring countries that are BTV-free, the development of diagnostic assays that are rapid, robust and able to differentiate BTV serotypes are essential.
BTV consists of a segmented dsRNA genome that encodes for 7 structural and 5 non-structural proteins. To-date there are 27 described serotypes of BTV that are determined by the neutralising antibody type-specificity to the outer capsid protein VP2, encoded for by Segment 2 (Seg-2) of the genome. A high level of genetic variation occurs between Seg-2 of different BTV serotypes which has subsequently been targeted for the development of singleplex real-time RT-PCR typing assays. While these assays have been shown to be specific, they are time-consuming and costly to perform individually. Therefore, the development of a multiplex real-time RT-PCR assay that retains a high level of specificity and sensitivity would be advantageous for the simultaneous and differential detection of multiple BTV serotypes.

Methods
Primers and probes targeting conserved regions of Seg-2 were designed to contain a distinct fluorophore and a non-fluorescent quencher for each BTV serotype (BTV-1, BTV-4 and BTV-8). Primer/probe ratios were optimised and the diagnostic sensitivity and specificity of the assay was evaluated using BTV RNA obtained from the Orbivirus Reference Collection at The Pirbright Institute. A compatible TaqMan® multiplex master mix was used in combination with the passive reference dye, Mustang Purple® for assay normalisation. All assays were performed using the Applied Biosystems 7500 Fast real-time PCR instrument.

Results
The development of a real-time RT-PCR multiplex assay allowed for the simultaneous detection of different BTV serotypes without compromising on accuracy and sensitivity. The assay is versatile and can be adapted for different serotype combinations due to the specific probe chemistries.

Conclusion
The multiplex assay is cost-effective, rapid and capable of high-throughput. This increased diagnostic capability will contribute to improved disease surveillance, particularly to BTV-free countries that risk the importation of infected animals from countries where multiple BTV serotypes may be circulating.

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Novel highly immunogenic strain of B.anthracis 363/11

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Background
The virulent strains of B.anthracis, which are able to evade immune response induced by live and chemical vaccines, are sporadically identified. In this case, massive continuous vaccination can potentially lead to selection of strains that threat public health and might cause disease in vaccinated animals. With the aim to develop a vaccine with a broad protective potential we identified and studied monoplasmid derivate of B.anthracis strain 363/11. The 363/11 strain was isolated from dead piglet and had low pathogenicity for laboratory animals (mice LD50 3,162*105 spores, guinea pigs LD >108 spores). Although the reference vaccine strain Sterne shows LD 1,6*102 and 8*105 respectively.

Methods
A comparative evaluation of protective properties and intensity of inducible immunity was studied in experiments on guinea pigs and sheep using capsular reference strains. The immunogenic and protective properties of the strains were studied according to the "Methodological recommendations for the selection of anthrax strains intended for the design of vaccines" (2006).

Results
Interestingly, that having low pathogenicity for laboratory animals novel strain has shown phenotype features similar to virulent B.anthracis strains: high proteolytic activity and α-hemolysin expression, protocatechuic acid synthesis. The B.anthracis 363/11 strain is unique and does not have any similar analogues within vaccine strains used in the Russian Federation. The typical vaccine strains have low enzyme activity and characterized by β-hemolysin expression. The phenotype difference might potentially help to fill the gap in vaccine induced immunity for epidemic strains that can escape from immune response. The vaccine strain Sterne has similar characteristics to virulent isolates; however, it cannot be used in the Russian Federation due to economic reasons. The results show that ImD50 of 363/11 for guinea pigs against capsuled strain 71/12 and Carbovax (200LD50) are 2,95*104 and 1,55*105 spores respectively; 100% survival rate of guinea pigs was achieved with 107 spores of B.anthracis 363/11 against 17JB and 71/12 strains (200LD50).

Conclusion
The preliminary results demonstrate the higher protective potential of B.anthracis 363/11 strain compared to vaccine strain 55-VNIIVViM, currently used in Russian Federation. Novel strain B.anthracis 363/11 showed acceptable protection against some epidemic strains, where 55-VNIIVViM did not. The results also suggest that novel strain B.athracis 363/11 is a potential candidate for vaccine development against anthrax.

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Simultaneous detection of AIV and NDV by droplet digital PCR (dd-PCR)

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Background
A droplet digital PCR (dd-PCR) evaluated for simultaneous detection of avian influenza virus (AIV) and Newcastle disease virus (NDV). In dd-PCR assay samples are partitioned into 20,000 droplets with target and background DNA randomly distributed among the droplets. After PCR amplification, each droplet provides a fluorescent positive or negative signal indicating the target DNA was present or not present after partitioning. At a certain dilution each droplet provides an independent digital measurement. Positive and negative droplets are counted in a reader and the absolute concentration of target DNA can be calculated.

Methods
The PCR assay presented here is developed using the one-step RT-dd PCR advanced kit for probes from Bio-Rad. Primers and probes for AIV and NDV were from Spackman E. et al 2002 and Wise MG. et al 2004, respectively. RNA from ten AIV strains (H1N1, H2N3, H3N2, H5N1, H5N3, H7N7, H9N2, H13N6, H14N5, H16N3) and from three NDV lentogenic strain LaSota/46 representing genotype II, the velogenic strains Herts´33 representing genotype IV and a pigeon paramyxovirus (PPMV) from Sweden isolated in 2007.

Results
Single dd-PCR and duplex dd-PCR assays were successfully used to detect all AIV strains or NDV strains tested. One-step RT dd-PCR assay was applied for the detection and absolute quantification of NDV and AIV and optimized using serial dilutions of clinical samples from Swedish outbreaks in 2007. Results showed good separation between positive and negative droplets. At the same time, each dilution was quantified. The duplex format was able to differentiate between AIV and NDV with absolute quantification of each virus. The copy numbers of each virus in the duplex dd-PCR were calculated.

Conclusion
A new assay for studying the population/copy number of two different viruses in the same sample was developed. The system could be used for broad detection of different organisms in the same sample and for study the population of each organism at the same time.

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Syndromic surveillance and pathogen detection using multiplex assays for respiratory infections in small ruminants

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Background
Several bacterial and viruses can infect the respiratory tract of small ruminants causing similar clinical signs. The differential diagnosis of respiratory diseases in small ruminants can be achieved using multiplex assays for an accurate identification of the causative agents.

Objective
The present study was aimed at developing molecular multiplex assays using different methods, such as real time PCR and microfluidics bead-based technology, applicable for the syndromic surveillance of respiratory infection in small ruminants. The targeted infections were those caused by Capripoxviruses (CaPVs), Peste-des-petits ruminants' virus (PPRV), Parapoxvirus, Mycoplasma Capricolum subsp. Capripneumoniae (MCCP) and Pasteurella multocida (PM). An internal control was included in order to determine the quality of samples being tested.

Methodology
Primers and probes were designed for the conserved regions of the genomes of all the targeted pathogens. The probes for real time PCR were labelled with compatible fluorescent dyes and quenchers, whereas for microfluidics bead based method, primers and probes were biotinylated, phosphorylated and C12 amino-modified accordingly. Total nucleic acid extraction procedures were evaluated to extract both DNA or RNA. The amplification protocols were optimized and the procedures were validated for the amplification of the above-mentioned pathogens in a single test (or tube).

Results
A one-step multiplex real time PCR method was developed to amplify four targets, CaPVs, PPRV, MCCP and PM in order to accommodate real time PCR platforms from different manufacturers and reduce complexity in performing the assay. This real time PCR method was highly specific and sensitive in detecting the targeted pathogens as well as co-coinfections. Out of 314 samples tested from different African countries, 80 samples were positive for PPRV, 50 for PM, 2 for CaPV and 8 were mixed infections of PPRV and PM. The same pathogens were included, and the panel was expanded with Parapoxvirus, an internal control, and tested in microfluidics bead-based method. The validated microfluidics bead-based method displayed a similar analytical sensitivity and specificity to the real time PCR based assay.

Conclusion
The real time PCR method is being implemented in routine diagnostics and surveillance of different veterinary laboratories in Africa and Asia for differential diagnosis of PPR. Microfluidics bead-based assays will extend the scope by allowing the screening of more pathogens. These two multiplex approaches facilitate the syndromic surveillance of respiratory infection in small ruminants in regions where several pathogens with similar clinical symptoms are present.

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Differentiation of sheep poxvirus vaccines from field isolates and other Capripoxvirus species

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Background
The genus Capripoxvirus (CaPV) within the family Poxviridae comprises three closely related viruses, sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV) causing sheep pox (SPP), goat pox (GTP) and lumpy skin disease (LSD) in small ruminants and cattle respectively. LSD has emerged in Europe in 2015 and first incursions of SPP in the European Union were reported in Bulgaria and Greece in 2013. Live attenuated SPPV vaccines are widely used in many countries to control SPP and GTP. With the increasing number of reports on SPP in previously vaccinated sheep herds, it is imperative to develop new diagnostic tools for differentiation of SPPV field strains from attenuated vaccine strains.

Objective
This work aimed at identifying appropriate diagnostic targets to develop assays for the rapid and accurate differentiation SPPV vaccine strains from SPPV field isolates and other CaPVs.

Methodology
To identify a suitable molecular target for the development of these assays, the full genomes of several SPPV vaccines strains and SPPV field isolates were compared. A unique 84-base pair nucleotide deletion located between the DNA ligase and the B22R gene was exploited to develop a gel-based PCR, and a region containing a 48bp deletion within the B22R gene of SPPV vaccines strains only, as well as species-specific nucleotide difference between SPPV field isolates, GTPV and LSDV, was targeted to develop a HRM assay.

Results
The gel-based assay was readily able to differentiate SPPV vaccines from field isolates. However, this method alone could not differentiate SPPV field isolates from GTPV and LSDV. In contrast, the HRM based method allowed the differentiation of SPPV vaccines from field isolates and further enabled the genotyping of capripoxviruses isolates. Out of 61 samples tested, we identified 4 SPPV vaccines, 14 SPPV field isolates, 11 GTPVs and 32 LSDVs. The two assays were both sensitive and specific and in agreement with the sequencing data of the tested samples.

Conclusion
The assays described herein are reliable and rapid methods for the differentiation of SPPV Vaccines from SPPV field isolates. While the gel based assay needs to be combined with capripoxvirus species-specific assays, the HRM assay stands alone as a tool to differentiate SPPV vaccines from field isolates and simultaneously genotype SPPVs, GTPVs and LSDVs. The methods are suited for routine use during outbreak investigations in both capripoxvirus enzootic and disease-free countries.

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Evaluation of Capripoxvirus surface proteins as antigens for the detection of antibodies using indirect ELISA

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Background
Lumpy Skin Disease (LSD), Sheeppox (SPP) and Goatpox (GTP) are contagious diseases of ruminants with a devastating impact on the livestock industry and trade, also affecting the living conditions of poor rural and small farmers. LSD, SPP, GTP were mainly confined to Africa, the Middle East and Asia, with some sporadic incursions of SPP in Greece and Bulgaria. However, in 2015 the first incursions of LSD occurred in the European Union. Due to their potential for rapid spreading, a highly sensitive and specific serological method for active and passive surveillance of SPP, GTP and LSD is needed. In addition, such a tool could serve for post-vaccination monitoring.

Objective
The aim of this work was to evaluate recombinants proteins of the capripoxvirus virion for use in an indirect ELISA (iELISA) to detect anti-capripox antibodies in vaccinated and naturally infected small ruminants and cattle sera.

Method
We have identified, characterized, expressed and purified capripoxvirus virion surface protein (CVSP) that react to positive SPP, GTP and LSD sera samples by Western Blot and iELISA. The iELISA was further optimised and evaluated using sera samples from vaccinated, experimentally and naturally infected sheep, goat and cattle.

Results
Twenty experimentally infected positive and 130 negative LSD, SPP and GTP sera samples were tested and correctly identified using the CVSP iELISA. We tested sera collected at multiple time points from several LSD experimentally infected animals. We observed a time dependant increased in anti-LSD antibody production after day 14 post infection. For specificity, five ORF positive sera were tested. None of the ORF positive sera was positive in the CVSP iELISA. A number of field sera collected during capripoxvirus outbreaks and vaccination campaigns from animals with known infection/vaccination status were tested by the CVSP iELISA and compared to the virus neutralization assay. The complete dataset and results of the field test evaluation will be presented.

Conclusion
As capripox diseases spread, and to strengthen disease surveillance and control programmes, there is an ever-increasing need for rapid and effective antibody detection assays. The CVSP tested in the present study demonstrated to be good antigen candidates for the development of sensitive and specific serological assays. The prototype iELISA developed has the potential to be used as an effective and rapid method for capripoxvirus antibody detection in vaccinated, experimentally and naturally infected sheep, goat and cattle sera.

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Development of an alternative multiplexed bead-based avian influenza H5 pathotyping assay

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Early warning avian influenza (AI) surveillance and rapid diagnosis are necessary to contain highly pathogenic H5 AI (HPAI) outbreaks. In this context, fast pathotyping is indispensable to allow the prompt implementation of restriction measures to limit virus spread. Currently, distinction of low and highly pathogenic AI (LPAI/HPAI) is mostly determined by sequencing the hemagglutinin gene cleavage site (CS). Here, an alternative specific multiplex bead-based luminex fast assay for H5 pathotyping is developed. Specific SNP (single-nucleotide polymorphism)-based primers for LPAI and HPAI CS were designed to determine the sample's molecular pathotype. The pathotype of all LPAI/HPAIs was predicted with a high degree of specificity. This alternative strategy may be considered for implementation in routine diagnostics and may be extended for AI neuraminidase (NA) subtyping.

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The influence of sample preparations on high-throughput sequencing detection of viruses in clinical samples

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Background
Viral metagenomics is a powerful tool not only for identifying potential causative agents of diseases but also for studying viral composition of biological or environmental samples. Detection of viral nucleic acids involves multiple steps such as sample homogenization, enrichment, extraction, amplification, library preparation, sequencing and data analysis. To enrich virus particles, different methods, such as filtration to remove bacteria, ultracentrifugation and/or precipitation to pellet out virus particles, and target-specific probes to fish out viral nucleic acids, may be used. The aim of this study is to investigate the possible effects of inclusion of ultracentrifugation as an additional sample preparation step on metagenomic detection of viruses in swine fecal samples.

Methods
Six swine fecal samples, of which three were in normal shape and three in semi-liquid form, were processed in duplicates by a routine metagenomic protocol (#1) to study fecal virome. The same samples were also prepared by the routine protocol plus an ultracentrifugation step aiming to enrich virus particles (#2). Nucleic acids were extracted by TRIzol combined with RNeasy Mini kit (Qiagen). A total of 24 libraries were prepared and sequenced using Illumina MiSeq Reagent Kit v3 (600-cycle). Reads were quality trimmed, assembled into contigs by Ray/2.3.1, which were then blasted against NCBI viral reference sequences. A minimal length of 100 nt, E-value of 1E-50 and identify percentage of 70 were applied to filter results in RStudio.

Results
The mean number of blast hits against virus reference genome over the six samples was 10.3 (protocol #1) and 8.7 (protocol #2). The ultracentrifugation step to enrich viruses seemingly did not improve performance. Regardless of the protocols, about one-third of the hits were from both duplicates and the rest were from only one of the duplicates. In the case of sample S3, not a single hit was from both replicates. On average, 22.6% of hits were identified by both protocols in duplicate while 77.4% were by one of them.

Conclusion
The routine protocol worked well for sample preparation and ultracentrifugation step did not improve the final result. The study demonstrates great variations in terms of number of hits against virus reference genome and highlights the need to include replicates in the whole procedure of metagenomics studies.

Acknowledgement
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Antipoxvirus compounds screening for veterinary medicine

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Background
Several viruses belonging to Poxviridae family are responsible for major human and animal diseases (Smallpox, Monkeypox, Sheeppox, Myxomavirus (MYXV)…). Although smallpox has been eradicated, it is not excluded that the virus is present in remote endemic areas, can reemerge during explorations of ice floes, or in case of bioterrorism. Hence, efforts have been intensified for the discovery of new therapeutic weapons against poxviruses, and in particular the development of anti-poxvirus compounds. Several antiviral molecules including cidofovir and ST-26 have been shown to be effective against vaccinia virus in humans. At present, there are no effective antiviral molecules for the treatment of poxviral infections in veterinary medicine. Our objective is to develop poxvirus-specific antiviral molecules by high throughput screening for veterinary purposes. We first chose to study MYXV, a Leporipoxvirus responsible for myxomatosis, a major infectious disease of the European rabbit (Oryctolagus cuniculus).
In France, heterologous (Rabbit Fibroma Virus) and homologous (attenuated MYXV SG33) vaccines are used to control myxomatosis. Both Vaccines are not completely effective and the current protocols requiring boost every six months, are exhausting and expensive. Therefore, the development of antiviral compounds could be interesting and particularly for pet rabbits.

Methods/Results
We first constructed two autofluorescent MYXV containing the ANCHOR TM system (1) from a wild strain (Toulouse 1) and a vaccine attenuated strain (SG33). The ANCHOR TM system is a new visualization system for DNA in living cells, developed by NeoVirTech. It is based on the insertion into the viral genome of an autofluorescent cassette composed of an ANCH sequence and a gene coding for the expression of the OR protein fused to a fluorescent protein. This system provides both quantitative and qualitative results on living cells in 24 hours, and is suitable for use with high-throughput microscopy. After validation of the virus constructions, the autofluorescence was checked in rabbit cells (RK13) by high resolution microscopy. Fine analysis of the MXYV viral cycle was carried out allowing the comparison of the infection kinetics of the different viruses. Then, FDA approved chemical library was screened to identify new antiviral molecules.

Conclusion/Discussion
These studies will give us a better knowledge of the poxvirus replication cycle, but also the establishment of a new animal model for the high-throughput screening of new classes of anti-pox compounds in the veterinary field.

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Circulation and impact of bovine herpesvirus 2 with diagnosis of BHV1 in France

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Background
Cross-reactions between bovine herpesvirus 2 (BHV2) antibodies and ELISA tests for the diagnosis of infectious bovine rhinotracheitis have already been shown to be responsible for BHV1 singleton reactors in certified BHV1-free herds. Thus, regions with high density of cattle and programs to control BHV1 are advised to check for prevalence of BHV2 and to perform relevant confirmatory tests for discrimination of such BHV1 singleton reactors. The aim of this study was to determine whether BHV2 was prevalent in France and to develop an alternative serological test including both viral genotypes.

Methods
A collection of 1083 individual sera collected from 55 French cattle herds located throughout the country were enrolled in this study. All sera were analyzed with 3 sets of BHV1 ELISA kits (whole-virus indirect tests, gB and gE blocking tests) and a BHV2 indirect ELISA kit (IDvet). Additionally, a panel of 116 false-positive singleton reactors from certified BHV1-free herds was screened with an "in house" indirect ELISAs established on new recombinant antigens derived from the gE protein of BHV1 and the gD proteins of both BHV1 and BHV2.

Results
The serological survey for BHV2 in French cattle herds revealed a between-herd prevalence of 55%. However, seroprevalence was not uniformly distributed throughout the country. Among BHV1-free herds, the mean reactivity for BHV1 indirect and gB ELISA kits was significantly increased in BHV2 seropositive herds, leading to the occurrence of BHV1 false-positive reactions. These data were consistent with the fact that 86 out of 116 (74%) false-positive singleton reactors from certified BHV1-free herds also tested positive with the BHV2 ELISA kit. In order to differentiate in a rapid and simply way these BHV2 cross-reactions from BHV1 infected animals, three recombinant antigens derived from the gE protein of BHV1 and the gD proteins of both BHV1 and BHV2 were developed and used independently as antigens in an indirect ELISA (r-ELISA). The performances of these r-ELISAs were evaluated using samples from the serological survey. The BHV2 r-ELISA and the combination of the two BHV1 r-ELISAs showed high specificity (100%) but lower sensitivity than those of commercially available ELISA kits. Notably, all false-negative samples (8 out of 128 sera) in the BHV1 r-ELISA corresponded to weak positive samples for gE blocking tests. The r-ELISAs were then used for screening the false-positive, BHV1 singleton reactors. Out of the 86 sera which were found positive with the BHV2 ELISA kit, 68 (79%) also tested positive with the BHV2 r-ELISA. In contrast, no sera were found positive with the combination of the BHV1 r-ELISAs.

Conclusion
These results highlighted the serological drawback of BHV2 circulation with BHV1 diagnosis and provided the basis for development of a multiplex test allowing discrimination between these two viruses.

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Validation of real-time PCR kits for specific detection of classical and African swine fever virus

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Background
Classical swine fever (CSF) and African swine fever (ASF) are highly contagious viral diseases with high economic impact. Although CSF and ASF are caused by unrelated viruses, both have similar clinical signs such as fever, ataxia, and severe depression in pig and wild boar. These diseases are notifiable to OIE and are present in many parts of the world including Asia and Eastern Europe. Moreover, ASF is reported in European Union, with recent dissemination in Hungary. This emphasizes the need for rapid, reliable and effective differential diagnosis tools for these diseases.

Methods
In this context, BioSellal decides to develop three ready-to-use real-time PCR kits: two duplex which detect either CSFV (FAM labelling) or ASFV (VIC labelling) with an internal positive control (Cy5 labelling), and one triplex that combined all three targets in the same reaction well. Validations of each Bio-T kit® were performed according to the specifications of the French National Reference Laboratory (NRL) for CSF and ASF (ANSES Ploufragan-Plouzané, France) which notably describes the panel of samples to be checked for inclusivity and exclusivity and the limit of detection of the complete method to be reached for each target. They include the general guidelines defined by the French AFNOR standard NF-U47-600-2.

Results
Bio-T kit® CSFV and Bio-T kit® CSFV & ASFV allow the specific detection of genotypes 1.1, 1.2,2.1 and 2.3 of CSFV while Bio-T kit® ASFV and Bio-T kit® CSFV & ASFV correctly detect ASFV genotypes I, II, III, IV, V, VI, VIII, IX, X, XVII and XXIII. Globally, PCR characteristics for the triplex are identical to those of the two duplexes: limits of detection were 20 copies/RT-PCR for CSFV and 5 copies/PCR for ASFV, and efficiencies were around 100% for both targets.
In combination with the BioExtract® Superball® extraction kit, reference samples at the detection level required by the French NRL for both targets were detected by all the three kits.
Finally, diagnostic sensibility and specificity were 100% respectively for each target by using the French NRL panel of samples for CSFV and ASFV and the European Union reference laboratory panel (CISA-INIA, Madrid, Spain) for ASFV.

Conclusion
The Bio-T kit® CSFV, Bio-T kit® ASFV and Bio-T kit® CSFV & ASFV are three effectives tools allowing the differential diagnosis of CSF and ASF. Moreover, the ready-to-use triplex one-step Master Mix could be an interesting tool to survey both diseases into regions where CSFV and ASFV are presents.

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Extented validation of ELISAs & qPCR for the diagnosis of African swine fever

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Background
ASF control and eradication programs require accurate and reliable diagnostic tests. IDvet offers solutions for antibody detection (indirect and competitive ELISAs) in serum, blood filter paper and meat juice samples, as well as a real-time qPCR. This poster shows complementary data for IDvet's ELISAs performance on wild boar samples, and qPCR validation data. Wild boar samples can be problematic for ASF diagnosis; previous studies (Dixon et al.) reported specificity issues with commercial ELISAs (Sp: 63 to 84%) whereas IDvet's iELISA had 100% specificity. The performance of the IDScreen® ASF competitive ELISA (P30 based – Sp: 100% domestic pigs – 97, 1% wild boar; Se: 95.8%) had not been extensively on wild board samples.

Methods
Diagnostic specificity of the IDScreen® ASF ELISAs on wild board was evaluated through the analysis of 255 wild boar samples coming from disease-free areas.
Diagnostic sensitivity of the IDGeneTM qPCR was evaluated through the analysis 162 field infected or exposed samples (tissues or blood) and 303 field samples (blood, organs, soft tick homogenates) from recent outbreaks (Eastern Europea, Africa). Theses sample were also tested with the UPL-qPCR and with the OIE qPCR. Comparative data with in-house PCR during routine diagnosis will also be presented.

Results
The measured specificity on the 255 wild boar samples was 100% for the Indirect ELISA and 98,4% for the competitive ELISA. Out of 162 field infected or exposed samples (tissues or blood) tested, 95% were positive with the IDGeneTM qPCR ; 91% with the UPL-qPCR and 67% with the OIE qPCR. 303 field samples (blood, organs, soft tick homogenates) from recent outbreaks (Eastern Europa, Africa) were also tested; the measured sensitivity was 98.6%.

Conclusion
The measured specificity were very high for IDScreen® ASF ELISAs reducing the risk of false positive reaction on wild boar samples. The IDGeneTM ASF is a ready-to-use qPCR assay detecting simultaneously ASFV and an endogenous internal positive control. Combined with magnetic beads extraction kit (MAGFASTTM, 20 minutes) and the IDEALTM automate, results may be obtained in less than two hours. The qPCR kit was validated by the EURL for ASF. All ASFV isolates tested were detected, regardless of the genotype. IDvet offers a full range of tools for accurate and rapid diagnosis of ASF.

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Poultry vaccines: innovative serological assays for diagnosis and vaccination monitoring for H9 and H5 avian influenza A

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Introduction
Influenza viruses belong to the family Orthomyxoviridae and infect a variety of human and animal hosts. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin (H) and Neuraminidase (N) antigens. Eighteen H antigens (H1 to H18) and eleven N antigens (N1 to N11) have been isolated. Most avian influenza viruses (H1 to 18 subtypes) are low pathogenic, such as H9, whereas some subtypes containing H5 and H7 are associated with highly pathogenic forms. Co-infection between avian respiratory diseases and low pathogenic H9 could lead to important losses in poultry flocks. For many years, inactivated vaccines based on circulating hemagglutinin or neuraminidase were developed to protect flocks against Influenza such as H5 and H7. As to control also H9 outbreaks, specific vaccines were developed leading to an increased need for rapid and reliable diagnostic and monitoring tools. Serological techniques are commonly used for disease monitoring. ELISA testing is an efficient and cost-effective method for the analysis of large numbers of samples, particularly in comparison with the Hemagglutination Inhibition Test (HI). As a result, IDvet has developed new tools to monitor vaccination uptake for H9 and H5 AI: ID Screen® Influenza H9 or H5 Indirect.

Material and Methods
ID Screen® Influenza H5 Antibody Indirect ELISA (H5 iELISA), based on H5 recombinant protein and ID Screen® Influenza H9 Antibody Indirect ELISA (H9 iELISA), based on H9 recombinant protein were used, in comparison with HI test.

Results
The H5 iELISA, based on a recombinant protein, shows high sensitivity and good correlation with the HI test. This ELISA is a suitable tool to monitor vaccination with recombinant vaccines such as rHVT-H5 and rVLP-H5 in chicken and duck samples. The test reach 90% of positivity after 2 to 3 weeks post-vaccination. After a challenge, the titers become higher and reach more than 22 000. What's more, this test may be used in combination with the ID Screen® Influenza A Nucleoprotein Indirect ELISA (NP iELISA), to detect natural infection in animals vaccinated with rHVT-H5 or rVLP-H5 vaccines alone (DIVA strategy). The H9 iELISA shows high sensitivity and good correlation with the HI test. This ELISA is a suitable tool to monitor H9 conventional vaccines.

Conclusion
To conclude, IDvet's kits highly correlate with HI tests and are the only commercial ELISAs able to detect H9 or H5-specific antibodies for diagnosis and monitoring of vaccination. It is also possible to make a DIVA strategy, in case of recombinant vaccines used alone, thanks to the use of NP iELISA.

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Improved detection of antibodies to PRRSV-US strains with the ID Screen® PRRS indirect ELISA

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Background
Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious and economically devastating porcine viral disease. PRRS mainly causes reproductive problems in sows and a respiratory syndrome. Two distinct genotypes of the PRRS virus (PRRSV) exist: European (EU-PRRSV, type 1) and American (US-PRRSV, type 2).
The ID Screen® PRRS Indirect ELISA is designed to detect antibodies directed against PRRSV in porcine serum and plasma.
In April 2018, IDvet improved the kit performance on US-type samples, while maintaining excellent sensitivity and specificity on European strains.

Methods
Diagnostic specificity was evaluated on 1844 samples, including 950 fattening pigs sera from herds known as PRRS negative for years and 894 breeding swine sera from European countries. Sensitivity was evaluated with:
– 8 reference sera from the International PRRSV PTS organised in 2016 by the Dutch Animal Health Service (GD Deventer, The Netherlands);
– 113 and 440 swine sera repectively from European and North-American (Canada: n=264; Mexico n=176) infected herds.
In a seroconversion study, eight 3-week old pigs were vaccinated with PRRS Porcillis vaccine by intradermic way and then challenged 31 day post vaccination (dpv) with a PRRSV Brittany strain (European type). The 8 pigs were bled 7, 14 and dpv. This study was performed by the French Reference Laboratory (ANSES, Ploufragan, France).
1528 sera from negative and infected herds were tested in parallel using the ID Screen® ELISA and another commercial ELISA, kit A.

Results
Measured specificity was 99.9 % (CI95%: 99.8–100.0%). The ID Screen® ELISA efficiently detected both EU and US-type samples. The percentages of correlation were 97.3% and 99.1% respectively with European and North American sera. The ID Screen® ELISA detected seroconversion between 14 and 21 dpv.
1516/1528 gave identical results on both tests. The percentage of correlation was 99.2% and the k coefficent (0.983 ; CI95% 0.974–0.992) indicated high correlation with Kit A.

Conclusion
The ID Screen® PRRS Indirect ELISA kit demonstrates high specificity and excellent performance on a reference panel of sera. It efficiently detects positive animals in the field and correctly identified both EU-PRRSV (type1) and US-PRRSV (type 2) strains and delivers similar results (Se, Sp, analytical Se) as kit A. As of batch D28, the ID Screen® ELISA demonstrates excellent agreement with kit A for both US-type and EU-type sera, while maintaining an excellent test specificity.

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A new pen-side test for the rapid detection of PPR virus in field conditions

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Background
Peste-des-petits ruminants (PPR) is a contagious disease affecting goats and sheep primarily in Africa, the Middle East and the Indian subcontinent. It is caused by the Peste-des-petits ruminants virus (PPRV), a species of the Morbillivirus genus. The disease is highly contagious, with approximately 80 percent mortality in acute disease. Detection of PPR virus can be useful to identify and monitor new outbreaks. The ID Rapid PPR Antigen test is a simple direct test (immuno-chromatographic assay) for the detection of all 4 lineages of the PPRV antigen in swabs that can be carried out at pen-side. Results are obtained in less than 20 minutes.

Methods
Ocular swabs were sampled from 70 goats and 6 sheep in a PPRV-free area without vaccination (Hérault, France). 22 ocular swabs sampled from animals infected with PPR virus having clinical signs when sampled, were tested with the ID Rapid® PPR Antigen. Results obtained were compared with RT-QPCR and the IDScreen® PPR Antigen capture ELISA. Analytical sensitivity was assessed by testing serial dilutions of inactivated PPRV and compared with commercially available lateral flow devices.

Results
All negative samples were found negative with ID Rapid® PPR antigen (specificity = 100%) and the two others techniques. All samples were found positive regardless of the technique tested, demonstrating the capacity of the pen-side test to identify and confirm PPRV infections (sensitivity = 100%). The ID Rapid® PPR Antigen has an equivalent sensitivity compared to the ID Screen® PPRV Antigen ELISA, and higher detection limit compared to other LFD.

Conclusion
The ID Rapid® PPR Antigen has an excellent specificity, successfully detected all the PPR virus isolates, regardless of the genotype tested, and can be used in field conditions without any specific laboratory equipment to confirm PPR diagnosis at pen-side.

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Validation of 2 new qPCRs for the detection of Capripoxviruses and differentiating LSDV infected from vaccinated animals

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Introduction
The genus Capripoxvirus (CPV) comprises three members namely, sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV) affecting sheep, goats and cattle, respectively. CaPV infections produce similar symptoms: severe and permanent hides damages resulting from skin lesions. Moreover, LSDV that recently merged into Europe, can causes temporary or permanent infertility in cattle leading to milk yield decrease and sometimes death. Serious economic losses can follow outbreaks that can have a high morbidity. Diagnostic tools are essential for the detection and the monitoring of LSD, and can play an important role to prevent the spread of the disease. IDvet developed two molecular diagnostic tools, the ID Gene™ Capripox Virus Triplex (IDCPV) for the detection of CPV and the ID Gene™ LSD DIVA Triplex (IDLSDIVA) to distinguish the LSDV field wild type strains from vaccine strains.

Material and Methods
The IDCPV and IDLSDIVA kits are real-time PCR kits. Both kits can be used to test ruminant whole blood, swabs (oral or nasal) and skin lesions. They simultaneously amplifies target DNA and an exogenous internal control to validate all analytical steps of the system. In addition, IDCPV also target an endogenous internal control that confirms the presence of cells and the quality of the sample. The qPCR kits were validated with two extraction protocols (automated and manual methods) in collaboration with the CODA-CERVA according to the French standard (NF U47-600-2). Specificity and sensitivity were evaluated through the analysis of 115 whole blood samples with IDCPV and 153 with IDLSDIVA from the field or from experimental infection (France, Germany, Belgium), previously analyzed with CODA-CERVA or FLI in-house methods.

Results and Discussion
The IDCPV and IDLSDIVA measured sensitivity was closed to 100% and the specificity was 100% regardless of the extraction method. The final multiplex CPV and LSD RT-qPCRs had an analytical sensitivity of less than 5 copies per PCR.

Conclusion
The IDCPV Triplex and the IDLSDIVA Triplex demonstrate an excellent specificity. These kits successfully detect CPV, wild type or vaccine strain either in LSDV vaccinated or infected animals, and have an equivalent sensitivity compared to CODA CERVA and FLI in house PCR assays. The DIVA RT-qPCR, distinguishing adverse reactions to LSDV vaccine from wild type virus circulation, is an essential tool in disease control. These ready-to-use kits, combined with magnetic beads extraction kit (MAGFASTTM, 20 minutes) and the IDEALTM automate, allow to obtain results in less than 2 hours.

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Validation of a new RT-qPCR kit for PRRSV detection

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Background
Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious porcine viral disease, causes economic losses in the pig industry worldwide. PRRS viruses (PRRSV) belong to the genus Arterivirus, family Arteriviridea, classified into two distinct genotypes: European (EU) and North American (NA). In 2006, a highly pathogenic NA strain (HP-PRRSV), characterized by high fever, high morbidity and mortality, emerged in swine farms in China. The ID GeneTM PRRSV kit is a triplex real time PCR assay that allows the detection and differentiation of both types of PRRSV. It simultaneously amplifies target RNAs of the European and North American types (including HP-PRRSV strain) and an exogenous internal control. This control validates all the analytical steps of the system allowing a reliable monitoring of the presence of the virus in swine herds.

Methods
The qPCR kit was validated in collaboration with the FLI Reference Laboratory in Germany (Friedrich Loeffler Institute). Panels of 14 reference, 82 field, 7 ring trial and 5 vaccinale strains was used to test the inclusivity study. Diagnostic sensitivity was evaluated through the analysis of 107 field infected or exposed samples (including 10 field samples of chinese HP-PRRSV strain) of various matrices (organs, sera, tissus homogenates and oropharyngeal fluids) previously analyzed with FLI in-house method. 25 negative samples were also used for validation.

Results
The inclusivity panels of strains were reliably detected, regardless of the genotype. This final multiplex PRRSV RT-qPCR had an analytical sensitivity of less than 10 copies per PCR for the EU type and 5 copies per PCR for the NA type. Closed to 100% of positive samples from both types were found positive with the IDPRRSV kit showing a high diagnostic sensitivity.

Conclusion
The ID Gene™ PRRSV Triplex PCR kit demonstrates high specificity and sensitivity on all matrices used for the detection of PRRSV by RT-qPCR. It efficiently detects positive animals in the field and correctly identifies all strains. Combined with magnetic beads extraction kit (MAGFASTTM, 20 minutes) and the IDEALTM automate, results may be obtained in less than 2 hours. The IDPRRSV kit is a ready-to-use, reliable tool for the detection and typing of PRRSV.

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Genotype detection of clinical isolates of (CAEV)/Maedi-Visna (MVV), using molecular beacons fluorescent probes

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Background
Caprine arthritis encephalitis virus (CAEV) is a serious threat to sheep and goats and could compromise the breeding economy, especially in those areas where the disease records high prevalence, such as Sardinia (Italy). During the 2005-2008 years, Istituto Zooprofilattico Sperimentale (IZS) della Sardegna carried out a serological survey aimed to study in deep the prevalence and the spread of disease, improving the knowledges about laboratory diagnosis. The results showed that CAEV infection was spread throughout the regional territory with a prevalence of positive farms around 80% and a seropositivity of more than 70% of tested animals. In the present work, a high specific molecular probe called "molecular beacon" has been evaluated in order to detect proviral DNA in infected blood cells. This procedure combines the great sensitivity of the polymerase chain reaction, the specificity provided by SRLV genotype molecular beacons, and the throughput of a multicolor fluorescence detection procedure.

Material and Methods
A total of 120 blood samples collected from farms located in the south of Sardinia with a clinical and serological diagnosis for CAEV infection have been analysed. Proviral DNA was obtained from buffy-coat by GeneProof Pathogen Kit (Brno-Czech Republic), 2 µl of DNA extract was used in a real-time PCR reaction by using the kit SYBR Premix (TaKara-Clontech®) with a LightCycler II Roche® apparatus, following the manufacture instructions. The PCR/Molecular Beacon target region was identified by multiple sequence alignment program Geneious Biomatters Ltd. Four different probes have been designed using two different SRLV genic regions: (i) gag gene, for typing genotypes B3 and E. (ii) gene for Pol protein as SRLV detection and (iii) goat 18S rRNA gene as amplification control. To evaluate the specificity and sensitivity of the MB probe, all samples were tested using a capillary sequencing method with ABI 310 (Applied Biosystems) and IN3 serological test.

Results
The test results showed a detection limit ranged from 1*103 to 5*103 genomes/µl. The 32% of samples were SRLV positive, while by serological test the positivity was assessed at 48%. The goats that resulted positive for SRLV, the 90% showed B3 genotype while the 10% was E genotype. These results were also confirmed using DNA sequencing procedure, detecting the Volterra, Fonni, Roccaverano and Seui subtypes, in according with previous studies.

Conclusion
The presented molecular approach by using these florescent circular DNA probes could be useful for a rapid and complete laboratory diagnosis of CAEV infection.

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Development of a new TaqMan based rRT-PCR for encephalomyocarditis virus (EMCV) detection

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Background
The encephalomyocarditis virus (EMCV) is a member of genus Cardiovirus, family Picornaviridae, and is a causative agent of myocarditis, encephalitis, and reproductive disorders, depending on host species and aging. It infects various mammals, including domestic, captive and wild animals, with zoonotic potential for humans; swine is the most receptive between farm species. The infection is widespread in many countries, both industrialized and not industrialized. Phylogenetic analyses show that viral strains detected worldwide are grouped into five distinguishable lineages, though the majority that includes all those from domestic species cluster in the two lineages A and B. In Italy, the disease is frequently confirmed in pig farms of highly-dense populated regions, where it causes fatal myocarditis. Molecular assays are the preferred diagnostic tools, therefore this work aimed to develop a one-step TaqMan-based real-time RT-PCR (rRT-PCR) able to detect EMC viruses belonging to lineages A and B, by targeting a portion of the well-conserved 3D-coding region.

Method
The optimal primers and probe, expected to detect any EMCV strain belonging to lineages A and B, were designed by the alignment of 3D-coding sequences obtained from GenBank and from 50 Italian isolates collected between 2013 and 2015. Analytical sensitivity was evaluated on ten-fold serial dilutions of virus grown on cell cultures with known infectious titre, while analytical specificity was verified by testing different swine viral pathogens, including other picornaviruses. A total of 266 heart homogenates from swine, previously evaluated with a routinely used conventional RT-PCR, were analysed to assess the diagnostic performances. Each sample originated from a different herd, including Italian farms with clinical suspect (n. 100) or healthy (n. 131), in addition to 35 positive samples from Belgium and Greece.

Results
The reaction efficiency resulted of 100% on average, with a detection limit of 100 TCID50/ml. No cross reaction was observed with SVDV, FMDV, enteroviruses, PCV2, PRRSV and ADV. The concordance between the rRT-PCR and the conventional assay was 100%, with 131 samples that scored negative and 135 positive in both assays. Moreover, 83 out of 100 Italian positive samples were submitted to virus isolation in BHK-21 cells and all the relevant isolates were confirmed EMCV-positive by ELISA and rRT-PCR, substantiating test effectiveness.

Conclusion
These data indicate that the newly developed real-time RT-PCR assay shows satisfying diagnostic specificity and sensitivity and is suitable for surveillance and diagnosis of EMCV-infections.

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Detection of Bombyx mori nucleopolyhedrovirus DNA by loop-mediated isothermal amplification (LAMP) assay

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Background
Bombyx mori nucleopolyhedrovirus (BmNPV, *Baculoviridae, Alphabaculovirus*) is one of the most significant viral pathogen of the silk moth (*Bombyx mori*). The virus causes the Grasserie disease or milky disease of silkworms. Larvae become inactive, swollen, and change colour. Eventually the integument ruptures, turbid haemolymph blows out, and the larvae die. The virus is very contagious and can cause severe losses in sericulture. Several methods are available for the definitive diagnosis of BmNPV infections, but they require laboratory conditions.

Methods
Silkworms showing signs of Grasserie disease were tested for BmNPV by transmission electronmicroscopy (TEM), as well as with end-point and TaqMan real-time PCR assays. BmNPV was identified by partial nucleotide sequence analysis. LAMP assay was developed for the rapid detection of the viral DNA from larva homogenates.

Results
In 2017 several silkworm rearers in Northern Italy experienced significant and unusual losses in the fifth instar larvae of their silkworm batches. BmNPV DNA was detected in the silkworm samples by PCR methods. Occluded virions with multiple nucleocapsids were detected by TEM. Phylogenetic analysis of partial genome sequences of ORF14 (envelope protein gene), however, revealed closer genetic relationship with BmNPV compared to the more virulent *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV). LAMP assay was designed for the gp41 gene of BmNPV. The assay was optimized and successfully applied for the detection of BmNPV DNA after alkaline hydrolysis of silkworm homogenates.

Conclusion
Our studies revealed that BmNPV caused mortalities in some Italian silkworm rearing. Considering the contagious character of the virus, rapid diagnostic methods could be useful tools for the quick detection of the BmNPV in the diseased larvae. The alkaline DNA purification and the LAMP assay can be performed by the rearers within a few hours and does not require specific machinery. Results can be evaluated optically. Therefore, this technique may be appropriate for monitoring of silkworm colonies for BmNPV infections.

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Lumpy skin disease – a new challenge to Georgia

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Background
Lumpy Skin Disease virus (LSDv) is a member of the Capripoxvirus genus of the Poxviridae. It is a transboundary disease of the cattle, which has recently spread out from Africa to Europe. Between 2012 and 2017 LSD has been reported from most of the Eastern European countries, such are Turkey, Greece, Bulgaria, the Balkans and the Northern Caucasus region. The disease causes considerable devastating economic losses. Therefore, Capripoxviruses are perceived as agents of agro terrorism.
In July of 2016 Lumpy Skin Disease was discovered at the border of Georgia and Azerbaijan and recently has become a new challenge for Georgia.

Methods
For epidemiological investigations the National Food Agency (NFA) has collected data and samples of suspected cases with clinical signs, which were transported to the laboratory with appropriate packaging in accordance of Biosafety guidelines.
All laboratory work was conducted according to the risk assessment required for working with the unknown pathogen. The laboratory diagnosis of LSD cases was based on virus detection in biopsy samples of skin nodules and EDTA blood samples. Molecular biology specialists extracted DNA from all clinical samples, investigated them using PCR and identified the agent. The qPCR methodology is Capripoxvirus specific; highly sensitive, fast and robust method for laboratory detection of all LSDv.

Results
According to research studies conducted by the laboratory of the Ministry of Agriculture of Georgia, 15 positive cases of LSD from 23 were confirmed in 2016 and 5 positive cases from 8 in 2017. Based on this laboratory investigation vaccination has been performed.

Conclusion
Based on the results of Laboratory investigation, we can conclude that it is important to study all risk factors of spreading the disease; studying of vectors and their seasonal dynamics; sharing the experience and updated information about LSD with trans-border regions, in order to develop laboratory and epidemiological surveillance.

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Ovine x mouse xenohybridomas for rabies virus antigens

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Introduction
Hybridoma biotechnology as a method of immortalization of cells – producers of Mab's serves perfectly well in many applications since 1975. Mouse myeloma model supply researches with large-scale production method – in vivo singenic ascetic tumors. Mouse Mab's are reliable tools with developed methods of purification and labeling for diagnostics. Xeno hybridomas production with mouse myeloma published for non-Mouse Mab's production, unsuccessfully for rabbit and human Mab's and successfully for ruminant and horse Mab's. Animal spleens and lymph nodes give a lot of antibodies producing cells-partners for fusion with myeloma cells, when taken from hyper immunized animals. Difficulties with large-scale production of such Mab's restrict their applications. Hear we have shown how to improve the situation by combination of ruminant Mab's with mouse anti-ruminant IgG Mab's.

Methods and Results
For production of ovine x mouse, xeno hybridomas we have used spleen of sheep immunized for production of precipitating serum with titer 1:128 in double immunodiffusion (ID) in agar gel. Cells from homogenized spleen were washed with RPMI-1640, 10% FBS and freeze in the cryo-protection media in liquid nitrogen by 108 cells aliquots. First fusion with Sp-2\0 mouse myeloma cell we made using rabbit anti-sheep/ovine IgG-HRP polyclonal conjugate for screening of Mab's. We selected two Mab's producer clones and their specificity was proved by immunodiffusion test conducted with concentrated cell culture or mouse brain antigens. Next fusion we made after 4 years, when we have produced specific ovine IgG detector – mouse Mab's to ovine IgG. Panels of mouse anti – ovine and anti-caprine IgG's Mab's were produced in our lab to escape cross reactivity of polyclonal anti-ruminant IgG HRP conjugates with bovine Ig's in fetal sera of cell culture fluids. Using monoclonal HRP-conjugates of anti-sheep IgG for screening, we successfully made sheep x mouse hybridomas. We have picked up 24 new clones for Rabies Virus antigens. Some of clones possess virus – neutralizing activity, that is were directed to surface virus antigen. To label Mab's of such xeno hybridomas we just add mouse anti ovine IgG Mab's HRP conjugate to cell culture fluid of xeno-Mab's. Combination of mouse and ovine Mab's could be applied for double Mab's sandwich ELISA's with excellent specificity and sensitivity.

Conclusion
Ovine Mab's can be useful for detection of antigens in many applications.

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Predictability of ionic liquid's virucidal potential in biological test systems

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Background
In a rapidly changing world where even the food industry is becoming globalised, the threat of virus infections is increasing. These facts highlight the necessity to develop new virucidal disinfectants. For three decades now, ionic liquids (ILs), organic salts comprising only ions, have emerged as a new class of pharmaceuticals and antimicrobials. Although recognition of the antimicrobial effects of ILs is growing rapidly, there is almost nothing known about their possible virucidal activities. Previously, our working group investigated the efficacy of various ILs, based on already known structure activity relationships (SARs) against the non-enveloped viruses P100 and MS2, whereas none of the tested IL toxicity SARs could be confirmed on these two model viruses and concluded that already known SARs cannot be readily transferred to viruses. Therefore, before starting again from the ground up, a systematic analysis of specific structural motifs of 27 ILs on three different biological test systems (viruses, bacteria and enzymes) was performed to gain information whether effects of these structure parts are present and if their virucidal potential is predictable.

Methods
The virucidal concentrations (using viruses P100, MS2 and Phi6), the minimum inhibitory concentration (using the corresponding bacterial host strains) and enzyme inhibition potential (using a qPCR assay) of 27 different ILs were determined. Comparison of all data was insightful in respect of predicting the toxicity of ILs on viruses.

Results
The results indicated that while the antibacterial and enzyme inhibition tests coincided with all investigated SARs, this was not the case for all tested viruses. No clear SARs for non-enveloped viruses P100 and MS2 could be identified, although some ILs were virucidal. Slight effects of the IL cations on virus P100 were recorded, in contrast to virus MS2 were no toxicity associated with IL cationic compartments could be observed. Apart of the outcome of the non-enveloped viruses, a correlation has been demonstrated between the effects of IL cations on enveloped viruses, bacteria and enzyme, while there was no observed effect of IL anion chaotropicity.

Conclusion
While virucidal effects of ILs on non-enveloped viruses occurred, previously described SARs are not applicable and future research must focus on identifying their actual mode(s) of action. However, a correlation has been demonstrated between the effects of ILs on enveloped viruses, bacteria and enzyme inhibition. These identified SARs serve as a sound starting point for further studies of ILs and strengthen their great potential as next generation virucides.

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Molecular characterization and phylogenetic relatedness of human and dog-derived Rabies viruses circulating in Cameroon between 2010 and 2017

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Background
Rabies is enzootic among dog populations in at least some administrative regions of Cameroon and the risk of human rabies is considered by public health authorities to be steadily high in Cameroon. However, the molecular epidemiology of circulating Rabies Virus (RABV) isolates has been hardly considered in Cameroon as well as in most neighboring Central African countries. Molecular data are of great interest for the optimization and evaluation of the interdisciplinary interventions related to rabies surveillance and control.

Material and Methods
To address this fundamental gap, 88 nucleoprotein (N) gene sequences of RABV, comprising 84 derived from dogs and 4 from humans, were determined from a collection of 112 brain specimens from rabid dogs and 9 skin biopsies (or saliva) from rabid humans in Cameroon from 2010 to 2017. Studied sequences were subjected to pairwise comparison and Maximum Likelihood-based phylogeny (inferred using the most complex GTR+I+Γ4 model) with homologous sequences retrieved from databases.

Results
All studied RABV isolates fell within the Lyssavirus species 1. As much as 83 of the 88 newly sequenced isolates were unambiguously classified as members of the worldwide distributed Cosmopolitan clade. The remaining 5 isolates belonged to the Africa-2 clade. Among the Cosmopolitan isolates, the Africa-1a lineage was strikingly more prevalent (81/83) whereas Africa-1b was exceptional. All Africa-1 isolates displayed 93.5–100% nucleotide (nt) and 98.3–100% amino-acid (aa) identities to each other respectively; while all 5 studied Africa-2 isolates shared 99.4–99.7% sequence similarities at nt and aa levels respectively. Interestingly, isolates of the most prevalent Africa-1a lineage segregated into three reliable region-specific variants. However, outstanding phylogenetic outcasts to the general pattern of geographic differentiation of RABV isolates indicated that importations from close regions (within the country) or from neighboring countries contribute to the maintenance of the enzootic cycle of domestic rabies in Cameroon.

Conclusion
This study provided the first insight into the genetic landscape of RABV involved maintaining the enzootic lifecycle of rabies in Cameroon. It also suggested that human-mediated movements of dogs may play a role in the propagation of domestic rabies in Cameroon.

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Replication of three different viral species in sheep respiratory ex vivo organ cultures (EVOC)

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Background

Pteropine orthereoviruses (PRVs) have been described as causes of respiratory infections in humans [1]. However, no data upon PRV replication in other mammalian hosts is available. Likewise, the transmission route of the recently emerged atypical BTV strains is obscure [2]. The present study aims to evaluate whether sheep respiratory ex vivo organ culture (EVOC) are able to support replication of bluetongue virus serotype 26 (BTV-26, atypical BTV strains [2]), BTV serotype 1 (BTV-1, classical vector borne BTV strains), the fruit bat borne zoonotic PRV [3] and parainfluenza 3 virus (PI-3 virus, employed as positive control).

Methods

EVOC were obtained from nasal, tracheal, bronchial mucosa and lungs of slaughtered 4-months old male lambs – serologically negative for BTV, PI-3 virus and PRV. After 24 hours post culture, EVOC were inoculated and incubated 1 hour with a dose of 10^3 $TCID_{50}$/ml of each virus. Furthermore, PRV was also inoculated with a dose of 10^5 $TCID_{50}$/ml. After washing with PBS, EVOC were placed in culture in 6-well plates up to 72 hours at 37°C in 5% CO_2. To evaluate viral replication, 200 µl of supernatant was collected and titrated at 1, 24, 48 and 72 hours post inoculation.

Results

Both BTV strains showed only minimal replication in the nasal mucosa. PRV showed a more sustained replication mostly in the upper respiratory tract at both doses. On the other hand, PI-3 virus replicated at higher magnitude with respect the other viruses in all EVOC.

Conclusion

Our results indicated that respiratory EVOC support viral infection and replication as demonstrated by the growth of a "pure" respiratory virus. PRV replication in the sheep respiratory EVOC suggests that viral infection may occur also in other species rather than humans. BTVs do not replicate in the respiratory tract of sheep and further efforts are needed to establish the transmission route of the newly emerged BTV strains. Overall, respiratory EVOC are of extraordinary importance for testing and characterize novel and emerging viruses circumnavigating the need for animal in vivo experiments.

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Virological surveillance of influenza virus type A, B, C, D in Italy

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Background

Influenza A, B, C and D viruses (IAV, IBV, ICV, IDV) are genus of Orthomixoviridae family and can cause influenza in humans and animals. IAVs infect humans, mammals and avian species. IBV is considered a common seasonal human pathogen but can occasionally infect pigs and seals, while ICV causes mild infection in humans. ICV has been isolated once from swine in China. IDV was first identified in 2011 in pigs with influenza-like illness but it was shown to be widespread and fairly common in cattle. IDV zoonotic role is currently under investigation.
AIM: To investigate the circulation of IAV, IBV, ICV, and IDV in cattle, swine and humans in Italy.

Methods

From 2015 onwards we performed a virological screening by real-time RT-PCR for IVs on respiratory samples collected in Northern Italy from cattle (IBV, ICV, IDV) and swine (IAV, IBV, ICV, IDV). Full genomes of IAVs and IDVs were sequenced.
Human samples collected from influenza-like illness cases in the framework of InfluNet (annual active surveillance of IAV and IBV in humans) were examined for ICV and IDV by real-time RT-PCR.

Results

Swine: we examined 856 herds and found 34% positive for IAV and 1.7% for IDV. IBV and ICV were not detected.
Cattle: we examined 888 herds and found 7.6% positive for IDV. IBV and ICV were not detected.
Humans: IAV or IBV were detected in 46.9% of analysed samples. 1491 respiratory samples that resulted IAV/IBV-negative were examined, and ICV was detected in 0.7% of cases. IDV was not detected.
Genetic analysis of IDV from swine and cattle confirmed the circulation of viruses clustering with D/swine/Oklahoma/1334/2011 while the genetic analysis of IAVs from swine showed a more complex situation with the circulation of multiple reassortant genotypes (H1N1, H1N2, and H3N2).

Conclusion

The study results showed that cattle are the most susceptible species to IDV infection whilst it was confirmed that IAV circulates with high prevalence among pigs. The high genotypic variability of Italian swine IAVs has undergone further progression. Circulation of ICV or IBV in cattle or swine was not demonstrated. The study did not prove IDV circulates among humans while ICV was detected only in 0.7% of respiratory illness. Despite the high circulation of IAV and IBV in humans, introduction of genes from human IVs to animal species was limited to H1N1pdm09 derived viruses detected in swine population.

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Analysis of bluetongue serotype 3 spread in Tunisia and discovery of a novel strain related to the bluetongue virus isolated from a commercial sheep pox vaccine

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Background
Bluetongue (BT) is one of the OIE-listed major diseases of ruminants.

Methods
Following the official report of BT virus serotype 3 (BTV-3) in a sheep in Cap Bon (Tunisia) [1], blood and serum samples of ruminants were collected from some areas of Tunisia to further investigate the presence of this virus in the country. A quantitative real time RT-PCR has been first developed for the detection and quantitation of BTV-3 RNA from field specimens.

Results
Out of 62 collected blood samples, 23 were shown to be positive for BTV-3 RNA. Isolation on cell cultures was also possible from six samples. Genome sequencing revealed the circulation of two unrelated western strains of BTV-3, one circulating in Cap Bon and neighboring areas, and the other circulating nearby the border with Libya. The presence of a putative novel BTV serotype (BTV-Y TUN2017) in sheep introduced from Libya to Tunisia, genomically related to the BTV strain contaminating a commercially-available sheep pox vaccine [2] and to BTV-26, has been also demonstrated.

Conclusion
This finding highlights the pressing need for a prompt production and release of a novel inactivated BTV-3 vaccine to be used in case of emergence or proactively in the areas of Southern Europe at major risk of BTV introduction. The assessment of a novel vaccine will certainly exalt the role and importance of surveillance activities and collaboration with Northern African countries.

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First detection of Crimean-Congo haemorrhagic fever virus in a tick from a migratory bird in Italy

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Background
Crimean-Congo haemorrhagic fever virus (CCHFV) is a vector-borne RNA virus belonging to the genus *Nairovirus* transmitted through tick bites or by direct contact with blood or other body fluids; it causes a severe illness to humans, while other vertebrates are asymptomatic reservoirs. The Balkan region has represented the western limit of detection for CCHFV in Europe until 2010, when the virus was found in Spain in a tick collected on a deer, while in 2016 two autochthonous human cases of CCHF were registered in the same area. The viral RNA sequences confirmed the African origin of the strain and support the hypothesis of infected tick dispersion through avian migration as a way of introduction. Indeed, infected ticks collected on wild migratory birds had previously been detected in Greece and in Morocco, indicating the role of birds in the spread of the virus.

Methods
We conducted our sampling session between March and May 2017 in the ISPRA bird ringing station on the island of Ventotene, in the Thyrrenian Sea, which is an important stop-over site for migratory birds in the Mediterranean. During the season, 15095 birds were captured, ringed and checked for parasite presence.
Ticks were identified, using both morphological and molecular tools, individually homogenized and tested to detect CCHFV specific RNA through Real Time RT-PCR assay. Sequencing of the positive sample was performed using NGS approach.

Results
A total of 620 ticks were found on 268 wild birds belonging to 28 different passerine species.
Viral RNA was detected in a nymph of the African tick species *Hyalomma rufipes (Ixodidae)*, collected from a Whinchat *(Saxicola rubetra)*, a small trans-Saharan migratory passerine breeding widely across Europe and wintering mainly in Central Africa.
Partial sequences of the two segments of CCHFV RNA obtained (S and M) confirmed the African origin of the virus.

Conclusion
Our finding represents the first detection of CCHFV in Italy and its presence in a tick carried by a migratory bird, confirming the potential virus dispersion over long distances. However, given the low percentage of parasitized birds and the even lower percentage of infected ticks transported, their role as a real hazard for human health should be better defined. Further field research is needed to improve our understanding on the potential risk of introduction and spreading of CCHFV in Italy.

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Detection and genotyping of hepatitis E virus in wild ungulates from North-Eastern Italy

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Background
Hepatitis E virus (HEV) is a zoonosis of concern for developed countries where the main route of transmission is the consumption of raw or undercooked meat from pigs, the main HEV host. Wildlife may also serve as HEV natural reservoir: wild boar (Sus scrofa) is the principal host, but infections were confirmed in red deer (Cervus elaphus) and roe deer (Capreolus capreolus). Therefore, infection in wildlife represents a possible risk of zoonosis, especially in regions as North-Eastern Italy where game meat consumption is increasing. Since in this area few data is available, new insights on HEV occurrence and strains molecular characterization were assessed in the present study.

Methods
With the collaboration of hunters, gamekeepers and veterinary services, liver, faeces and blood samples were collected in 2017 from the three ungulate species previously mentioned, during hunting season and population control plans. In wild boars, bile and muscle tissue were also sampled. All samples were georeferenced to allow comparison in distribution between different areas across Veneto and Friuli Venezia Giulia regions. Serological analyses were performed using commercial ELISA kits on sera and/or meat juice. Livers, bile and/or faeces were analysed with an ORF-3 targeting rRT-PCR protocol, whereas ORF2 HEV regions of positive samples were sequenced and phylogenetically analysed.

Results
Samples from 482 wild boars, 160 roe deer and 97 red deer were collected. Both serological (25/145) and virological (12/138) positives were evidenced in wild boar only, clustering in a small area (Euganean Hills – province of Padua). In a neighbouring hilly area (Berici Hills – province of Vicenza) only 1/131 wild boar tested positive serologically, while no virological positivity was recorded. Phylogenetic analyses showed that HEV isolates sequenced belong to genotype 3, clustering together with a similarity ranging between 99.7–100%, and grouped with HEV detected in wild boars in central Italy (92%) and in a human case from Vicenza (91%) in 2012.

Conclusion
Genetic characterization of the HEVs showed that they clustered with viruses previously identified in wild boars and humans in Italy. However, the low similarity observed compared to all the sequences publicly available makes it difficult to pinpoint how these viruses entered the region. Although a more efficient sampling plan is needed in the other territories to assess the absence of HEV in wildlife, these preliminary results allow identifying the Euganean Hills as the most suitable area for further investigations on HEV at the wild boar/domestic swine/human interface.

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Mammalian Orthoreovirus reassortant isolated from diarrheic pigs: Prevalence in the Netherlands and propagation in cell culture

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Background
Next generation sequencing revealed a novel Mammalian Orthoreovirus (MRV) reassortant in a fecal sample of diarrheic pigs diagnosed PCR-positive for the coronavirus (CoV) porcine epidemic diarrhea virus (PEDV). This MRV reassortant contained an unique nucleotide sequence of the sigma 1 segment (S1). The S1 segment codes for the attachment protein on the surface of the virus and largely determines the cell, tissue and host tropism of MRVs. The phylogenetic distance of this novel S1 segment to S1 segments of MRV serotype 3 strains isolated in the USA, China and Italy from faeces of pigs diagnosed positive for PEDV, was high. The amino acid sequence deduced form this novel S1 segment showed the highest identity to S1 segments of MRV serotype 2 strains isolated from bats and from humans with respiratory disease due to severe acute respiratory syndrome (SARS CoV).

Methods
Infection experiments were performed with the novel MRV reassortant in porcine cell lines originating from lungs, intestines, and kidneys. Using quantitative RT-PCR (qPCR) tests for PEDV and MRV, the prevalence of MRV in swine fecal samples collected from farms with diarrheic pigs in the Netherlands, including samples of pigs diagnosed positive for PEDV, was studied. For MRV positive fecal samples, the sequence of the S1 segment was determined.

Results
The susceptibility of cell lines originating from lung, intestine, and kidneys for infection with this novel MRV reassortant, and the growth characteristics of this MRV reassortant in these cells will be presented as well as the data of the PEDV and MRV qPCR screening of fecal samples and sequence data of the S1 segment present in samples scoring PCR-positive for MRV.

Conclusion
The prevalence data of PEDV and MRV will reveal whether or not simultaneous PEDV and MRV infections in diarrheic pigs occur, as was observed for SARS CoV and MRV serotype 2 strains in humans with acute respiratory syndrome. Together with the growth characteristics in different cells, S1 sequence comparisons will provide insight in the tissue tropism of the novel MRV reassortant and of MRV strains circulating in the pig population in the Netherlands.

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The investigation of the prevalence of BTV infection in some domestic ruminants in Eastern Mediterranean region, Turkey

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Background
Bluetongue is an arthropod-borne disease affecting wild and domestic ruminants although the clinical disease is present mostly in sheep, cattle and goats. The disease occurs worldwide and is caused by bluetongue virus (BTV), which belongs to the genus Orbivirus within the family Reoviridae with 27 serotypes currently described and at least three putative new serotypes. BTV circulation in Turkey was first reported in 1944 in Hatay province, and several serological and virological surveys indicate that the BTV infection is widespread, although with varied prevalence. Given this background, this study was carried out to determine the epidemiology of BTV infection in cows, sheep and goats herds with abortion and/or congenital abnormalities problems in Eastern Mediterranean Region, Turkey.

Methods
This study was conducted during the 2015 and 2017 in Hatay, Kahramanmaraş and Osmaniye provinces in the Eastern Mediterranean region of Turkey. The study focused on herds where abortion and/or congenital abnormalities cases were reported. The prevalence of BTV was investigated using serological and virological methods in unvaccinated domestic ruminants. With this purpose, totally 1156 serum samples, blood with EDTA (n=1414) and vaginal swab (n=165) from sampled animals that had aborted and/or healthy appearance and tissue samples of aborted foetuses (n=25) were collected from three provinces. A commercial competitive ELISA kit was used for the detection of BTV antibodies in sera samples. Real time RT-PCR assay targeting the VP7 protein was used to detect viral RNAs present in blood with EDTA, vaginal swabs and tissues.

Results
The rates of antibody for BTV were determined as 47,65% (426/894) in cows, 43,51% (114/262) in sheep and 38,01% (165/434) in goats. Overall seropositivity rate of BTV infection was found as 44,33% (705/1590). No BTV viral nucleic acid was detected in any of the collected samples.

Conclusion
In this study, we found the seroprevalence of BTV infection was high in sampled animals. BTV seroprevalence rate can change depending on locations and seasons. No BTV viral nucleic acid was detected in any of the collected samples in this sampling term, however, BTV-4 outbreak was reported in Osmaniye province located in the same region in October 2017. Therefore, the results of this study will be beneficial for understanding the BTV epidemiology and control of BTV in Turkey. It is think that mathematical models based on approved reliable data from each region and estimates of disease outbreaks will guide the application to prevent disease in the future.

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Physiological and immunological responses to *Culicoides sonorensis* blood feeding: A murine model

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Background
Hematophagous Culicoides spp. biting midges are of great agricultural importance as livestock, equine, and wildlife pests, and as vectors of the orbiviruses bluetongue, epizootic hemorrhagic disease, and African horse sickness. To obtain a blood meal, midges deposit saliva containing allergens, proteases, and anti-hemostatic factors, into the dermis to facilitate feeding. Infected midges deposit virus along with the myriad of salivary proteins during feeding. The extreme efficiency, with which midges are able to successfully transmit orbiviruses resulting in productive infections in immune-competent animals, is not clearly understood. One reason is that much is still unknown about the effects of midge feeding on mammalian hosts; both the physiological trauma of the bite and responses to saliva deposited during feeding. Of particular interest are the first few hours and days after the bite; a critical time period for any midge-transmitted virus to establish a localized infection and disseminate, while avoiding the hosts' immune responses.

Methods
A mouse/midge feeding model using colonized *Culicoides sonorensis* midges was used to characterize innate mammalian immune responses to midge feeding. Histological changes in the skin, lymph node cell populations and cytokine expression profiles were examined during the first three days after midge feeding. Both in vitro and in vivo studies were conducted to specifically examine the effect of saliva on mast cell activation and consequent physiological and immunological responses.

Results
Culicoides midge feeding elicited a potent pro-inflammatory Th-mediated cellular response pattern with significant mast cell activation, subcutaneous hematomas, hypodermal edema, dermal capillary vasodilation, and rapid infiltration of leukocytes to the bitten areas. Mast cell activation, triggered by bite trauma and specifically by midge saliva, was key to physiological and immunological responses and the ability of midges to feed to repletion.

Conclusion
Midge feeding causes physiological changes and immunological responses highly favorable to rapid infection establishment and systemic dissemination of midge-transmitted orbiviruses. Recruitment of leukocytic cells to bitten skin brings susceptible cell populations in proximity of deposited virus within hours of feeding. Infected cells would drain to lymph nodes, which become hyperplastic in response to saliva, and result in robust viral replication in expanding cell populations and dissemination via the lymph system. Additionally, saliva-induced vasodilation and direct breaches in dermal capillaries by biting mouthparts exposes susceptible vascular endothelial cells, thereby providing immediate sites of virus replication and a dissemination route via the circulatory system. This research provides insights into the efficiency of Culicoides midges as orbivirus vectors.

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Apparent re-emergence of Schmallenberg virus in Northern Ireland

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Background
Schmallenberg virus (SBV) emerged in Germany in 2011. Offspring of infected ruminants can display congenital malformations including scoliosis, arthrogryposis, hydrocephalus, torticollis and cerebellar hypoplasia. Many offspring are stillborn. SBV was detected in N. Ireland in stillborn ruminants in 2013 by real-time RT-PCR. Subsequently, SBV was not detected until 2017 in an isolated case. Since the beginning of 2018 there has been a marked increase in PCR positive cases in stillborn lambs in N. Ireland. This study details increased detection of SBV in N. Ireland and initial sequence analysis of the hypervariable region (HVR) in selected cases.

Methods
As of late-April 2018, 70 cases of stillborn ruminants, where there was suspicion of SBV involvement, were tested by RT-PCR. Nucleic acids were isolated from tissues of stillborn calves', lambs and 1 stillborn goat submitted to the Agri-Food & Biosciences Institute for necropsy. Tissues assessed included brain, spinal cord, lung, liver and kidney. Viral nucleic acids were extracted using the MagAttract 96 cador pathogen kit (Qiagen). Nucleic acids were analysed using the AgPath-ID One Step RT-PCR kit (Ambion) combined with an SBV S-segment TaqMan® assay. Partial sequencing of the M-segment of SBV was carried out on selected cases. Sequence data was analysed and aligned using Geneious software (Biomatters). Amino acid sequence of the HVR located in the M-segment was compared with that of previously sequenced SBV isolates.

Results
Of the 70 suspect cases described, 27 tested positive for SBV (26 lambs & 1 calf). Detection of SBV was not limited to spinal cord and brain, with positive results in soft-tissues. In some cases detection was only in viscera rather than exclusively or in tandem with CNS tissues. Comparative analysis of the HVR fragments from select cases (n=8) including one from 2013 and 2017, suggests that the SBV detected is ~96% similar in this region.

Conclusion
In the years following detection in N. Ireland, submission levels of for SBV testing by RT-PCR have fluctuated, adding skew to detection rates. However this recent upsurge in positive cases impresses upon us the possible cyclical nature of this infection, resulting from reduced herd immunity and vaccine uptake. Continued monitoring is prudent considering the subtle but evident genetic malleability of this virus. Considering N. Ireland covers an area only 1/25th that of Germany, this is also a timely reminder of how an arboviral infection, vectored by indigenous arthropods could spread rapidly within a naïve population.

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First isolation of infectious hematopoietic necrosis virus (IHNV) in Finland

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Background
Infectious hematopoietic necrosis is a fish disease notifiable to the European Union and the World Organisation for Animal Health. The causative agent, infectious hematopoietic necrosis virus (IHNV), is a single-stranded RNA virus belonging to the genus Novirhabdovirus, family Rhabdoviridae. IHNV causes clinical disease and mortalities in several salmonid species, including Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss), and the virus is considered to be an economically important pathogen worldwide. Until recently, Finland, like other Nordic countries, has maintained an official IHNV-free status. However, in November 2017, IHNV was isolated for the first time in Finland from farmed rainbow trout.

Methods
Virus isolation from pooled organ samples from fish that were sampled and studied according to the national surveillance program for fish diseases was carried out using sub confluent monolayer cell cultures of bluegill fry (BF-2) and epithelioma papulosum cyprini (EPC) cell lines. Cell cultures that showed cytopathic effect were further tested for the presence of IHNV using ELISA, real time RT-PCR targeting the N gene, and RT-PCRs targeting the N and G genes of the IHNV genome. PCR positive samples were subsequently sequenced.

Results
Cytopathic effect typical for IHNV was detected on the EPC cell line from two cultured samples. Both samples were from the same marine fish farm located in Northern Ostrobothnia. The viruses were identified as IHNV in ELISA, real time RT-PCR, RT-PCRs, and sequencing.

Conclusion
This was the first isolation of IHNV in Finland. Immediately after the first detection of the virus, examination of fish farms and other locations that had contact with the IHNV positive farm started. By the end of January 2018, IHNV was detected from altogether six fish farms or ponds located in Northern Ostrobothnia, Northern Savonia, and North Karelia. In all cases, the virus was diagnosed from rainbow trout.

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Bat lyssaviruses in Finland

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Background
European bat lyssavirus type 2 (EBLV-2) was first detected in a Daubenton's bat in Finland in 2009[1] and in active surveillance project antibodies were detected in Daubenton's bats[2] in Turku area in the South west part of the country. In 2016, a new case of EBLV-2 was detected in a Daubenton's bat, circa 100 km from the previous case[3]. There was no data on the presence of EBLV-2 or other lyssaviruses in the Finnish bat population in other parts of the country.

Methods
A dead bat found in Eastern Finland in the municipality of Leppävirta, in the village of Kotalahti was send for autopsy. Smears were prepared from brain tissue for fluorescent antibody test (FAT), rabies tissue culture infectious test (RTCIT) and RT-PCR from RNA extracted from the brain tissue, targeting the nucleoprotein (N) gene was performed.

Results
Based on FAT, the possibility of lyssavirus infection could not be ruled out. No viral growth was visible after four consecutive cell passages; RT-PCR produced positive bands of expected sizes on the agarose gel. Phylogenetic analysis of the obtained 1370 nt N-gene sequence revealed that the virus differed from other known lyssaviruses. It shared the highest nucleotide identities of 81.0%, 79.7%, 79.5%, and 79.4% with KHUV, ARAV, BBLV, and EBLV-2, respectively. The bat was identified as a Brandt's bat (Myotis brandtii). Since the bat was found dead, no information on whether it displayed clinical signs of rabies were available. The virus was designated as Kotalahti bat lyssavirus (KBLV), a tentative novel member of the genus Lyssavirus.[4]

Conclusion
Even though bat lyssavirus infections seem to be rare in Finland, all bats should be assumed to be potentially infected with lyssaviruses.

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Phylogenetic analysis of alphacoronaviruses from five different species of bats in Denmark

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Background
Bat populations worldwide harbour numerous viruses, some of which are known to be or potentially pathogenic to other animal species or humans. Therefore, it is important to monitor the populations and characterize these viruses. In this study, we investigated bat populations in Denmark for the presence of coronaviruses (CoVs). Previously, we have reported results from bat sampling in 2013–14 (Rasmussen et al. 2015); these sampling activities have continued biannually into 2017. This larger dataset of partial CoV sequences provided the basis for phylogenetic analyses and comparisons to sequences obtained from bats in other European countries.

Methods
Faecal samples were collected from species-identified bats captured in different geographical regions of Denmark between 2013 and 2017 with a focus on the important hibernacula in Mønsted limestone mines. The samples were screened for CoVs using a combination of 3 pan-CoV RT-PCR assays. Amplicons from positive samples were sequenced using Sanger sequencing and analysed using CLC Main Workbench. The nucleotide (nt) sequences from the 3 different assays were 130, 208 and 395 nt in length after primer trimming and they were aligned with reference sequences. Phylogenies were constructed using the Jukes-Cantor Neighbor-Joining method with 1000 bootstrap replicates and a minimum bootstrap value of 50 for phylogenies based on the 208 and 395 nt long sequences and a minimum value of 20 for the phylogeny based on the 130 nt long sequences. Nucleotide sequences were translated to the predicted amino acid sequences and compared.

Results
In total, 271 faecal samples were collected from 10 different species of bats, with the majority of samples collected from M. daubentonii. CoVs were detected in samples from 5 different species and the overall prevalence of CoVs in the samples was 21.4% (95% confidence interval [17–26%]). All generated coronavirus sequences fell into the genus Alphacoronavirus. Phylogenetic analysis revealed a species-specific clustering with the samples from M. daubentonii showing a close resemblance to coronavirus sequences obtained from the same species of bat in Germany and the United Kingdom. Likewise, the predicted amino acid sequences were distinctly different for each species.

Conclusion
This study presents the first phylogenetic analysis of CoV in Danish bats. Our results show that several distinct alpha-CoVs are present in the Danish bat population and that they are apparently species-specific.

Reference
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Virome of Croatian bat populations

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Background
Emerging infectious diseases (EIDs) continue to arise. Approximately 60–80% of them is zoonotic and presents a great public health threat worldwide. More than 70% of EIDs are wildlife-borne. With almost global distribution bats are increasingly recognized as source of EIDs, particularly those caused by RNA viruses.
The increase in human population, environmental destruction and agriculture intensification lead to crossing the roads between bats and humans with possible pathogen spillover. Accordingly, the understanding of the virome present in wildlife is very important for the prevention and control of wildlife-borne EIDs. Numerous studies have focused on zoonotic bat viruses in tropics, but little is known about the presence of such viruses in European bats. The only known zoonotic disease associated with bats in Europe is rabies. The main goal of this study was to determine the prevalence of rabies in Croatian bat species through serological and virological survey of captured bats. Furthermore, by using metagenomic approach we were also focused on detection of other viruses, especially those with zoonotic potential.

Methods
Bats were trapped by using mist net or hand net. Bat samples were collected in Continental and Mediterranean parts of Croatia. Blood was collected on small pieces of filter papers and tested for neutralizing antibodies with modified fluorescent antibody virus neutralization (mFAVN) test. Oropharyngeal swabs were taken from each bat for detection of lyssavirus RNA using conventional RT-PCR and for further virus isolation in the case of positive RT-PCR results. All oropharyngeal swabs, individual fecal samples and guano under colonies were analyzed using Illumina sequencing. After sampling, all bats were successfully released at location of their capture. Capturing, handling and sampling of bats were approved by the State Institute for Nature Protection.

Results
All oropharyngeal swabs were found negative for the presence of lyssavirus RNA. In total, 20 sera out of 350 showed to have detectable levels of neutralizing antibodies against EBLV-1. Metagenomic analysis revealed abundance of bacteriophages and insect viruses. Among vertebrate viruses, contigs and sequences assigned to circoviruses, bat bocavirus, bat adenovirus, bat alphacoronavirus, picobirnavirus, paramyxovirus, herpesvirus and Rhinolophus ferrumequinum retrovirus were found.

Conclusion
We have indirectly confirmed the presence of lyssavirus in Croatian bat population. Metagenomics revealed bio insecticide role of bats in Croatian ecosystem. No direct relationship with the other known human or animal pathogens was found. Currently it is presumed that bats pose low risk for the general public.

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The emergence of porcine epidemic diarrhoea in Croatia: molecular and serological investigation

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Background
Porcine epidemic diarrhoea (PED) is an emergent/re-emergent viral disease (family Coronaviridae, genus Alphacoronavirus) of pigs with worldwide importance. Clinical appearance is characterized by acute diarrhoea, vomiting and dehydration in all age categories with possible high mortality in suckling piglets. The disease got a lot of media attention in 2013 when it emerged in the USA causing heavy production losses (highly pathogenic non-S-INDEL genotype). Since 2014 PED has re-emerged in Europe spreading around western European countries, but causing milder consequences. The causative agent responsible for European outbreaks (apart from Ukraine) is S-INDEL PEDV genotype which represents a variant of non-S-INDEL genotype having insertions and deletions in spike (S) genomic region. Croatia had been considered free of PED until 2016 when the first outbreak was reported in Osijek-Baranja County. In the present study we report molecular characterization of the first Croatian PEDV strain and following serological investigation of sows in eastern Croatia.

Methods
In April 2016 we received small intestines from two 8-10 days old diarrhoeic piglets. The holding of origin was located in Osijek-Baranja County and reported 20–30% mortality in suckling piglets. Initial diagnostics included detection of PEDV by two real-time RT-PCR protocols (N and S genome fragments) together with TGEV and RVA real-time RT-PCRs. NGS sequencing was performed on Illumina MiSeq platform and Sanger sequencing on conventionally amplified complete PEDV S gene (Geneious and MEGA7 software for data analysis). Serological investigation was carried out in 2017 on 266 sow sera collected from 39 holdings in Osijek-Baranja and Vukovar-Srijem Counties by commercial ELISA test (ID Screen® PEDV Indirect ELISA test; IDVet, France).

Result
The presence of PEDV genome was confirmed by both real-time RT-PCR protocols (TGEV and RVA were excluded). NGS sequencing was rather unsuccessful as majority of virus related reads corresponded to bacteriophages and only four reads were PEDV related. However conventional RT-PCR and Sanger sequencing resulted in a complete S gene sequence sharing high identity with S-INDEL genotype strains from Germany, Austria, Slovenia, Italy and Romania. Serosurveillance resulted in three positive holdings (7.7%), located in both counties with 15.4% of positive sows. Seroprevalence within a positive holding went up to 82.8%.

Conclusion
PEDV has emerged in Croatian pig population causing moderate losses. Expectedly the circulating strain was a member of S-INDEL genotype. Serological surveillance revealed additional three holdings of the same enterprise that didn't previously reported PED, demonstrating the importance of strict biosecurity measures.

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Development of real-time PCR kit for specific detection of porcine circovirus type 2 and type 3

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Background

While porcine circovirus 2 (PCV2) is a well-known virus associated with different symptoms in pigs collectively named porcine circovirus disease (PCVD), PCV3 is an emerging virus, identified in USA, Brazil, China, Poland, Republic of Korea and more recently in Italy and Thailand. The exact pathogenicity of PCV3 is still unknown, but high levels of viremia were reported in sick animals with severe respiratory, skin disease or reproductive disorders. Quantitative and specific diagnostic tool for porcine circovirus are essential for the distinction between PCV2 and PCV3 and could play an important role to clarify PCV3 pathogenicity.

Methods

In this context, BioSellal decides to develop a triplex ready-to-use real-time PCR kit which allows the specific detection of PCV2 (FAM labelling) and PCV3 (VIC labelling) with an exogenous internal positive control (IPC, Cy5 labelling). Bio-T kit® validations were done according to the AFNOR standard NF U47-600-2 that defines the general requirements and recommendations for the development and the validation of PCR tools in animal health. For blood sample, the Bio-T kit® PCV2 & PCV3 allows the relative quantification of the viral load of each target thanks to a reference material set at the interpretation threshold (MRSI). For PCV2, this threshold was set at 10^6 GE/ml according to previous publication (Olvera et al., J Virol Methods. 2004 Apr;117(1):75–80) and was extrapolated at the same level for PCV3. A ready-to-use material set at the level of MRSI could be provided by BioSellal.

Results

Analytical specificity for PCV2 was confirmed on 25 PCV2 positives samples provided by GD Animal Health (Ring Trials, Netherland). For PCV3, 6 blood samples coming from Spain was found positive for PCV3 with the Bio-T kit® PCV2 & PCV3 and confirmed by SANGER sequencing. The PCR limit of detection were determined at 10 copies/PCR for both targets. The linearity range was 10^6 to 10 for PCV2 and PCV3 with PCR efficiencies around 100% for both targets.

Conclusion

The Bio-T kit® PCV2 & PCV3 enables rapid and automated differential detection of PCV2 and PCV3. Moreover, the use of a common and ready-to-use MRSI will improve the accuracy of qPCR quantification of PCV2 and PCV3 viral loads, regardless of the extraction method and thermocycler used, and thus the overall consistency of these measurements between different laboratories. Finally, the Bio-T® PCV2&PCV3 kit could be a very interesting tool to investigate more precisely the pathogenicity of PCV3.

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Identification of mosquito species present in the United Arab Emirates and investigations on their virus-carrier status

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Background

The United Arab Emirates (UAE) are a hub for tourism and trading, and a high number of laborers from South-East Asian countries are employed. Imported Dengue cases are frequently diagnosed. In certain countries of the Arabian Peninsula, such as Yemen and Saudi Arabia, Dengue as well as Chikungunya are still endemic. It is therefore of utmost importance to know whether transmission-competent mosquito species are present in the UAE. Interestingly, there is no such publication available. The objective of this study is therefore a survey of the mosquito population at various sites throughout the UAE. Furthermore, these mosquitoes will be tested for the presence of viral nucleic acids.

Methods

Adult females were sampled using two trap designs: BG Sentinel trap (BioGents, Regensburg, Germany) and a CDC miniature light trap (J.W. Hock, Gainseville, FL, USA). Both trap types were run overnight, baited with CO_2 (dry ice), "BG Lure", or light-only, respectively. In the mornings, trap contents were transferred to –80°C until identification. Mosquitoes were sorted on dry ice using a stereoscopic microscope, and pooled by species in pools <30 individuals. Species identifications were based on published morphological characters. Further molecular identifications will be performed on selected samples using cytochrome oxidase 1 gene primers, a mitochondrial genomic region frequently used in taxonomic barcoding. A variety of virus-specific RT-PCRs and RT-qPCRs will be applied to the mosquito pool suspensions in order to identify nucleic acids of viruses which might be present in the samples.

Results

Study Sites: Sites were chosen in three geographic locations in the UAE: Al Ain, Dubai, and Wadi Wurayah in Fujairah emirate. At Al Ain, sites included the date palm oasis, lake Zakher, and Al Ain zoo. In Dubai traps were placed at Ras al Khor Wildlife Sanctuary and Qudra lakes. The trapping sites represent several different habitats. Trapping was carried out from 30.01.–11.02. and 16.04.–02.05.2018. In total, 1,142 mosquitoes were trapped and morphologically typed. Various Culex, Anopheles and Ochlerotatus species were identified. At the time of abstract submission the molecular barcoding of the mosquito species as well as the molecular investigations for virus nucleic acids are in progress. The results will be presented at the Conference.

Funding

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Serological evidence of Usutu virus infection in Moroccan horses

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Background
Usutu virus (USUV) is a mosquito-borne flavivirus belonging to the Japanese encephalitis virus serocomplex, causing a mosquito-borne viral infection that can affect birds as a primary host and humans and horses as incidental dead-end hosts. The virus was discovered in South Africa in 1959, and named after a river in Swaziland, it has been reported in several African countries including Morocco. In Europe, in 2001, the circulation of USUV was reported for the first time in Vienna, Austria where it caused a significant mortality within wild birds' populations particularly Eurasian blackbirds (Turdus merula). The USUV is closely related to West Nile Virus (WNV). As Morocco is characterized by the presence of wetlands with wide range of migrotary and resident birds involved in the introduction and the spread of some zoonosis and in order to deepen our knowledge about the circulation of USUV and its co-circulation with WNV in Morocco a retrospective serological survey was conducted to determine the seroprevalence of USUV.

Methodology
A Total of 634 Moroccan horses originating from different ecoregions sampled during 2011 and 2016 were tested for anti-USUV antibodies by VN assays, the USUV strain used has been isolated in Teramo by the OIE's Reference Laboratory for WNV disease from a sick Austrian horse. The sera were re-tested for anti-WNV antibodies.

Results
Serological studies demonstrated the presence of USUV antibodies in Moroccan horses. USUV-neutralizing antibodies were detected in 138 with an overall prevalence of 21.77%. Co-infection with WNV was observed in 124 horses (20%).

Conclusion
Even though the fact that there has never been a reported USUV epidemic in Morocco unlike other countries, the current study confirms the circulation of USUV besides WNV in different provinces in country. Consequently, a continuous monitoring of USUV circulation based on sero-surveys and clinical investigations is necessary.

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Diversity of paramyxovirus-related sequences identified in South African rodent populations

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Background
Paramyxoviruses include some long established human and animal pathogens such as measles- and canine distemper virus, as well as some recently emerged ones such as the henipaviruses, which have a severe impact on veterinary and human health. Over the past three decades, an increasing number of novel paramyxoviruses, some of which demonstrate the ability to cross the species barrier and infect other terrestrial mammals, including humans, have emerged from wildlife reservoir hosts. Species-rich small mammals, especially rodents, are notorious for harbouring numerous different viruses and have been implicated as hosts for paramyxoviruses. This study aims to describe the diversity of paramyxovirus-related sequences identified in South African rodent populations.

Methods
Rodent specimens were obtained from collaborators who conduct field work in various locations within South Africa. Kidney specimens were firstly homogenised, then total RNA extracted, followed by the random synthesis of cDNA. A highly sensitive and broadly reactive PCR assay targeting a conserved region within the L-gene of paramyxoviruses was used for screening. Positive PCR products were sequenced and analysed by generating sequence contigs using Geneious R11. Sequences were then aligned to known and previously identified paramyxovirus-related sequences obtained from Genbank, for phylogenetic analysis.

Results
To date, 173/573 (30.2%) individuals from 11 different rodent species (D. auricularis, M. coucha, M. natalensis, M. verreauxii, M. unisulcatus, M. namaquensis, O. irroratus, R. dilectus, R. pumilio, S. campestris, X. inauris), representing 3 rodent families (Muridae, Nesomyidae and Sciuridae) were identified as hosts for paramyxoviruses.
Preliminary phylogenetic analysis of study sequences shows that they cluster with previously identified paramyxovirus sequences within the morbillivirus genus and unclassified paramyxoviruses.

Conclusion
In order to better equip ourselves against potential zoonotic risks, the identification of animal species associated with potentially zoonotic agents and the characterisation of the novel viruses these animals may harbour are vital steps in the development of control strategies. The prevalence and diversity of paramyxoviruses in South African small mammals are severely understudied and poorly understood. This study aims to address these shortcomings.

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Seroprevalence for West Nile virus (WNV) and Saint Louis virus (SLEV) in equids from the Northeast Brazil

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Background
Arboviruses have a great impact on public and animal health, among them the flavivirus family encompasses some of the most threatening viruses responsible for large outbreaks worldwide. The Northeast Brazil was the epicenter of the Zika virus (ZIKV) outbreak and where most cases of the congenital syndrome were reported. It still debated whether cross-reactivity among ZIKV and other Flaviviruses might play a role on the disease pathogenesis. Most of people infected with both WNV and SLEV do not develop disease and the symptoms are difficult to distinguish between other arboviruses and usually associated with fever, encephalitis and neurological disease. Both viruses have been described in horses in Brazil.

Methods
Two set of sera samples from equids from Northeast Brazil were used. The first set includes 159 equine samples from the state of Pernambuco. The second set comprises 110 equid samples collected on the Island of Fernando de Noronha, which is located on the Atlantic Ocean and is migration route of several bird species. Neutralizing antibodies against WNV and SLEV were detected by plaque reduction neutralization test (PRNT75) using viral chimeras where WNV and SLEV structural proteins (prM and E genes) were built on YFV backbone (YFV 17D vaccine strains). Chimeras were cultured in BHK-21 and VERO cell lines and previous titrated by plaque assays.

Results
Neutralizing antibodies against WNV and SLEV were found respectively in 32 and 22 samples (out of 159) in the first set. Analysis of the second set of samples are still ongoing and antibodies against WNV were found in two samples (out of 110).

Conclusion
Our results shown the Results here are preliminary so far but show the circulation of WNV and SLEV in the Northeast Brazil. Further studies should focus on the epidemiological factor, molecular characterization and implications concerning animal and public health.

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The virome of *Culex quinquefasciatus* mosquitoes in Brazil

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Background
Mosquitoes are the major arthropod vector for arboviruses such as, Dengue, West Nile virus, Rift Valley fever virus and yellow fever virus. With increasing global temperature and population densities, mosquito-borne viruses are an emerging global health concern both in animals and humans. Intensified mosquito surveillance and new sequencing technology have resulted in discoveries of a multitude of novel viruses in mosquitoes, including so-called insect-specific viruses (ISVs). These are strictly restricted to arthopods and are unable to replicate in vertebrate cells and have been in focus for their potential use to study virus evolution and utilization as a biocontrol agent. In this project, we aim to characterise the virome of *Culex quinquefasciatus* mosquitoes from Brazil.

Methods
Culex quinquefasciatus were collected in Cargagutatuba, São Paulo Brazil and 72 mosquitoes were shipped to Sweden in TRIzol. At the Swedish University of Agricultural Sciences, mosquitoes were homogenised and RNA extracted and further processed with Ribo-Zero gold rRNA removal kit and Ovation RNA-seq system. The material was sequenced with the Ion Torrent PGM platform and the raw data was analysed with a metagenomics pipeline, including quality control, de novo assemble and homology search.

Results
Annotation with Diamond resulted in over 20 different viruses in various taxa including the near complete genome (10823bp) of the ISV Culex flavivirus (CxFV). BLASTn analysis of the CxFV consensus sequence showed that it have a 90–99% nucleotide identity to other CxFV and display closest similarity to CxFV isolated in Brazilian and Mexican *Culex quinquefasciatus*. Several identified viruses belonged to the family Orthomyxoviridae, for example, 59 singletons/contigs mapped to the Quaranjavirus Wuhan mosquito virus 4 (APG78242) with an amino acid identity of 90–100% and 374 singletons/contigs mapped to Wuhan mosquito virus 3 (AJG39091) 50–70% amino acid identity. However, the majority of annotated viruses were unclassified RNA viruses often displaying a low amino acid identity to known viruses.

Conclusion
This metagenomic study of the Brazilian *Culex quinquefasciatus* virome showed that mosquitoes are indeed an extensive reservoir of a broad range of viruses. We recovered the almost complete genome from a CxFV virus and also identified, for example, different strains of the Wuhan mosquito virus (3, 4, 5, 6 and 9) and an unclassified virus in the family Narniaviridae. Majority of the annotated viruses was unclassified without an order or family. A follow up study including more mosquitoes could allow complete genomes of these viruses and enable virus isolation for further evaluation.

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Epidemiologic investigation and possible vector identification of Akabane virus infection in domestic ruminant species (cows, sheep and goats) in Eastern Mediterranean region, Turkey

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Background
AKAV belongs to Simbu serogroup of Orthobunyavirus genus in the family of Peribunyaviridae, is an arthropod-borne virus (arbovirus) that has potential to transmit by a hematophagous arthropod vector, the Culicoides biting midge. AKAV infection has been associated with abortions, stillbirths, premature births and congenital abnormalities in sheep, goats and cattle. This study was carried out to determine the epidemiology of AKAV infection in herds with abortion problems and identification of the possible vectors that are responsible for transmission of this disease in Eastern Mediterranean region, Turkey.

Methods
This study was conducted during the months of October 2015 and November 2017 in Hatay, Kahramanmaraş and Osmaniye provinces in the Eastern Mediterranean region of Turkey. The study focused on herds where abortion and/or congenital abnormalities cases were reported. To aim this, sera (n=1590), blood with EDTA (n=1414) and vaginal swab (n=165) samples from cows, sheep and goats that had aborted and/or healthy appearance and tissue samples of aborted foetuses (n=25) were collected from three province. A commercial ELISA kit was used for the detection of AKAV anti-G1 antibodies in sera samples. In addition, light traps were set up to collect Culicoides, believed to carry the infections, were caught and identified. Real time RT-PCR assay was used to detect viral RNAs present in blood with EDTA, vaginal swabs, tissues and Culicoides samples. Genetic characterization of the local AKAV field viruses was conducted by sequencing of AKAV S segment.

Results
The rates of antibodies for AKAV in cattle, sheep and goats were determined as 44,74% (400/894), 22,90% (60/262), 14,51% (63/434), respectively. AKAV viral nucleic acid was detected at a rate of 1.12% (9/799) for cows and 1.73% (5/288) for sheep. Eleven different Culicoides species were detected in the area and C. schultzei was found as the dominant species. AKAV viral nucleic acid was detected in C.schultzei, C.longipennis and C.circumscriptus.

Conclusion
Higher sero-prevalence obtained for cattle has considered as an indicator of the fact that they were more likely to be infected with AKAV than other species. According to the results of phylogenetic analysis, it has been determined that two different AKAV genogroups (Ib and II) circulate and C.schultzei, C.longipennis and C.circumscriptus were proposed as possible vectors of AKAV infection in this region. In addition, AKAV infection plays an important role in the abortion cases of domestic ruminants in sampled provinces and therefore, a control program is needed to restrict AKAV infection.

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Serosurvey and molecular investigation of tick-borne encephalitis virus (TBEV) in northern Turkey

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Background
Tick-borne encephalitis (TBE) is a viral zoonotic disease endemic in many regions of Eurasia. Tick-borne encephalitis virus (TBEV) is a zoonotic Flavivirus maintained in a cycle involving small mammals as reservoir and amplifier hosts and ixodid ticks as vectors.

Methods
In this study, the hard ticks and serum samples collected from ruminants (cattle, sheep and goat) in middle Black Sea region of Turkey where Tick-Borne human cases such as Crimean–Congo hemorrhagic fever (CCHF) were observed in the past years were surveyed for the presence of RNA and specific IgG antibodies from TBE virus (TBEV). A total of 2508 ticks were collected between March and July of 2016 and 2017 from 509 sheep (530 pools), 93 cattle (97 pools) and 106 goat (118 pools) grazing in middle Black Sea region. In addition, a total of 460 serum samples were collected from cattle, goat and sheep in the same region and the sera were analysed for the presence of antibodies to TBEV using an enzyme-linked immunosorbent assay (ELISA).

Results
Nine tick species were identified and the most abundant were Haemaphysalis sulcata 28.9% (724/2508) and Rhipicephalus turanicus 28.7% (719/2508). No TBEV genomic RNA was found in tick samples. However, eighty-three of 460 cattle, goat and sheep blood serum samples tested were antibody-positive for TBEV by ELISA. Serological examination of serum samples for anti- TBEV infection revealed that TBEV IgG antibody was present in 61 of 198 (30.8%), 7 of 115 (6.1%) and 15 of 147 (10.2%) cattle, goat and sheep, respectively. Positivity rates for the provinces varied and were as follows: Samsun 12.7%, Sivas 35.2% and Tokat 13.2%.

Conclusion
While we did not detect TBEV RNA in tick species, the presence of TBEV antibodies was confirmed by ELISA surveyed in this study, continued efforts may reveal more information about the role of different tick species in TBEV maintenance and/or dissemination in the region.

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Serological evidence of Lineage -1 West Nile virus infection in Ardahan Province, Northeast Turkey

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Background
West Nile virus (WNV) is one of the important arthropod-borne pathogen leading cause of serious illness in human and animals in many parts of the world. WNV, a positive sense single-stranded RNA enveloped virus of the genus Flavivirus, family Flaviviridae, causes epidemics and/or epizootics of encephalomyelitis and mortality in certain domestic and wild birds. During peak infection periods, as governed by favorable climatic conditions, adult female mosquitoes carrying WNV can transmit the virus to humans, horses, and other mammalian species, which are all considered to be dead-end hosts. The presence of WNV in humans and animals in Turkey were reported in various studies during the last 15 years.

Methods
In this study, 1800 horse serum samples collected from Ardahan Province in the Northeast Turkey were screened by using the virus neutralisation test with NY99 strain of WNV, known to be lineage 1, for WNV serology.

Results
Overall seroposivitiy rate was found 0.11% (2/1968) and was also confirmed by 90% endpoint plaque reduction test (PRNT90). Neutralising antibody titers of positive samples were determined as 1/40 and 1/80.

Conclusion
This study showed the serological evidence of the lineage-1 of WNV in horses in the Northeast Turkey and the results indicated that WNV was in circulation in Turkey.

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The serological evidence of West Nile virus Infection in backyard hens in Western Anatolia, Turkey

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Background
West Nile virus (WNV) is one of the important arboviral pathogen being a zoonosis. Human's horses and dogs are susceptible species and migratory birds play an important role in the transmission of WNV. Domestic birds are important to public health because they can be infected by a number of pathogenic microorganisms that are transmissible to humans. Among the most important of these diseases are emerging infectious diseases that are newly recognized or previously known diseases that appear in a new population or are rapidly increasing in incidence or geographical area. West Nile virus has a wide geographical range that includes portions of Europe, Asia, Africa, Australia (Kunjin virus) and North America. West Nile virus (WNV), genus Flavivirus, family Flaviviridae, is an "old-world" arbovirus, transmitted mainly by infected mosquitoes. This study was designed to investigate antibodies against WNV in backyards hens considered as sentinel animals.

Methods
A total of 480 chickens from 6 different provinces (Mugla, Izmir, Aydin, Afyonkarahisar, Kutahya and Manisa) selected from the western Anatolia region, 80 serum samples per province, were collected by sampling 40 sera in spring as well as 40 sera in fall. Serum samples were tested using competitive ELISA methods.

Results
12 out of 480 (2.5%) were found to be seropositive for WNV. All of seropositive were detected among backyards hens from two provinces (Mugla and Izmir) which are located seaside and climate conditions are more suitable for vector insects. Positive results were obtained in both seasons.

Conclusion
The results showed that both WNV was in the circulation and hens were infected. The results also indicated that there was WNV infection risk for people living in the same areas by the way of mosquitoes biting.

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Serological status of West Nile virus in a private small scale thoroughbred horse farm in Turkey

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Background
West Nile virus (WNV) which is one of the important arthropod-borne pathogens transmitted by mosquitoes is the member of Flaviviridae and is also leading cause of serious neuroinvasive illness and death in humans and some animal species including horses, dog as well as birds.

Methods
In this study, sera and whole blood samples collected from 30 horses in a private thoroughbred horse farm in Corlu District of Tekirdag in Thrace region of Turkey were studied. Serum samples were tested by using competitive-ELISA in order to investigate in terms of WNV IgG as well as WNV IgM. The whole blood samples were tested using real-time RT-PCR to investigate whether there had been an active WNV infection in horses.

Results
Neutralising antibodies against WNV were found in 5 out 30(2.5%) serum samples. No WNV IgM could be determined in all of the serum samples. In addition to, all of the blood samples were negative with real-time PCR. The findings indicated that WNV is in circulation in Thrace Region.

Conclusion
WNV seropositivity in horses that are accepted among sentinel animals may indicate a risk of WNV infection for people working on the same farm as well as people living in the same district.

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Potential implication of promiscuous-landing *Musca domestica* L. flies in lumpy skin disease virus spread in Russian cattle

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Background
Since 2012, lumpy skin disease virus has been spreading from the Middle East to south-east Europe and Russia. Although vaccination campaigns have managed to contain LSDV outbreaks, the risk of further spread is still high. The most likely route of LSDV transmission in short distance spread is vector-borne. Several arthropod species have been suggested as potential vectors, but no proven vector has yet been identified.

Methods
To check whether promiscuous-landing synanthropic flies such as the common house fly (*Musca domestica*) could be involved, we carried out entomological trapping at the site of a recent LSDV outbreak caused by a vaccine-like LSDV strain.

Results
The presence of vaccine-like LSDV DNA was confirmed by the assay developed in this study, the assay by Agianniotaki et al. 2017 and RPO30 gene sequencing. No evidence of field LSDV strain circulation was revealed. In this study we discovered that *M. domestica* flies carried vaccine-like LSDV DNA (Ct >25.5), whereas trapped stable flies from the same collection were negative for both field and vaccine LSDV. To check whether flies were contaminated internally and externally, 50 randomly selected flies from the same collection were washed four times and tested. Viral DNA was mainly detected in the 1st wash fluid, suggesting genome or even viral contamination on the insect cadaver. In this study internal contamination in the insect bodies without differentiation between the body locations was also revealed, however, the clinical relevance for mechanical transmission is still unknown.

Conclusion
Further work is needed to clarify a role of *M. domestica*, in the transmission of LSDV. To our knowledge, this is the first report demonstrating that an attenuated LSD vaccine strain has been identified in Russian cattle.

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Usutu virus-associated mortality in captive azure-winged magpie nestlings

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Background
Usutu virus (USUV), a mosquito-borne flavivirus, emerged in 2001 in Vienna causing high mortality mainly in blackbirds. In the subsequent years the virus spread in Europe, leading to numerous deaths of wild and captive-held birds. Since the first outbreak, surveillance for USUV has been carried out in Austria.
In 2017, within a group of captive azure-winged magpies (Cyanopica cyanus; Corvidae; native to eastern Asia), which were kept for cognitive research purposes a higher nestling mortality compared to previous years in the first and second clutch of eggs was observed. These birds were kept in groups and originated from zoo populations in Europe or had hatched directly at the facility. They were housed in an aviary in an open courtyard in the city of Vienna.

Methods
Four dead nestlings were subjected to thorough post mortem investigation, RT-PCR and sequencing.

Results
The most prominent macroscopic finding in all animals was a severely enlarged spleen. Pathohistological examination revealed a non-purulent myocarditis (3/4) as well as liver (4/4) and spleen (2/4) necrosis. Virological tests using RT-PCR were positive for USUV and negative for West Nile virus. The performed immunohistochemistry showed abundant amounts of USUV antigens in many organs in three nestlings. The complete genome sequence of the USUV of one bird one and partial nucleotide sequences (E, NS5 and 3'UTR regions) of the others were determined. Phylogenetic analysis revealed the closest genetic relationships with USUVs of the "Africa 3" genetic cluster.

Conclusion
Until recently only USUVs of the "Europe 1" and "Europe 2" genetic clusters had been detected in Austria. This study provides for the first time evidence of USUV-associated nestling mortalities, presence of USUV in a corvid species, as well as the introduction of a new USUV strain in Austria.

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Determining the host range of Neoromicia capensis coronavirus by using viral pseudo particles

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Background
NeoCoV is a coronavirus that was discovered in the South African bat species Neoromicia capensis. It is 85.5% genetically identical to Middle East respiratory syndrome coronavirus (MERS-CoV) and believed to be a possible ancestor. The host-switching events that gave rise to present-day MERS-CoV are unclear. With the coronaviral spike protein being responsible for host cell attachment and entry, its characterisation is key to studying host range. This study aims to determine which animal species NeoCoV as well as MERS-CoV and severe acute respiratory syndrome coronavirus (SARS-CoV) can infect by using viral pseudo particles that express these three viruses' spike proteins.

Methods
The laboratory-based study is being conducted at the Division of Medical Virology at Stellenbosch University. Viral pseudo particles bearing NeoCoV, MERS-CoV and SARSCoV spike proteins were constructed using BHK-21 cells transfected with plasmids and a recombinant vesicular stomatitis virus (VSV) pseudo typed system that expresses GFP upon infection. The pseudo particles were harvested and are being used to attempt infecting cell lines of different mammalian origin. GFP expression is monitored semi quantitatively by fluorescence microscopy and the rate of infection is determined by calculating the percentage of cells expressing GFP.

Results
Two cell lines have been infected, namely Vero E6 cells and N. capensis kidney cells. The NeoCoV pseudo type had the highest infection rate in Vero E6 cells but was quantitatively less in comparison to SARS-CoV in N. capensis kidney cells - an unexpected result seeing as NeoCoV was first detected in N. capensis. Another surprising discovery was that MERS-CoV was the least successful in infecting N. capensis kidney cells, even though it is more closely related to NeoCoV than SARS-CoV is. Experiments using three additional cell lines (Camelus dromedarius kidney, Pipistrellus pipistrellus kidney and Vero EMK cells) are being performed but will be presented at the congress.

Conclusion
Thus far, results indicate that NeoCoV replicates more efficiently in primate cells than bat cells.

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Production of a vaccine against avian influenza from locally isolated viral strains/control of their safety and efficacy

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Background
Avian influenza is highly contagious viral disease, the causal agent is type "A" influenza virus which can infect several kinds of pet birds as well as the birds of ornament and the wild birds. There are several strains classified in two categories: the highly pathogenic strains (IAHP) and the low pathogenic strains (IAFP). The genetic variations of the virus of avian influenza have an impact on the protective capability of the vaccinal strains because of the use in the field of strains that are quite genetically distant from circulatory ones.

Methods
This job aims to produce a vaccine against Avian influenza in Morocco, from locally isolated viral strains, as well as the control of their safety and efficacy. The clinical samples of chicken are constituted of tracheal bottlebrush, Bottlebrush Cloacal and Lungs, having been collected enters 2/4/2016 and 3/15/2016 in different regions of Morocco (Meknès – Fès – Nador – Berkane). The virus was identified by qRT-PCR.

Results
The results of this technology showed that on 14 samples 8 was exclusively positive H9 with Cts who varies between 12.18 and 34.12, while 3 samples were at the same time positive H9 and NDV (Cts between 12. 49 and 29. 31) and 1 only sample was positive Inf A (Ct=32. 58). This virus was isolated on egg SPF and characterized by sequencing of the complete genome. The vaccine was produced by using an automated system , by respecting requirement of the Good Manufacturing Practice and using strains adapted to the local pathology, identified and characterized by the reference laboratory of CDC ATLANTA; It is about a pure strain endowed with a good contagious title allowing so a good vaccinal formulation.

Conclusion
To limit the impact of the avian Influenza virus H9N2, it is necessary to Consolidate surveillance on a national scale (poultry units, wholesale markets of poultry etc.), to use vaccines based on strains adapted to the local pathology, to reinforce the measures of biosecurity and hygiene at the level of pet poultry, including the means of transport of poultry and to adopt adequate and compatible programmes of vaccination in epidemiological situation of pet birds (proportion of maternal antibodies).

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External quality assessment of Rift Valley Fever virus diagnostic capacities in veterinary laboratories of the Mediterranean and Black Sea regions

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Background
The MediLabSecure project, supported by the European Commission (DG-DEVCO), aims at enhancing the preparedness and response to viral threats by establishing a one-health network of laboratories (human virology, animal virology and medical entomology) and public health institutions in 19 non-EU countries of the Mediterranean and Black Sea regions (http://www.medilabsecure.com/). Since 2014, the animal virology network has organized several training courses focused on the diagnosis of Rift Valley Fever virus (RVFV). To evaluate the capacity of the beneficiary laboratories to incorporate the diagnostic techniques, an external quality assessment (EQA) was organized in 2017 by INIA-CISA, as coordinator of the animal virology network.

Methods
The EQA was composed of: (a) a molecular panel of 10 sheep samples spiked with different concentrations of inactivated RVFV and (b) a serological panel of 10 inactivated sheep sera with different RVFV antibody titers. Seventeen laboratories participated in the exercise. For the molecular diagnosis, a specific real-time RT-PCR for RVFV detection[1] was applied. The serological panel was analysed using a commercial competitive ELISA kit for the detection of specific RVFV antibodies (ID Screen Rift Valley Fever Competition Multi-species, IDVet). Positive controls, reagents and kits were delivered to all the participants.

Results
The overall results obtained upon analysis of the molecular panel were satisfactory: 70.6% (12/17) of the labs reported 100% of correct results and 94.1% (16/17) were able to correctly identify at least 80% of the panel. The number of labs with correct results increased as the expected Ct value of the sample decreased (higher viral load).
Regarding the serological panel, the results were highly satisfactory since 94.1% (16/17) of the labs were able to correctly identify 100% of samples using the competitive ELISA. The only incorrect result was obtained in one of the samples with low antibody titer.

Conclusion
The results of this EQA demonstrated the competence of most of the participant labs (infrastructure and expertise) to face the molecular and serological diagnosis of RVF and also proved the good performance of the diagnostic techniques applied in diverse labs using different equipment.

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Acknowledgements
We are grateful to the MediLabSecure animal virology labs for their participation in this EQA. This project was funded by the EU Commission (DEVCO: Grant IFS/21010/23/194).

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External quality assessment of West Nile virus diagnostic capacities in veterinary laboratories of the Mediterranean and Black Sea regions

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Background
The MediLabSecure project aims at enhancing the preparedness and response to viral threats by establishing a one-health network of laboratories in 19 non-EU countries of the Mediterranean and Black Sea regions (http://www.medilabsecure.com/). Since 2014, the animal virology network has organized several training activities focused on the diagnosis of West Nile virus (WNV) and related flaviviruses. To evaluate the capacity of the beneficiary laboratories to incorporate the diagnostic techniques, an external quality assessment (EQA) was organized in 2017 by INIA-CISA as coordinator of the animal virology network.

Methods
The EQA comprised: (a) molecular panel of 10 horse and avian samples spiked with inactivated WNV, Usutu virus (USUV) or other flaviviruses and (b) serological panel of 10 horse sera with different antibody titers. Seventeen laboratories participated in the exercise. For the molecular diagnosis, a pan-flavivirus conventional RT-PCR (Scaramozzino, J.Clin. Microbiol 2001) and a triplex RRT-PCR for simultaneous detection of WNV lineages 1 and 2 and USUV (Del Amo, J.Virol.Methods 2013) were applied. The serological panel was analysed using two commercial ELISA kits (INgezim WN compac and INgezim WN IgM, INGENASA) for detection of global and IgM WNV antibodies, respectively. Positive controls, reagents and kits were delivered to the participants.

Results
As regards the molecular panel, the overall results were satisfactory, especially given the technical complexity of the exercise and the beginner level of some participants. Although some limitations arose when applying conventional RT-PCR methods, most of the labs demonstrated their capacity to simultaneously differentiate WNV L1, L2 and USUV (88.2% of the labs were able to identify the three viruses in at least 70% of the panel). Results of the serological panel were highly satisfactory as 93.75% of the labs were able to correctly identify 100% of samples using the blocking ELISA. The detection of IgM positive sera was also adequate although some mistakes were observed in samples with low antibody titers. Overall, both PCR and ELISA methods showed excellent specificity, while some expected variations regarding sensitivity occurred with weak positive samples.

Conclusion
The results of this EQA demonstrated the competence of most of the participant labs (infrastructure and expertise) to face the molecular and serological diagnosis of WNV and also proved the good performance of the diagnostic techniques applied in diverse labs using different equipment.

Acknowledgements
We are grateful to the EU Commission (DEVCO: IFS/21010/23/194) for funding and to the MediLabSecure animal virology labs for their participation in the EQA.

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Detection of influenza D in respiratory disease samples from Northern Irish cattle

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Background
Influenza D virus (IDV) is a newly described member of the Orthomyxoviridae family, initially identified during a 2011 outbreak of respiratory disease in North American pigs. Cattle were subsequently shown to be the main reservoir of the virus and accumulating evidence suggests a role for IDV in bovine respiratory disease complex.

Methods
During the winter of 2017/2018, cattle submitted to the Agri-Food & Biosciences Institute for post-mortem with confirmed respiratory disease were tested for the presence of IDV. Viral nucleic acids were extracted from nasal swabs, trachea and lung tissues and analysed using a real-time RT-PCR TaqMan assay based on the PB1 gene. Partial sequencing of the IDV P42 gene was carried out on selected cases. Sequence data was analysed and aligned using DNAStar Lasergene 15 software and phylogenetic trees constructed using maximum likelihood in MEGA6.

Results
Of 102 cattle with confirmed respiratory disease, 7 tested positive for IDV (6.9% prevalence). Virus was detected in both the upper and lower respiratory tract. Lung tissues from IDV positive samples were negative for the presence of bovine herpesvirus 1, bovine respiratory syncytial virus, bovine viral diarrhea virus and parainfluenza virus 3 by immunofluorescence. Histological analysis of lungs from IDV positive samples revealed pathological features including necrosis, neutrophil infiltration of alveolar spaces, fibrosis, congestion, oedema and haemorrhage. Samples having CT values <20 were successfully sequenced and shown to cluster with European isolates of the D/swine/Oklahoma/1334/2011 clade.

Conclusion
To date, IDV has been detected in North America, Mexico, Japan, China, France, Italy and the Republic of Ireland. This study is the first to identify IDV in UK cattle herds. The presence of IDV in respiratory disease samples supports a role for this virus in bovine respiratory disease complex.

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Detection of Peaton virus in Israel

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Background
Simbu serogroup is one of the largest serogroups within the Perbunyaviridae family, comprising at least 24 viruses antigenically differing, but serologically related viruses which are transmitted mainly by Culicoides biting midges. Akabane and Shuni viruses, two members of this serogroup have been previously isolated from malformed ruminants in Israel. A recent retrospective serosurvey revealed that Israeli' ruminants were exposed to several additional Simbu viruses including the species Shamonda, Sathuperi and Peaton. These viruses are considered potentially teratogenic agents in ruminants and seem to have been circulated in Israel. In April 2017, an apparently healthy one-month-old male calf was transferred to the Kimron Veterinary Institute. A few days later, the calf was reported to be poorly responsive and not capable to feed on its own. Upon clinical examination, weak calf syndrome (WCS) was observed.

Methods
Viral RNA was extracted from the calf's brain and testis tissues and from the Cerebrospinal fluid (CSF). RNA of the small, medium and large segments of Simbu serogroup viruses was amplified and sequenced using previously described primers and methods. Phylogenetic analysis for each segment was performed using maximum likelihood, implemented in PhyML.

Results
The calf's post-mortem examination showed micro-hydranencephaly pathology. CSF oozed out while opening the calf's skull, with engrossed internal cisterns refilled with an excessive volume of CSF. Partial nucleotide sequences of the small, medium and large segments of Simbu serogroup viruses were identified from the calf's CSF and testis. Phylogenetic analyses showed that the sequences detected in the Israeli calf were virtually identical to Peaton virus.

Conclusion
The effective detection of PEAV genomic fragments is of great epidemiological importance as it shows for the first time that PEAV is present in Israel. The discovery of PEAV genomic fragments in the calf's testis might also suggest a possible role in virus persistence. Finally, the findings presented here strengthen the probable circulation of other Simbu serogroup viruses in Israel as was previously proposed. Even though a recent serosurvey indicated the presence of PEAV in Africa, this is the first genomic detection of this virus outside South-East Asia and Australia. Thus, our results are of paramount importance to the potential spreading of different Simbu viruses into the European continent.

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Flaviviruses in Serbia – current situation

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Background
The aim of this work is to summarize the data about the presence and prevalence of some zoonotic flaviviruses that circulates in Serbia in a last decade.

Results
After some historical data, the first data on presence of WNV in Serbia was obtained by testing of horses in northern part of the country. The presence of WNV antibodies was detected by ELISA/PRNT in 12% (46/349), in 28.6% (72/252), in 49.2% (64/130) and in 46.9% (45/96) blood sera of horses sampled during 2009/2010, 2011, 2012 and 2013. WNV circulation was also detected by ELISA/PRNT in 5/92 (5.4%) WNV antibody positive blood sera and in 8/82 (9.8%) WNV RNA positive tissues from 134 wild birds (46 species) collected during 2012 in northern part of Serbia. In 2010 and 2013, 3/50 (6%) and 28/306 (9.15%) sampled mosquito pools tested positive for WNV RNA. Also, 5.04% (17/337) human blood sera tested WNV antibody positive by ELISA in 2010. Human WNV clinical outbreaks were recorded each year, starting from 2012, when first human epidemic was recorded. National WNV surveillance programme funded by Veterinary Directorate is established from 2014, and was successful in detection of the WNV presence in sentinel animals, wild birds and mosquitos before human outbreaks in each season. After several decades without data, presence of TBEV was demonstrated by real-time RT-PCR, conventional RT-PCR and sequencing in Ixodes ricinus ticks collected during 2014 and 2015 (prevalence 2% (1/50) and 6.6% (30/450) at 2 out of 17 tested localities). In addition, low prevalence of anti-TBEV antibodies were detected by ELISA in human blood sera collected in the same period (0.37%; 1/267). One TBEV isolate from I. ricinus tick was sequenced and typed as Western European subtype. For USUV presence, in total 109 pools of Culex pipiens mosquitoes were tested from the territory of Vojvodina Province, northern part of Serbia. The presence of USUV was detected by real-time RT-PCR and RT-PCR in 2.75% (3/109) of tested samples. Two of this isolates were sequenced and typed as EU linage 2 USUV strains. The obtained results confirmed the first findings of this virus in mosquitoes and specific antibodies in human's blood sera in Serbia in 2015.

Conclusion
To understand the true public health concern of different flaviviruses in Serbia, detailed seroepidemiological, clinical and virological research are required.

Acknowledgments
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The airway epithelium maintains the barrier function after influenza virus infection despite the extensive loss of ciliated cells

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Background
The airway epithelium is the primary barrier to infection by respiratory pathogens. The main strategy of influenza viruses to overcome the defense mechanisms of the host is to infect the epithelial cells. However, the virus-host interactions in the respiratory epithelium during long term influenza virus infection are not well characterized.

Methods
We established an air-liquid interface (ALI) culture system to analyze the infection of differentiated airway epithelial cells by influenza viruses.

Results
Porcine ALI-cultures were sensitive to infection by swine and human influenza viruses. Release of virus at a high level was observed for up to eight dpi. Infection was characterized by a dramatic change of the epithelium. A large number of ciliated cells were lost due to virus-induced apoptosis. As a consequence, the thickness of the epithelial layer was reduced. However, the epithelial cell layer remained intact and there was no decrease of the transepithelial electrical resistance (TEER) and the tight junction (TJ) network was not destroyed. Our findings are explained by the regeneration of epithelial cells to compensate for the loss of ciliated cells. Basal cells had started to differentiate into specialized cells. The early differentiation process was sufficient to maintain the barrier function as indicated by the TJ network and the TEER. However, the differentiation process had not yet proceeded to the generation of ciliated cells. During differentiation, the epithelial cells showed different surface properties as compared to well-differentiated cells. The latter cells were characterized by the presence of α2,6-linked sialic acid, whereas basal cells mainly contained α2,3-linked sialic acid. Lectin staining indicated that both linkage types are present on the surface of regenerating cells. Because of the different surface markers, regenerating and well-differentiated airway epithelial cells may have different susceptibilities to infection by viral and bacterial pathogens.

Conclusion
Taken together, the ALI culture system allows to analyze the regeneration of airway epithelial cells after influenza virus infection. These cells will be valuable to study viral-viral and viral-bacterial co-infections.

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The use of insect-specific viruses to understand the transmission of pathogenic arboviruses

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Background
Arthropod-borne viruses (arboviruses) are a growing global problem which can cause disease in both humans and animals. In recent time increasing number of outbreaks of pathogenic arboviruses have been seen both globally and in Europe. Much research has been performed regarding the disease-causing properties of arboviruses in their vertebrate host, but little is still known about the viruses' interaction with their vector and the determinants of host range restrictions. In this project, insect-specific viruses (ISVs) and arboviruses within the Bunyavirales order will be used to study these topics.

Methods
Adult mosquitoes have been collected in Sweden and Brazil. Genetic characterization of the virome using high-throughput sequencing, and attempts for virus isolation directly from the mosquitoes are currently being done. Apart from virus isolation, molecular tools will be used to create infectious clones of a selection of the identified viruses. A collection of members of the Bunyavirales, both detected in our studies and in others, will be used.
A large-scale approach to investigate the effect that ISVs have on their vector will be taken by investigating the effect that ISV infection has on RNAi, on the vector transcriptome and proteome. Similar studies will be done using arboviruses. The factors restricting ISVs from infecting vertebrate cells will also be determined.

Results
The work on creating ISVs from infectious clones is ongoing and a clone of Herbert virus (HEBV), an insect-specific bunyavirus, has been made. As the cloned HEBV genome segments are under the control of a T7 polymerase promoter the T7 polymerase gene has been inserted into the pIZT/V5 expression vector. The HEBV segment plasmids as well as the T7 expression vector are transfected simultaneously in to a mosquito cell line to obtain live virus.

Conclusion
Arboviruses cause significant morbidity and mortality in both humans and animals worldwide. In recent years, newly discovered arboviruses such as Schmallenberg has emerged, but also known arboviruses, such as Zika virus, have caused outbreaks with a new set of pathologies. Despite this, little is known about what drives viruses to affect new hosts and what is required for ISVs to switch from single to dual/multi host tropism. For this, both fundamental and applied research is needed to provide knowledge to be used in the prevention and control of future outbreaks.

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Co-localization of Middle East respiratory syndrome coronavirus (MERS-CoV) and dipeptidyl peptidase-4 in the respiratory tract and lymphoid tissues of pigs and llamas

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Background
Middle East respiratory syndrome coronavirus (MERS-CoV) threaten animal and human health. The present study investigated for the first time the co-localization of the MERS-CoV and its receptor dipeptidyl peptidase-4 (DPP4) across respiratory and lymphoid organs of experimentally MERS-CoV infected pigs and llamas by immunohistochemistry (IHC). Also, scanning electron microscopy (SEM) was performed to describe the ciliation integrity of respiratory epithelial cells in both species.

Materials and Methods
Two-month-old pigs and 6 to 8-month-old llamas were intranasal inoculated with 107 50% tissue culture infective dose of MERS-CoV. Four pigs were euthanized on day 2 post-inoculation (p.i.), and 4 animals of each species were sacrificed on day 4 p.i. Finally, 6 pigs and 4 llamas were euthanized on day 24 p.i. Necropsies were performed and the co-localization of the MERS-CoV/DPP4 across respiratory and lymphoid organs of pigs and llamas was analyzed by double IHC. Formalin-fixed samples of nasal turbinate, trachea and lung were also used for SEM studies.

Results
In pigs, on day 2 p.i., MERS-CoV/DPP4 co-localization was detected in epithelial cells of medial turbinate and bronchus-associated lymphoid tissue. On day 4 p.i., the virus/receptor co-localized in frontal and medial turbinate epithelia and cervical lymph node in pigs; however, many infected-cells did not display DPP4 in their surface. Infected-epithelial cells were distributed unevenly through the whole nasal cavity and cervical lymph node in llamas. MERS-CoV viral nucleocapsid was mainly detected in upper respiratory tract sites on days 2 and 4 p.i. in pigs and 4 p.i. in llamas. While pigs showed severe ciliary loss in the nasal mucosa both on days 2 and 4 p.i. and moderate loss in trachea on days 4 and 24 p.i., ciliation of respiratory organs in llamas was not significantly affected.

Conclusion
The present work provides evidence that MERS-CoV preferably infects respiratory epithelial cells expressing DPP4 in llamas, supporting that DPP4 is necessary for virus entry. However, the role of DPP4 in regulating virus entry in respiratory organs of pigs and lymphoid tissues of both species may not be sufficient. Although pigs showed a significant expression of DPP4, the number of cells permissive for MERS-CoV in this species was lower than that of llamas. Since a very low amount of MERS-CoV antigen was found in nasal turbinates of pigs, the severe ciliary loss was probably due to a bystander effect.

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Molecular characterization of recent Spanish West Nile virus strains

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Background
West Nile Virus (WNV) is a zoonotic flavivirus, which is maintained in the nature in an enzootic cycle between mosquito-vectors and birds. Humans, horses and other mammals are also susceptible to the infection. WNV is spread worldwide. In Europe, its incidence and geographical distribution have increased in the last decades, with a significant number of outbreaks, mostly caused by WNV lineages 1 (L1) and 2 (L2). Focusing on Spain, WNV L1 has been circulating in the south of the country at least since the early 2000's. First sequence data pointed out to a single virus introduction[1]. The aim of this work was to perform a molecular characterization of recent Spanish isolates and to elucidate their molecular relatedness with other WNVs from the Western Mediterranean (WMed) region.

Methods
A panel of WNV RNAs obtained from infected wild birds and horses identified in different provinces of Spain over 2010–2016 were analysed. Viral genome regions coding for membrane (M), envelope (E) and non-structural NS1, NS2 and NS3 proteins, selected based on divergence degree, were sequenced. Phylogenetic analyses of M-E genome region of representative WNV isolates and amino acid sequence analyses were carried out.

Results
Initial phylogenetic analysis placed all WNV Spanish sequences within WNV L1, creating a monophyletic group together with other isolates from the WMed region. Indeed, most Spanish isolates grouped in WMed-1 cluster, while only the earlier ones (2007–2008) belonged to WMed-2 cluster (as defined by Sotelo et al.)[1] and a single case from 2011 was placed separately. More in detail, three subgroups were differentiated within WMed-1 cluster, all identified in the same Southern province (Cádiz). At the amino acid level, unique substitutions were found in 2007–2008 strains, which were absent in more recent Spanish isolates and other Mediterranean ones[1].

Conclusion
Different WNV L1 variants, having a WMed common ancestor, have been circulating in Southern Spain, at least since its first isolation in 2007, as it is occurring in other Mediterranean countries. Interestingly, one variant showed the ability to spread northward affecting wild birds and horses. WMed-2 cluster seems to have extinguished, as no more recent strains gathered in it. Full-genome sequencing will provide more information to elucidate the epidemiological dynamics of WNV in Spain and in the Mediterranean region.

Reference
[1] Sotelo et al., J. Gen. Virol. (2011), 92, 2512–2522.

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Validation of ready-to-use real-time PCR kits for specific and reliable detection of lumpy skin disease (LSD) and distinction with Neethling vaccine strain

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Background

Lumpy skin disease is a viral notifiable disease initially enzootic to Sub-Saharan Africa. It has been detected in Turkey in late 2013 and spread to Europe through the Balkans. In 2016, a total of 7,483 outbreaks have affected Greece, Bulgaria, Macedonia, Montenegro, Albania and Serbia. In this emergency context, BioSellal decides to develop a ready-to-use real-time PCR kit for the rapid and specific detection of LSDV towards the other Capripoxviruses (Sheeppox, Goatpox). Since vaccination with a live attenuated LSDV strain (Neethling strain) was mainly used in Europe and significantly reduced the number of outbreaks (385 in 2017), we decide to develop a DIVA PCR kit to distinguish between wild strains and Neethling vaccine strain.

Methods

BioSellal has developed two kits:
– Bio-T kit® Lumpy Skin Disease allowing the detection of all LSDV strains (FAM) and an Internal Positive Control (Cy5)
– Bio-T kit® LSDV-DIVA which detects in the same well all LSDV strains (FAM), only field LSDV strains (VIC) and an Internal Positive Control (Cy5)

Validation of each Bio-T kit® was done according to the French standard U47-600-2 and an evaluation of the Bio-T kit® Lumpy Skin Disease was performed by the former reference laboratory of Pirbright Institute (UK).

Results

The results of the validation of the Bio-T kit® Lumpy Skin Disease were the following:
PCR Characteristics
– Inclusivity validated on 54 isolates, from Africa and from outbreaks in Europe; improvement in detectability compared to panCapripox PCR from Bowden et al.
– Exclusivity assessed on Goatpox and Sheeppox viruses
– Limit of Detection 3.125 GE/PCR
– Limit of Quantification 10 GE/PCR
– Efficiency 113%
– Domain of Linearity between 10 to 1.10^6 GE/PCR
Complete Method Characteristics
– Limit of Detection on whole blood 3,000 GE/ml
– Limit of Detection on skin lesions 50,000 GE/g
– Diagnostic sensitivity on 20 Blood,19 Skin 100%
– Diagnostic specificity on 20 Blood,19 Skin 100%

As expected, the Bio-T kit® LSDV-DIVA doesn't recognize the Neethling vaccine strain on the VIC channel.

Conclusion

Thanks to their complete validation, Bio-T kit® Lumpy Skin Disease and Bio-T kit® LSDV-DIVA allow an efficient and reliable survey of the spread of LSD in Europe. In France, to anticipate LSD spread and to respond to the propositions of the French platform for animal health surveillance, Bio-T kit® Lumpy Skin Disease was easily implement and accredited by Cofrac in a French analysis laboratory.

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An interdisciplinary approach addressing ticks and tick-borne diseases of cattle in Uganda

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Background

Ticks are the most important arthropod vectors and transmit a wide range of pathogens. Tick-borne diseases (TBDs) cause substantial morbidity and mortalities in humans as well as domestic and wild animals and are thus a major threat to both cattle and human health. Here we present findings from an ongoing project with the overall purpose to improve cattle health through increased understanding of the microbial community in cattle and ticks in Uganda. Understanding the complex interactions within the microbiome is of great importance for understanding how tick-borne pathogens spread and cause disease. Metagenomics, a powerful tool for pathogen detection and for making in-depth analysis of the microbial community provides this possibility. The microbiome of ticks, either questing in close proximity to cattle or feeding on cattle, will be characterized and compared with the cattle microbiome.

Methods

In this study, we have collected blood samples from 500 cattle and >7500 ticks in the five districts: Kasese, Soroti, Moroto, Gulu and Hoima, covering five agro-ecological zones in Uganda. In total, 250 animal caretakers/owners have been interviewed using a structured questionnaire delivered electronically on a tablet. Questions covered topics such as knowledge about TBDs, use of protective measures against ticks and TBDs, and interactions with wildlife. In one village we have also performed an in-depth study of cattle owner's local knowledge of disease causing pathogens and ticks.

Results

Thick and thin blood smears were examined microscopically for haemoparasites. Haemoparasites detected include: Theleria spp. in 9.8% (49/500), Anaplasma spp. in 1.4% (9/500) and Trypanosoma spp. in 0.8% (4/500) of the blood samples examined. Preliminary results from the tick species morphology indicate ticks from following genera: Rhipicephalus (seven species), Amblyomma (two species) and Hyalomma (two species).

Conclusion

These combined quantitative and qualitative data will be analyzed during spring and we aim to present results on:
– What types of ticks and TBDs are present across Uganda
– Qualitative and quantitative data on cattle owner's knowledge of ticks and TBDs
– Data on how actual presence of pathogens correspond to animal owners' knowledge about the topic, and implications for veterinary advice.

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Development of a method for concentration and detection of infectious salmon anaemia virus from seawater

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Background
Atlantic salmon (*Salmo salar*) is the biggest farmed marine species in Norway and provides export earnings. Despite these contributions, significant losses of fish during seawater phase production persist. The causes of these high mortalities have yet to be fully unravelled. Changes in farm practices, increased fish densities, poor quality of smolts have been proposed as major contributory factors. Yet, a recent study carried out by the Norwegian Food Safety Authority concluded that "fish diseases" constituted the most important causes. Virus transmission and disease control strategies are important issues in Atlantic salmon health management. Seawater represent Atlantic salmon natural environment. Thus, water from the fish environment could be used as indicator for evaluation of potential virus transmission. Currently, infectious salmon anaemia virus (ISAV) transmission in Atlantic salmon farms are largely surveyed using traditional manual methods. These methods are selective, are limited to *in vivo* sampling of live fish for identification of the virus. Development of a non-invasive test to confirm the presence of the virus in fish environment will serve as an early warning. The aim of this study is to establish a non-invasive method for detection of infectious salmon anaemia virus by sampling fish environment for the purpose of virus detection without sacrificing live fish.

Method
The study is based on concentration of ISAV in seawater through filtration and adsorption to charged membrane filter, before detection and quantification of the virus with reverse transcriptase quantitative (qPCR). In this study, sixty Atlantic salmon (weighing 100 g) post smolts were bath challenged with high and low dose of ISA-Glesvær isolate respectively. Seawater and Atlantic salmon tissue samples were collected from control, low and high dosage tanks for eighteen days and analysed by histology, immunohistochemistry and PCR.

Results
Mortality was 100% for high dose and 97% for low dose tanks. Analysis of samples revealed clinical features-ascites, petechiation and haemorrhagic liver necrosis was observed in fish from high dose (days 9–24) and low dose (days 16–32) tanks. Mid kidney and gill samples was positive by PCR from day 8–32 post bath challenge. Seawater from high and low dosage ISAV tanks were also positive by PCR from days 8–32 post bath challenge.

Conclusion
The correlations of results from clinical signs, tissue and seawater samples provided evidence that suggest the method can be applied for risk assessments and/or for early warning system in salmon farms.

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Empirical tool to estimate the costs to be incurred in case of ASF outbreaks eradication

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Background
African Swine Fever (ASF) is a highly contagious viral disease, for which notification to the Competent Authorities of the National Veterinary Service is required.
Originating from Africa, ASF has reached Europe for several decades. Since 1978, ASF virus reached Italy (Sardinia), too, where it has assumed endemic features.
Recently, the disease has attracted ever increasing attention because the fact that since 2007, year of its appearance in Georgia (Poti), it has rapidly spread to the north (Russia) until reaching EU Territory. The interaction between the domestic pig population and the different wild boar populations makes hard prevention and control measure management. The aim of our work was the creation of a simple, empirical tool that would provide the possibility to have an approximate estimation of the direct costs of management / eradication of a potential African Swine Fever outbreak.

Methods
Reference was made to the Italian Guide Lines for Epidemic Emergencies and to the Manual for Classical and African Swine Fever, in order to outline in terms of cost, everything provided for, in the above mentioned procedures (people, vehicles, equipment, etc.). Microsoft Excel models were used to allow a real-time calculation of the unit cost of the various envisaged items, in relation to current market prices and/or employment contracts or services (National Agreements). A research of the unit cost of each item of expenditure was, then, performed in different scenarios (rural and commercial farms).

Results
The resulting product is a "compound spreadsheet" able to provide the "estimate cost" of the management/eradication of ASF outbreak, on the basis of information about pig population and involved farm size. The simulated scenarios clearly highlight the significant unitary financial burden especially for the rural farms.

Conclusion
The economic damage that ASF can be able to cause is significant, both as a direct damage to the affected farms and the territory and as indirect penalties due to unavoidable commercial "limitations". The economic information could positively influence the managerial optimization of this emergency. The potentially inferable data can be able to stimulate improvements in the optimization of the "Early Warning" and "Prompt Reaction" phases (to be reviewed in "peace time"). The creation of the calculation model on the provisions of the "National Handbook for Epidemic Emergencies" and the "excel" format make our product adaptable to the management/eradication of any infective disease outbreak where "stamping out" policy is foreseen.

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Establish well-differentiated airway epithelium models from harbor seals to study the course of infection by viral pathogens

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Background
For decades, massive infections by influenza viruses, phocine distemper virus, canine distemper virus, and phocid herpesviruses in harbor seals (hs) have been reported. Lack of a suitable cell culture system comprising polarized hs airway epithelial cells limits the investigation of viral replication.

Method
To study the effects of virus infection in the harbor seal airway epithelium, we applied an ex vivo precision-cut lung slices (hsPCLS) technique, and in vitro primary trachea epithelial cell cultures (hsPTEC) to analyze virus-induced effects on the respiratory epithelium.

Results
PCLS contain airway cells in the original setting including ciliated cells, goblet cells, basal cells and sub epithelial cells. Therefore, this culture system is suitable to study the cell tropism of the above-mentioned viruses. PTEC of harbor seals could be maintained for three passages. They express sialic acids on the cell surface which is consistent with the high susceptibility of harbor seals to infection by influenza viruses. Furthermore, we established an air-liquid interface culture system for well-differentiated harbor seal tracheal epithelial cells (hsALI) which contains ciliated and mucus producing cells.

Conclusion
These culture systems will be invaluable tools to study the entry route and the cell tropism of microbial respiratory pathogens of harbor seals.

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Prevalence determination by using a non-fully characterized diagnostic test

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Background
OIE recommends that the characterization of newly developed diagnostic test procedures, for which no gold standard is available, can be performed by latent class analysis (LCA). However, there is no acceptance in practice for this approach, which is due to the fact that there is no published theoretical basis available that could be used for calculating true solutions. A system of equations was derived from axioms, from which two facts can be derived: First, in general there is no single solution for LCA problems, which could partly explain the variety of reported solutions for the same problem. Second, the unknown prevalence from the sample used for the calculation is the decisive criterion for a true solution to an LCA problem. This results in the principle indispensability of a gold standard. However, if a gold standard is not available, this fact implies the need for meaningful "substitute knowledge" and resulting procedures that could assist on finding the true prevalence.

Method/Result
The finite set of discrete prevalence information inherent in a natural way in a finite sample taken out of a population (discrete/quantized prevalences) and weak knowledge about the prevalence forms the basis for a procedure to limit the complete set of quantized states of prevalence to a few consistent quantum states.

Conclusion
The proposed approach is exemplified by data from a paper by Clegg et al. (2011) by identifying possible numbers of bovine tuberculosis cases in relation to two populations examined using the skin test.

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Survey of the Hungarian PRRSV diversity in 2016

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Background
A PRRSV survey was conducted in 72 industrial pig farms in 2016. The goal of this work was to determine the current occurrence and diversity of the virus, and to better assess the epidemiologic status of the Hungarian PRRSV isolates.

Methods
30 blood samples were collected from each farm. Samples were pooled into pools of 5. Diagnostic primers were designed to the conservative part of ORF7. PCR was executed with Qiagen OneStep RT-PCR kit and was tested on agarose gel. For the phylogenetic comparison ORF5 was amplified with four sets of primers with Qiagen OneStep RT-PCR kit. In the case of unsuccessful attempts SuperScript IV reverse transcriptase was used to generate cDNA, and the ORF5 gene was amplified with Phire Hot Start II DNA Polymerase. PRRSVs were isolated from the blood samples on primary porcine alveolar macrophages. For full genome sequencing DNA library was prepared using the NEBNext® Fast DNA Fragmentation & Library Prep Set for Ion Torrent with the Ion Torrent Xpress barcode adapters. Library was sequenced with an Ion Torrent PGM sequencer.

Results
46 pig herds from the 72 were diagnosed as positive for PRRSV. Sixty ORF5 sequences were achieved from 38 positive farms. More than one virus pool was sequenced successfully from 22 farms and at least 11 farms were infected with two relatively distant PRRSV. All samples proved to be type 1 PRRSV and were grouped within European subtype 1. The majority of the sequences can be grouped in five clusters: at least four of them can be fit into established clads. One cluster of samples was similar to the Amervac vaccine, other clusters were similar to a PRRSV isolate from Spain from 2003 (Spain 28/2003) and a Belgian isolate from 2010 (08V194). The closest relative of the third cluster is a Belgian isolate (08V204). The forth cluster is similar to isolates from South Korea, while the closest relatives of the fifth cluster are isolates from the Czech Republic. Twelve viruses were isolated from different farms for further investigation from which the complete sequence of five isolates were determined, analyzed and compared to other sequences.

Conclusion
The fact, that Hungarian PRRSV strains show high genetic diversity implies multiple viral introduction events from surrounding central European and western European countries. Despite the diversity, virus subtypes that are characteristic to Eastern Europe are currently not present in Hungary.

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The results of AMR study of *Mycobacteria bovis* strains isolated in the Central part of Russia and Siberia region

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Background
Tuberculosis (TB) is a serious public health problem worldwide. Its situation is worsened by the presence of drug-resistant strains of tuberculous mycobacteria which threaten TB-control and prevention efforts. There is limited information available on the level of antibiotic resistant mycobacteria in the Central part of Russia and Siberia region. Our study aimed to investigate resistance to the first and second-line anti-TB drugs of mycobacterial strains isolated from tuberculin-positive animals.

Methods
Drug-resistance of mycobacteria was determined by the absolute concentration method on the LJ-medium (indirect method of determining drug-resistance after the isolation of mycobacterial culture).

Results
We demonstrated that 60% of mycobacterial cultures were resistant to isoniazid, 40% – to rifampicin and ethambutol and 30% – to streptomycin. All mycobacterial strains tested were resistant to ethionamide, 40% of mycobacterial strains – to paraaminosalicyl acid, 60% – to kanamycin, 30% – to capreomycin and 20% – to ofloxacin. It was established that 40% of mycobacterial strains were mono- and 50% – polyresistant. 40% of M.bovis strains were defined as multidrug resistant (resistance to 2 main anti-TB drugs isoniasid and rifampicin). In the Nizhny Novgorod region, almost all M.bovis strains were resistant to most anti-TB-antibiotics. At the same time, in the Moscow region, about 50% of M.bovis strains were resistant to such antibiotics, as isoniazid, streptomycin, ethambutol, kanamycin, oflaxacin and capreomycin. In the Moscow region resistance to ethionamide was revealed in all M.bovis strains. In the Republic of Sakha (Yakutia) the studies of non-tuberculous mycobacteria revealed resistance to rifampicin, ethionamide and PAS in all strains tested. In the Moscow region all non-tuberculous mycobacteria were found to be resistant to the majority of antibiotics commonly used in clinical practice. In the Ryazan region resistance to isoniazid, kanamycin and ethionamide was observed in all non-tuberculous mycobacterial strains, 50% of these strains were resistant to streptomycin, capreomycin, PAS.

Conclusion
We hope that the up-to-date data presented in this study will help to understand the current drug-resistant/MDR-TB situation in Russian regions. Our findings showed that isoniazid, ethambutol, kanamycin, and ethionamide should be considered ineffective for TB therapy. We strongly suggest that a wider set of surveillance sites is needed to get a more realistic view of the MDR-TB in the Central part of Russia and Siberia region.

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Enzootic bovine leukosis: Italy has been declared as officially free

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Background
Bovine Leukaemia Virus (BLV) is the causative agent of Enzootic Bovine Leukosis (EBL) an infectious disease worldwide distributed in cattle population. Most of European Union (EU) countries have already eradicated the EBL by virtue of eradication plans based on the active serological survey of susceptible population and the "test and removal" strategy (for seropositive animals); this approach was supported and financed by the EU as provided by a specific legislation (council directive of 64/432/EEC). In this contest, EBL is still persisting in several European countries, including Italy, despite the application of a national eradication plan since the 1996.

Methods
The rules of Italian eradication plan are reflecting the European legislation: all bovines, aged more than one year from breeding herds, should be serologically tested twice a year or annually in case the herd already demonstrated to be EBL free; the seropositive cattle should be promptly removed. While the Italian northern regions eradicated the disease in a reasonable time, in the "South" the breeding conditions and other social/economic factors have hindered the complete application of the plan. For these reasons the Italian Ministry of Health implemented, in the recent past, more relevant measures to efficiently fulfill the eradication process. Currently the epidemiological situation shows the persistence of few EBL clusters in a limited number of Italian regions (Lazio, Puglia, Campania, Sicilia); anyway the risk of viral spread in the rest of the country should be considered as negligible because the adopted strict rules in terms of biosecurity.

Results
The data extracted from VET-INFO (the national platform of veterinary informative system for the management of animal population and the control of the animal diseases) allowed to demonstrate that Italy achieved the compliance with the European rules: the prevalence of BLV outbreaks is now computable under the threshold of 0,2% (of controllable herds). In fact the Commission Implementing Decision (EU) n. 2017/1910 of 17 October 2017 declared Italy as an officially enzootic-bovine-leucosis-free Member State as regards bovine herds.

Conclusion
Consequently, the eradication plan could be considered as out of date and a less expensive surveillance plan could be implemented to assure the maintenance of the free status. A consistent part of the saved resources will enable the Ministry of Health and the involved Regions to adopt specific plans to eradicate the EBL even in the remaining persistent clusters of infection.

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Hepatitis E in Vojvodina Province, Serbia – an epidemiological overview and associated risk factors for humans and animals

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Background
Hepatitis E virus (HEV) is a causative agent of hepatitis E infection outbreaks. It was identified that human and swine HEV strains are genetically closely related, suggesting the enzootic potential of the virus. Here are presented the results of HEV investigations conducted in pigs, humans and surface water in the Vojvodina Province, Serbia.

Methods
We examined antibody prevalence in pig and human sera samples by in house ELISA (based on the use of recombinant HEV gt3) and the presence of HEV gt3 RNA in swine feces and in surface and sewage water samples by RT-qPCR. To identify if HEV infection is present in pigs in Serbia, 30 swine feces from 6 different farms (five pooled feces samples per farm) were analyzed and sequenced for the first time during 2009. Furthermore, from 2010 to 2013, HEV seroprevalence was tested in 315 backyard pigs, 300 pigs from three large commercial farms and in 200 sera from Serbian blood donors. Additionally, from 2012 to 2014, HEV RNA was examined in 108 samples of surface and municipal sewage waters.

Results
HEV RNA was detected in 70% (21/30) feces samples from six farms with the prevalence in range from 20% to 100%. The established overall seroprevalence in backyard and farm pigs was 34% (109/315) and 44.66% (122/300), respectively. HEV seropositivity significantly varied between herds and swine categories. In total, 15% (30/200) of tested sera from Serbian blood donors reacted positive. HEV RNA was identified in 2.77% (3/108) of tested water samples.

Conclusion
Our results show that hepatitis E infection is widespread in Vojvodina Province in pigs, humans and surface water. Among HEV seropositive blood donors, 43.33% were pig owners and 93.33% people lived in villages. Based on the obtained results we can conclude that farmers, who are in direct contact with pigs, are considered as particularly vulnerable population. Further research should be focused on the assessment of risk factor indicators, such as the level of biosecurity measures, HEV seroprevalence among farm workers and veterinarians, the use of waste waters from farms for irrigation and swine feces as a source of manure. It is necessary to recommend biosecurity and safety programs with the aim to prevent further transmission of hepatitis E from animals to humans.

Acknowledgment
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West Nile virus in north-eastern Italy: overview of surveillance activities in 2017

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Background
West Nile virus (WNV) re-emerged in north-eastern Italy in 2008, after ten years from its first appearance in Tuscany. In 2009, a national surveillance programme was implemented, and re-modulated since 2011 in Veneto region, north-eastern Italy. Hereby we illustrate the results of surveillance activities in Veneto region in 2017.

Methods
The surveillance plan active in Veneto since 2011 aims at early detecting WNV seasonal reactivation to inform Public Health control measures. A risk-based approach is applied to define the area where WNV could be more likely circulate, accounting for the epidemiological situation of the previous year. Active surveillance is performed on a representative sample of residential horses (i.e. animals that were not moved in the past 3 months) by searching IgM antibodies, indicating a recent infection with WNV. Entomological surveillance is based on bi-weekly mosquito captures and PCR testing pools of the known vector species of WNV *Culex pipiens*, *Ochlerotatus caspius*, and *Aedes albopictus mosquitoe*s. Passive surveillance is performed on wild birds found dead in surveillance area.

Results
From July to October 2017, 2272 equine sera were screened (1859 collected from 542 premises and 413 at slaughterhouses), leading to detect 61 IgM sero-positive equines in 30 holdings. Mosquitoes were captured by CDC-CO2 or gravid traps at 54 sites spread out through the flatlands of Veneto region. Collected mosquitoes were identified, pooled by species/date/location and examined by real-time RT-PCR. A total of 128,428 mosquitoes of 15 species were collected, and 2508 pools examined. Twenty-two *Culex pipiens* pools tested positive for the presence of WNV. Surveillance on non-migratory wild birds allowed detection of the virus only in two seagulls and one sparrow, of 216 birds sampled. The WNV belonged to the lineage 2.

Conclusion
WNV has been annually detected in Veneto since 2008, indicating that the virus has become endemic in the region. The evolution of the epidemiological situation prompts for accurate surveillance measures, especially during the seasonal period favorable to mosquitoes. The implementation of a protocol combining IgM screening of horses with surveillance on mosquito vectors proved to be valuable for early detecting WNV circulation. In fact, entomological and veterinary surveillance have been included as triggers for enhancing screening activities in humans. A multidisciplinary approach, also in the light of the One-Health initiative, is paramount to support the implementation and strengthening of preventive measures to reduce the risk of spill-over to humans, also in the light of the One Health initiative.

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Spatiotemporal characteristics of LSD epidemics in Samara region in 2016–2017

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Background
Lumpy skin disease (LSD) is a pox-like disease affecting cattle. LSD virus belongs to genus Capripoxvirus. LSD was endemic in most African countries but since 2012 it has spread in the Middle East including Israel and Turkey, and since 2015 in Europe, Trans-Caucasian region and Russia. The main route of LSD introduction and transfer is the transmission of infected animals. Local spreading is due to mechanical vectors, blood-sucking insects.

Methods
This study provides spatiotemporal characteristics of two LSD epidemics in Samara region in 2016-2017. For this, we analyzed official reports of regional veterinary authorities, weather records and other data in relation to LSD outbreak dynamics.

Results
After first officially registered LSD outbreak in Dagestan in 2015, the epizooty was spreading rapidly and by October 2016 covered North Caucasian, Southern and Central Federal Districts of Russia. In 2017, the disease was introduced into the Volga Federal District. In October 2016, an LSD outbreak occurred at Northeast of Samara region. This outbreak lasted since mid-November affecting 5 individual farms in neighboring villages, where after the disease cases were not reported in the region since August 2017. In August 2017, the disease was reported in 6 other districts of Samara region adjacent to Saratov region and Kazakhstan at South and to Orenburg region at East. A total of 29 outbreaks were reported. The average incidence in commercial herds (with more than 50 animals) was 0.3%. Veterinary service implemented quarantine, restrictive measures and treatment activities. The analysis of the situation with due consideration of possible spreading factors and relationship of epizootic peaks with weather records suggests that the LSD spreading in the region in 2016-2017 was most likely mediated by insect vectors, particularly Stomoxys calcitrans, which are active just at this time. Cattle contacted with insects at pastures. It should be noted that stock density in infected areas was as low as 2 to 8 animals per 100 hectares of farmland that probably restricted virus spreading. Epizooty of 2017 was faded in September that was also related to decreasing in daily mean temperature and the lack of insects.

Conclusion
Therefore, in the case of an outbreak at the end of a season of insect flight, with low daily mean temperature and low stock density, and provided quarantine maintenance, the disease has no chances for local spread even without vaccination. Thus, animal transmission (human-mediated spread) remains to be the main factor of spreading.

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Prevalence of Brucellosis in humans during the last decade in the Country of Georgia

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Background
Brucellosis is one of the most widespread and debilitating zoonotic disease and remains the most important health problem worldwide. It is responsible for enormous economic losses and considerable human morbidity in endemic areas and is one of the most prevalent zoonosis in Georgia.
The main objective of this study was to analyze human brucellosis cases in the last ten years and help to identify the tendency of the disease.

Methods
Tendencies in the epidemiology of human brucellosis in Georgia were investigated by analyzing national surveillance data (2008–2017) complemented by questionnaires.
Confirmation of diagnosis was performed by slide (Huddleson), standard tube (Wright) agglutination test and/or by enzyme-linked immunosorbent assay (ELISA) to detect Brucella-specific antibodies and/or by bacteriology test for culture isolation.

Results
During 2008–2017 the incidence rate of brucellosis varied from 3.8 to 5.7 cases per 100,000 population in Georgia and the year 2014 had the highest incidence rate (6.6), supposedly because of some active surveillance activities in several regions. A total of 1896 cases of brucellosis were registered from 2008 through 2017 with 81.65% (n=1548) male and 18.35% (n=348) female. The most common jobs of the cases with brucellosis were shepherds (35.3%) and farmers (60.02%) and the most frequent risk factor was a contact with sick animals and consumption of unpasteurized milk and dairy products (66.67%). The most affected regions were Kakheti and Kvemo Kartli. The total of 84 outbreaks were reported during 2008–2017 and in all cases source of infection was contact with domestic animals and consumption of unpasteurized dairy products and undercooked meat. The highest incidences of this disease were in patients who were in the 15-19 years age group (10.6%) and the lowest incidences were in the 1–4 years age group (1.9%). During 2008-2017, of the 2098 sera tested for brucellosis 20% (419/2098) were positive by ELISA, slide and tube agglutination at the NCDC, but by blood culturing 7.11% (65/913) B. melitensis and 1.5% (14/913) B. abortus strains were isolated and confirmed.

Conclusions
This Review summarizes the situation of Georgia in regard to human brucellosis. It is necessary to conduct preventive measures against brucellosis through the cooperation between the public health and veterinary network. It should be mentioned that from 2016 active vaccination campaign for brucellosis started in animals by the National Food Agency throughout the country and which will help to reduce the prevalence of brucellosis in Georgia.

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Case report – Human anthrax meningitis in the Black Sea coast, Country of Georgia

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Background
Anthrax is a widely distributed endemic infection in Georgia, historically affecting nearly all territories within the country. Ecological conditions in Georgia support persistence of B. anthracis, the causative bacterial agent of anthrax. A substantial number of veterinary-acquired human cases are registered annually in Georgia and the disease remains a significant threat to human health.

Objective
This is a report of the human case of anthrax meningitis registered in the Black Sea region of Georgia. Epidemiological and laboratory investigation of the case are presented.

Materials and Methods
A 59 year old male with multiple skin lesions on left arm, enlarged left armpit lymph-node, and 38°C fever was presented to the clinic. Patient was in coma during admission to the clinic. Cutaneous anthrax complication with meningitis was suspected and laboratory diagnostics were performed on swab, CSF and blood samples. Additionally, the following tests were performed: blood test, CRP, chest X-Ray, cerebrospinal fluid (CSF) biochemical analyses.

Results
Epidemiological data have indicated that this case was linked to the processing of dead cattle in Adjara region. A patient did not seek treatment for few days. Bacteriological investigation demonstrated a presence of B. anthracis from swab, CSF and blood samples. An isolate of B. anthracis was obtained and confirmed by qPCR.
The patient was treated by antibiotic according to the national guideline of anthrax. On the 3rd day after admission, the patient died. The final diagnosis in medical record was "Anthrax cutaneous, Anthrax meningitis".

Conclusion
We report a rare case of anthrax meningitis arising after a complication from the cutaneous form. The case was fatal despite recommended antibiotic therapy due to the delayed treatment. Development of meningitis should be considered in all forms of anthrax.

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Feline retroviral infections (FeLV and FIV) and their effect on the bacterial blood pathogens detected in cats in the Czech Republic

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Background
Feline retroviral diseases are represented by Feline Leucosis Virus (FeLV) and by Feline immunodeficiency virus (FIV). These viruses cause diseases with nonspecific clinical signs. FeLV is primary manifested by depression of bone marrow, formation of lympho sarcomas and reproductive failures. FIV is presented by chronic infections like diarrhoea, weakness, lymphadenopathy, or chronic respiratory disease. Chronic stomatitis and gingivitis are also very typical. The aims of the study were a detection of important feline retroviral pathogens like FeLV and FIV by nested RT-PCR or by SNAP tests from feline blood, and to detect haemothropic bacterial microorganisms like Mycoplasma haemophelis and Mycoplasma haemominutum and introduction of molecular biological methods like nested RT- PCR or PCR and sequencing.

Methods
A total of 75 feline blood samples were investigated by nested RT-PCR and by SNAP tests to detect FeLV, and 64 samples for the presence of FIV. There were tested 45 outdoor, 7 indoor and 23 sheltered cats. The samples originated from cats with acute or chronic forms of different types of disease or from healthy animals. DNA and RNA were extracted by commercial extraction kits according to manufacturer´s instructions: Viral RNA by Nucleo Spin® RNA II kit, bacterial DNA Nucleo Spin Blood DNA (M-N), common extraction (DNA/RNA) High Pure viral nucleic acid kit (Roche). Bacterial DNA and viral RNA were detected and amplified by PCR in Thermal Cycler, followed by visualisation in agarose gel. DNA sequence: The part of genome of feline haemothropic bacteria was sequenced. Resulting DNA sequences were compared with DNA sequences of reference strains from the Gen Bank database. The sequence analyses were performed by Geneious programme.

Results
Cats infected by FIV and FeLV are easily infected by feline haemothropic bacterial pathogens. Out of the total of four FeLV positive cats two of them were also infected by M. haemominutum and one was infected by M. haemofelis. Out of a total of six FIV positive cats two of them were infected by M. haemominutum and one by M. haemofelis. DNA sequencing confirmed the positivity of PCR in 100%.

Conclusion
Mycoplasma haemofelis and Mycoplasma haemominutum can be the agent of feline anaemia, particularly in immunosuppressed animals due to retroviral co-infection.

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Laboratory identification and characterization of Equine gamma herpesviruses in the Czech Republic

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Background
Equid gamma herpesvirus 2 and Equid gamma herpesvirus 5 (EHV-2 and EHV-5) are found in the horse population worldwide. Virus EHV-2 is associated primarily with keratoconjunctivitis and EHV-5 with multinodular pulmonary fibrosis. In addition, equine gamma herpesvirus (γEHV) infections are mentioned in association with respiratory symptoms, fever, depression, decreased performance, and reproductive disorders.

Methods
The duplex qPCR method was designed and used for quantitative laboratory detection of γEHV. In the group of 34 Old Kladruber foals exact time of primo infection was screened during 30 weeks of their life. In a group of 160 patients of Eqine Clinic at the VFU Brno, the presence of gamma herpesviruses in relation to clinical disease has been investigated. A total of 16 oligonucleotide primers were designed in the region encoding gB of EHV-2. The resulting PCR products of 4 virus strains were subjected to NGS (New generation sequencing).

Results
The probable age of foals primo infection was determined as week 6 in EHV-2 and week 30 in EHV-5. Virus excretion occurred in foals for several weeks with gradually decreasing titres. All the foals experienced the infection by one or both the viruses by the 30th week of age.
In 160 suspected patients, the EHV-2 was shown to be positive in 42%, EHV-5 in 19% and coinfection in 9% of cases. The highest rate of EHV-2 positivity showed samples from a group of patients with reproductive problems. Viruses were detected also in eye / respiratory diseases: EHV-2 in 51%/36% of cases, EHV-5 in 27%/20% of cases and coinfection in 14%/10% of cases respectively. The Sequences of 4 positive samples in the gB gene of EHV-2 were obtained by NGS and served to phylogenetic analysis of Czech strains of the virus. A total of 10 different variants of virus were distinguished in those 4 samples.

Conclusion
The occurrence of Equine gamma herpesviruses is thoroughly investigated in this work. It becomes the basis for further research focused on the influence of genetic variability of EHV-2 strains on the virus virulence.

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Longitudinal study on epizootiology and genetic variability of equine arteritis virus in hucul horses

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Background
Equine arteritis virus (EAV) is one of the most important causative agents of respiratory and reproductive diseases in equine populations around the world. Persistently infected stallions play a key role in the epizootiology of equine viral arteritis. Those stallions constantly shed virus through semen for prolonged period of time that could last many years. Additionally, new viral strains could emerge in persistently infected stallions. The aim of the study was to monitor EAV seroprevalance in the selected hucul horse stud in Poland in the 9 years period, identify persistently infected stallions and analyse genetic variability of EAV isolates emerging during persistent infection using Next Generation Sequencing (NGS).

Methods
In total 261 serum samples from 107 horses and 87 semen samples from 37 stallions have been collected and analysed between 2008 and 2017. Serum samples were tested using virus neutralization test, whereas semen samples by real time RT-PCR with primers specific to the N gene of the EAV. Six isolates from 3 persistently infected stallions were analysed using NGS.

Results
EAV specific antibodies were found in 117 (44.8%) serum samples: 71 (69.6%) from stallions and 46 (28.9%) from mares. Significant increase in percentage of seropositive animals (from 32.2% to 72.8%) was observed between the first (2008-2012) and the second (2013–2017) half of study period. Genetic material of EAV was detected in 40 (46%) semen samples collected from 7 stallions. Six full genome sequences of EAV isolates were acquired from persistently infected stallions using NGS with average coverage from 11.4x to 536.6x. Homology of consensus sequences varied from 94.97% to 99.62% in isolates originating from different stallions and from 99.4% to 99.6% for isolates acquired with 2–3 year interval from the same stallions. Multiple (84 to 237) variable sites were detected in genome of each of the EAV isolates.

Conclusion
Increase of the seroprevalance observed in the analysed herd was probably associated with the spread of the virus from persistently infected stallions. All EAV isolates were closely related to each other and belonged to EU1 phylogenetic subgroup. NGS sequencing not only allowed for the identification of whole genomes of EAV isolates but also confirmed high diversity of pseudospecies population of the virus and enabled for identification of its major genetic variants.
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Investigation on the occurrence of small ruminant lentiviruses among wild ruminants

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Background
Small ruminant lentiviruses (SRLVs) are the prototypic animal lentiviruses of domestic sheep (maedi visna virus) and goats (caprine arthritis encephalitis virus), representing the largest natural reservoir of lentiviruses. Different reports have shown that SRLV are causing natural cross species infection in wild ungulates following contacts during free grassing season in wilderness area or during the sharing of breeding areas. The aim of this study was to assess the infection with SRLV in wildlife ruminants from Poland.

Methods
Blood was collected during hunting season from 559 wild ruminants, including red deer (Cervus elaphus), roe deer (Capreolus capreolus) and fallow deer (Dama dama) from different geographical places in Poland. Each serum sample was tested by four indirect ELISAs, based on protein G-peroxidase, as conjugate and on multi-epitope recombinant antigens (SU1/Gag/SU5), representing subtypes A1, A13, B1 and B2 of SRLV, circulating in Poland (Olech et al. 2018). Cut-off values for positive samples in each ELISA were calculated on the basis of scatter plots of S/P value of tested samples, using STATISTICA software. DNA was extracted from whole blood of animals showing the seropositivity to SRLV antigens and a 625 bp fragment of gag gene was amplified by PCR, cloned into pDRIVE plasmid vector and sequenced. The resulted sequences were analysed by the Geneious Pro 5.3 software and phylogenetic tree was constructed using the neighbor-joining method.

Results
26 out 559 (4,65%) sera were classified as positive for different SRLV subtype-derived antigens. Fifteen and two sera reacted with antigen representing subtypes B1 and B2 while 10 and 8 sera reacted with antigen representing subtypes A13 and A1, respectively. Out of 26 sera only 1 reacted with all four antigens; 2 samples reacted with 3 and 2 antigens while remaining 21 samples reacted with one antigen only. SRLV-related sequence was successfully amplified from DNA of 1 red deer and it was clustered to subtype A12, with mean nucleotide genetic distance of 7,8%. Amino acid sequence of the immunodominant epitope 2 of the capsid protein differed from the reference strains by 8 aa substitutions.

Conclusion
These results may suggest the existence of new SRLVs reservoir among wildlife ruminants from Poland. This data may be useful in planning control measures to reduce the prevalence in sheep and goats and open the way for further isolation and characterization of SRLV infecting wild ungulates.

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Asinine herpesvirus-3 (Equine herpesvirus-8) associated neurological disease in a donkey

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Background
A 30-year-old female donkey with Pituitary Pars Intermedia Dysfunction (PPID) was referred to the Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht) for losing weight despite good appetite and treatment for PPID. Twelve days after returning home the mare developed nasal discharge, the next day she was found recumbent and was only able to stand up with manual assistance. The next day the mare showed severe tetraparesis, ataxia, hypotonia of anus, tail and bladder and she became completely recumbent. Equine herpesvirus myeloencephalopathy (EHM) was suspected and to confirm the diagnosis a deep nasal swab was taken. Because of the poor prognosis the mare was euthanized.

Methods and Results
The nasal swab was tested for EHV-1 using specific real-time PCRs (primers and probes according to Hussey and others 2006) with a strong positive result (Ct value of 15 corresponding with 106–107 TCID50/mL). Since this was a remarkably strong positive result for a nasal swab the DNA extraction and PCR were repeated the next day with the same result. Then virus isolation on equine dermis cells was performed and alphaherpesvirus-like cytopathic effects were observed. The virus was clearly identified as EHV-1 using EHV-1 and EHV-4 specific monoclonal antibodies (Allen and Bryans 1986) in an Immuno Peroxidase Monolayer Assay (IPMA). After a third passage on equine dermis cells 100% CPE was obtained in 2–3 days. The identity of this isolate was again confirmed as EHV-1 by testing the third passage supernatant in the real-time EHV-1 specific PCR (Ct 16.5), but sequencing identified the virus as AsHV-3 (EHV-8).

Conclusion
Equine and asinine herpesviruses can be so closely related that misdiagnosis may occur. The specificity of diagnostic reagents like MAbs and primers and probes has to be evaluated regularly taking new findings into account; since complete genome sequences are available for these closely related alphaherpesviruses primer and probe sequences used in diagnostic PCRs should be checked in silico and experimentally for cross-reactions between EHV-1, EHV-8 and EHV-9 and redesigned if necessary; the association of AsHV-3 in a donkey with EHM-like neurological disease has not been described before and adds to the spectrum of disease manifestations in donkeys due to herpesvirus infections; immune suppression due to aging and PPID may have increased the susceptibility of this donkey to AsHV-3 reactivation or infection with a high level of virus multiplication and neurological disease.

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Development of an equine myeloid cell culture system for equine infectious anaemia virus (EIAV) isolation

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Background
Equine infectious anaemia virus (EIAV) belongs to the lentivirus genus of the Retroviridae family. EIAV is the aetiological agent of equine infectious anaemia, an episodic disease that persists for the life of the host, and is a notifiable disease in the UK, the EU and towards the World Organisation for Animal Health (OIE) for international trade. Macrophages (MF) have been identified as host cells for EIAV but the technically demanding nature of isolating equine MF has led to much EIAV research being conducted using cell lines that are less susceptible and may cause more viral adaption. Therefore an approach of monocyte isolation and differentiation was implemented to apply monocyte-derived cells that best supported EIAV replication.

Methods
Monocytes were isolated from whole blood collected from healthy horses using a Ficoll gradient and purified via magnetically activated cell sorting. Monocytes were plated into a 24 well plate at a density of 1x106 cells per well for differentiation into EMDM (equine monocyte derived macrophages) and MoDC (monocyte derived dendritic cells). To drive MoDC differentiation 4µg IL-4 and 500U GM-CSF were used, to drive EMDM differentiation 10% autologous horse serum and 1µg M-CSF was used. EMDM were incubated at 37°C, 5% CO2 for 3 days and MoDC for 4 days to differentiate. The Wyoming strain was used as a control and post mortem tissues from two British cases, one asymptomatic and one symptomatic, were used as test inoculum.

Results
The differentiation of macrophages had little effect on virus replication while dendritic cells appeared to be unable to support efficient replication. Variation was seen between different monocyte isolations so the effect of single cytokines was tested, with IL-4 found to improve EIAV replication reproducibly. The Wyoming strain was successful inoculated and cultured. The system was used to successfully isolate virus from the symptomatic horse tissue however unsuccessful from the asymptomatic case.

Conclusion
A reproducible method for the isolation of EIAV was developed which allowed the successful isolation and culture of a symptomatic British field samples. However, further work is required in order to conduct isolations from a range of additional clinical sample with focus on viral isolation from asymptomatic cases.

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Molecular epidemiology of bovine viral diarrhea virus in Slovenia: Data collected over twenty years

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Background
Monitoring of bovine viral diarrhea (BVD) started in Slovenia in 1994. According to data, collected between 1996 and 2003, about 30% of breeding cattle herds was infected with BVD. Since 2014, according to the national legislation, a voluntary program for the first time allowed the certification of bovine viral diarrhea virus (BVDV) free status for cattle herds. In 1997 the first BVDV strain from Slovenian positive herd was genetically characterised as subtype 1d. Vaccination against BVD was never practised in Slovenia. The present work represents data collected within molecular epidemiological studies over the last twenty years.

Methods
Cattle serum and tissue samples, delivered into our laboratory between 1997 and 2018 for diagnosis of BVDV, were tested with different polymerase chain reaction (RT PCR) methods. Three hundred and forty three BVDV positive samples were further sequenced and genetically analyzed in 5' non-coding and Npro regions of BVD genome by direct Sanger sequencing from PCR products. The complete genomes of three representative BVDV strains were sequenced.

Results
The BVDV positive results were obtained in 1,32% of tested samples. According to the phylogenetic comparison of 343 BVDV positive samples, originated from 146 different positive herds, seven subtypes of BVD (1a=1, 1b=22, 1d=90, 1e=8, 1f=217, 1g=4 and 1h=1) were identified. The Npro region allows the further discrimination of genetically closely related BVDV strains. Three complete genomes of Slovenian BVDV isolates which are available since 2017 (1f strain SLO/1170/2000; KX987157, 1d strain SLO/2146/2002; KX577637, 1e strain SLO/2407/2006; KY849592, have 92%, 87% and 90% nucleotide homology to the closely related strains in GenBank, respectively) represent important new data regarding genetic variability of BVDV strains circulating in Europe.

Conclusion
Two predominant subtypes (1f and 1d) are present in Slovenia, with several sub lineages and clear evidence for long time persistence of the same strains of BVDV in region. In general, only one strain of BVDV was detected in infected herds and transmissions between different cattle herds are therefore evident. The identification of new subtypes (1a, 1e, 1h) is probably the result of trans-border transmission of BVDV infected animals, but these new subtypes were found only in limited number of infected herds.

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Does vaccination of pregnant cows against bovine viral diarrhea viruses confer foetal protection against HoBi-like pestivirus infection?

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Background
HoBi-like viruses are an emerging group of pestiviruses, which similar to bovine viral diarrhea virus types 1 and 2 can cause reproductive disorders in cows and establish persistent infections in their offspring. Previous studies demonstrated that very low neutralising titres are raised against HoBi-like pestivirus in animals immunised against extant BVDVs. However, it is currently unknown whether this poor cross-neutralisation in vitro reflects a lack of cross-protection in vivo. The aim of this study was to assess the foetal cross-protection induced by a vaccination against challenge with HoBi-like pestivirus.

Methods
Five pestivirus seronegative cows were vaccinated with a modified live BVDV vaccine with foetal protection claim between 32 and 106 days before artificial insemination (AI); additional four cows were not vaccinated. Seroconversion was evaluated by means of a commercial ELISA antibody test. All cows were challenged with HoBi-like strain Italy-1/10-1 between 82 and 89 days after AI. Viremia and viral shedding were evaluated on blood and nasal swabs, respectively, using a commercial ELISA antigen test, a panpestivirus real-time RT-PCR assay and a specific HoBi-like RT-PCR protocol. At birth, the same specimens from calves were collected along with ear notches, tissue samples from dead animals and vaginal swabs of the dams.

Results
All vaccinated cows seroconverted against pestiviruses, while control animals remained seronegative until the challenge. Viremia started in 3 unvaccinated cows between day post-infection (dpi) 4 and 6. Simultaneously, RNA was detected in nasal swabs of all unvaccinated cows, and in 3 vaccinated animals. One aborted foetus from an unvaccinated cow was not recovered, while the 3 control animals gave birth to virus positive, putative persistently infected (PI) calves. One vaccinated cow aborted but the foetus tested negative, and the other 3 gave birth to healthy, pestivirus-negative calves. The remaining cow delivered a virus positive calf. Vaginal swabs resulted positive in the cows that delivered positive calves. All positive samples were confirmed to contain the HoBi-like strain by means of the specific RT-PCR assay.

Conclusion
This pilot study suggests that the vaccine tested might not confer full foetal protection against HoBi-like pestiviruses, since one of the vaccinated animals generated an infected calf. A longer interval between vaccination and AI could explain the failure of foetus protection in this immunised animal. Further studies are needed to confirm these findings, thus better elucidating the potential existence of cross-protection of BVDV vaccines against HoBi-like pestivirus infection.

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Comparative analysis of bovine and caprine herpesvirus 1 infections in goats

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Background
BoHV1 is responsible for infectious bovine rhinotracheitis. Some species, including goats, are considered as potential reservoir of BoHV1. Goats are the natural host of CpHV1 virus which is closely related to its bovine counterpart. This study aimed to increase the knowledge of the behaviour of CpHV1 in its natural host and the outcome of BoHV1 infection in goats.

Methods
Two groups of animals, each composed of three non-pregnant goats and six goats at 90-days of pregnancy, were inoculated intranasally with the same infectious dose of either BoHV1 or CpHV1 reference strains. Four additional goats were mock infected and kept as negative controls. Kinetics of clinical, virological and serological features were analyzed over a period of 36 days post-infection (dpi) for non-pregnant animals, up to the expected date of parturition for pregnant animals.

Results
Seroconversion and virus excretion were detected in all virus inoculated animals. No evidence of infection was observed in negative controls. All CpHV1 infected goats showed a strong transient increase in temperature at 5 dpi, in association with slight to severe nasal discharges. Abortion occurred at different dpi in 5 out of 6 infected goats. In contrast, no clinical signs were observed in BoHV1 infected goats. CpHV1 infected goats shed virus continuously in nasal secretions from dpi 2–36, with a maximum viral load at 6 dpi. Intermittent virus shedding was detected in vaginal secretions from all of these animals, with a peak of viral excretion preceding the abortion. Virus shedding in BoHV1 infected animals was intermittent, far lower than in CpHV1 infected animals, and limited to the nasal mucosa. Neutralizing activities were correlated with the intensity of viral excretion, CpHV1 infected goats developed neutralizing antibodies earlier and at higher titres than BoHV1 infected goats. While serological patterns to the gB protein were similar between CpHV1 and BoHV1 infected goats, detection of antibody responses to the gE protein greatly differed between the two viral infections and among the blocking ELISA tests. All CpHV1 infected goats were found positive with two ELISA tests but negative with the last one. Out of the 9 BoHV1 infected goats, only 5 were found positive, 3 of them being positive with no more than one test.

Conclusion
These results confirmed that goats are susceptible to both viruses, despite evidence for virus-host co-evolution. Importantly, we showed that both infections cannot be differentiated using BoHV1-gE blocking ELISA kits, which must be considered in epidemiological studies.

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Genotyping and phylogenetic analysis of bovine viral diarrhea virus in metropolitan France

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Background
Bovine viral diarrhea (BVD) is a major pathology in bovine herds in Europe. Clinical signs of BVD include fetal death, abortions and diarrhea. BVD is caused by the bovine viral diarrhea virus (BVDV). This pestivirus is classified into two genotypes: BVDV-1 and BVDV-2. About twenty subgenotypes have been described for the genotype 1 (1a, 1b, 1c…). The three subgenotypes described for BVDV-2 are: 2a, 2b, 2c. Different countries in Europe have developed strategies for the eradication of BVD which are based on the elimination of persistently infected animals from bovine herds. However the success of those strategies depends on the efficiency of serological and molecular tools used for the diagnosis of BVD. Those tools must be in line with the epidemiologic situation of the virus on the fields. The goal of this study was to characterise the viral strains of BVDV circulating in metropolitan France.

Methods
Viral RNA was extracted from 102 sera and ear notch samples of persistently infected animals using the QIAamp Viral RNA extraction kit (Qiagen Inc.) according to the manufacturer's instructions. The samples were collected from different geographic origins (29 departments of France) during a period ranging from 2008 to 2016. Extracted viral RNA (5 µl) was analysed by RT-PCR using the kit One step RT-PCR (Qiagen Inc.), the primers 5'-ATGCCCWTAGTAGGACTAGCA-3' and 5'-TCAACTCCATGTGCCATGTAC-3' and PCR conditions designed by Vilcek et al. (1994) in order to amplify the 5' UTR region of pestivirus. PCR products were sequenced using the Sanger method. The nucleotidic sequences were analysed using the Vector NTI software. A phylogenetic tree including BVDV reference strains was established according to the neighbor joining method in order to classify the subgenogroups identified.

Results
An amplicon of 288 bp was obtained following PCR reactions. The analysis of the nucleotidic sequences obtained indicates that all the viral isolates belong to the genotype 1 of BVDV. The two dominant subgenotypes were 1e and 1b. The genotype 1d was also noticed. Only one isolate of each subgenotype k and l was identified. No evidence of BVDV-2 was found in this study.

Conclusion
The results indicate a clear dominance of the genotype BVDV-1. The absence of detection of BVDV-2 suggests a low prevalence of this strain in France although a largest screening should be considered to confirm this hypothesis. These results are important because they may help to adapt the diagnostic tools for the surveillance of BVD in France.

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Digging out a 25-years old astrovirus encephalitis case in a sheep by archive mining

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Background
In mammals, the small, positive-sense single-stranded RNA astroviruses are known as being mostly enterotropic and host-specific. In recent years, however, they were identified several times in central nervous system tissues of humans, minks, cattle, sheep and pigs with different patterns of inflammatory disease of that organ system. We lately reported such neurotropic astroviruses, amongst which bovine astrovirus CH15 (BoAstV-CH15) in two cows and ovine astrovirus CH16 (OvAstV-CH16) in a sheep. Both viruses were very close to one another on the genetic level, raising the question of potential inter-species transmission of this specific virus species. The aim of this study was to screen small ruminants' brain tissues from our archive by immunohistochemistry for BoAstV-CH15/OvAstV-CH16.

Methods
We selected 48 historical cases of small ruminants (34 sheep, 14 goats) with moderate to severe nonsuppurative encephalitis from our archive and screened formalin-fixed, paraffin-embedded brain samples of these animals by immunohistochemistry for the capsid antigen of BoAstV-CH15/OvAstV-CH16.

Results
One sheep, which died in 1992 with neurological symptoms, showed strong immunostaining for BoAstV-CH15/OvAstV-CH16 in various brain regions. Positive cells, which based on morphological criteria were exclusively neurons, were not always co-localized with the strongest histopathological lesions.

Conclusion
Even if neurological infection events appear to be rare, our study indicates that the astrovirus species BoAstV-CH15/OvAstV-CH16 or genetically similar viruses existed in Switzerland already more than two decades ago. The fact that the antibodies used (originally raised against the bovine astrovirus strain BoAstV-CH15) reacted in an ovine host underlines the close relationship of the bovine and ovine isolates of this virus species. Unfortunately, all epidemiology and pathogenesis aspects of this virus still remain unknown. This is the fourth report of astrovirus-encephalitis in sheep worldwide.

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Analysis of the serological response and intra-species and cross-species transmission after experimental infection of sheep and goats with Belgian small ruminant lentivirus strains

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Background
Maedi Visna virus (MVV) in sheep and caprine arthritis encephalitis virus (CAEV) in goats were longtime considered as two distinct species-specific pathogens. Recent phylogenetic studies however classified MVV as genotype A and CAEV as genotype B strains in the group of the small ruminant lentiviruses (SRLVs). Limited evidence is nowadays available that these viruses are able to cross the species barrier. These retroviruses are mainly transmitted vertically via milk and colostrum but horizontal transmission between animals in close contact has also been described. Data on the transmission efficiency of SRLV is however scarce. To address these knowledge gaps, we performed an experimental in vivo infection of sheep and goats with genotype A and B strains.

Methods
6 sheep and 3 goats and 3 sheep and 5 goats were intratracheally inoculated with a genotype A or a genotype B strain, respectively, that were isolated from a Belgian sheep and goat. At 4 weeks post infection, groups of 3 inoculated sheep or goats with one SRLV strain were moved to separate pens and 3 contact sheep or goats were added to evaluate potential intra- and cross-species transmission. Oral and nasal swabs and blood were collected every two weeks until 4 months post-inoculation (pi) and then every month until the end (9 months in total) of the experiment. All collected sera have been tested in the ID screen ELISA (Idvet). Leucocytes pellets and swabs were prepared to be tested in qPCR.

Results
At the time of writing (5 months pi), 14/17 inoculated animals have seroconverted. After inoculation with the genotype A strain, 5 sheep seroconverted between 6 and 16 weeks (median: 6; mean: 10). One goat also seroconverted at 9 weeks. After inoculation with the genotype B strain, 3 sheep seroconverted between 4 and 11 weeks (median: 8; mean: 7.7) and 5 goats seroconverted between 2 and 8 weeks (median: 4; mean: 4.6). No statistical differences in time to seroconversion were found between sheep and goats inoculated with a genotype A or B. Until now, no SRLV transmission to contact animals has occurred yet.

Conclusion
These preliminary results confirm that genotype A and genotype B strains can cross the species barrier and no clear difference in infection efficiency can be deduced from the observed time to seroconversion. Furthermore, the current absence of SRLV detection in contact animals suggests an inefficient horizontal transmission under experimental conditions. Further analyses are currently ongoing.

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Detection of PPR virus nucleic acid in the body excretions of experimentally infected goats

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Background
Peste-des-petits ruminants virus (PPRV), causes an acute contagious disease of small ruminants and places a huge disease burden on agriculture in many developing countries. The morbidity rate can reach 100% with a high case fatality rate in the acute form of disease. The clinical signs and mortality can vary considerably depending on the virulence of the viral strain. During the acute stage of disease, animals show pyrexia (up to 41°C) that may last for 3–5 days and that can be accompanied by depression, anorexia and dryness of the muzzle.

Methods
In this study, the presence or absence of PPR nucleic acid was evaluated in a variety of body excretions (ocular, nasal, oral and faecal material) in experimentally infected (intra-nasally) goats (n=14). Viral material was measured with real time RT-PCR.

Results
PPRV disease progresses with the development of lacrimal, nasal and mucosal discharges and viral nucleic acid was detected in excretions in experimentally infected goats as early as 4 days following infection using various molecular and virological techniques.

Conclusion
The excretion of PPR virus in different body excretions may help to determine out the most infectious period of infection that may pose a threat of transmission of the disease to in contact animals.

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An investigation on the molecular epidemiology of Neboviruses in Turkey

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Background
Caliciviruses are important viruses of humans and animals. In cattle, at least three distinct genera (Nebovirus, Norovirus, and Vesivirus) of caliciviruses have been detected but only neboviruses and noroviruses have been associated with enteric replication and with enteric symptoms, on the basis of either experimental infections or observational studies. The aim of this study was to investigate the prevalence and genetic characterization of nebovirus in diarrheic calves in Turkey.

Methods
In this study, we tested 167 fecal samples from diarrheic calves housed in family (n=48) and organized (n=11) farms located 10 different provinces between 2002 and 2016 for the presence nebovirus. For this purpose, viral RNA was extracted by using Trizol LS Reagent and then Nested-PCR targeting RdRp gene region was carried out. After that, the complete capsid gene of detected neboviruses was amplified by RT-PCR by using the specific primer pairs. The sequences were compared with cognate sequences available in GenBank by using the MEGA 6.0 software.

Results
Among the 167 samples from calves, 37 were positive for nebovirus. Nebovirus positive samples were obtained from calves housed in 7 organized and 11 family farms. Phylogenetic analysis of RdRp gene region of nebovirus indicated that all sequenced neboviruses (n=20) were related to the reference strain Bo/Nebraska/80/US. However, using the primer targeting complete capsid gene region only two Nebovirus strains were able to be amplified. These strains were clustered in lineage 3 and 4 in the phylogenetic tree. According to the Simplot analysis, the strain in lineage 4 was determined as a recombinant strain.

Conclusion
As a result, neboviruses were designated having an important role in new-born diarrhea alone or along with other enteric pathogens for both small and organized farms. Also, this study is the first to show the presence of nebovirus in diarrheic calves along with the recombinant strains of neboviruses in Turkey.

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Detection of viral agents associated with respiratory disease in calves

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Background
The bovine respiratory disease complex (BRDC) is associated with several viral pathogens such as bovine respiratory syncytial virus (BRSV), bovine herpesvirus type-1 (BHV-1), parainfluenza virus-3 (PI-3), bovine coronavirus (BCoV), and adenovirus (BAV), etc. and some bacterial agents. Whereas BRSV infection could mostly cause is a mild disease in adult cattle, it could be quite severe in calves and even leads to outbreaks and deaths. Therefore, the molecular characterization of causative agents is important for the understanding of its epidemiology and the production of a biological product such as a vaccine. In this presentation, it is aimed to detect and characterize possible causative agents of respiratory disease in calves.

Methods
In this study, nasal swabs samples from 28 calves at different ages, with respiratory disease symptoms, were examined for possible etiological agents such as BRSV, BAV, BCoV, BHV-1, and PI-3 virus. After the extraction of viral nucleic acids by using Trizol LS protocol, PCR and RT-PCR were carried out by using suitable primer pairs for each pathogen. Moreover, positive samples for BRSV were analyzed for the G gene region. PCR products with expected size were sequenced and the phylogenetic tree was constructed using MEGA 6 Software.

Results
Out of 28 nasal swab samples, 7 for BRSV, 2 for BCoV and 1 for BAV were detected as positive, while they were negative for other tested pathogens. Currently, the molecular characterization was successful for only BRSV samples. BRSV positive samples were subjected to RT-PCR of G gene for the detection of subgroup and three samples were amplified and sequenced. The phylogenetic tree based on the sequencing data showed that our BRSV strains are clustered in genetic subgroup III.

Conclusion
As a conclusion, BRSV, BCoV, and BAV were determined as the viral causative agent, in acute respiratory disease, along with or without other possible agents which were not investigated in this study. Further studies on the isolation and molecular characterization of field viruses are likely to provide a more detailed data on the molecular epidemiology of BRDC and the choice of vaccine to control the infection.

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Characterisation of a first bovine parainfluenza virus 3 isolate detected in cattle in Turkey

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Background
Viruses and bacteria in combination with stress factors have an important role in bovine respiratory disease and the febrile diseases induced are commonly known as "Shipping Fever". The Bovine respiratory diseases complex (BRDC) is a major health problem for cattle worldwide. It consists of bovine parainfluenza virus type 3 (BPIV-3), bovine herpes virus type-1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), all leading to severe respiratory disease in cattle. BPIV-3 is an enveloped, nonsegmented, negative sense, single stranded RNA virus belonging to the Respirovirus genus in the family Paramyxoviridae. In this study we report (i) the first isolation of BPIV-3 from cattle in Turkey and (ii) present the results of sequence and phylogenetic analysis of the complete BPIV-3 genome.

Methods
A respiratory disease outbreak on a cattle farm in Northern Turkey presented with respiratory tract symptoms and severe pneumonia symptoms among 20 calves. Eight calves died and lung specimen of one carcass were analysed for bacteria and for viruses of the Bovine respiratory diseases complex. Virus isolation was performed on MDBK cells and subsequent whole genome sequencing and phylogenetic analysis were conducted. A BPIV-3 RT-PCR positive sample of passage 4 was sequenced using Illumina chemistry and the MiSeq platform.

Results
Microbiological analysis was negative but antigen detection ELISA and RT-PCR results indicated the presence of Bovine parainfluenza virus (BPIV). Virus isolation succeeded on MDBK cells and subsequent whole genome sequencing and phylogenetic analysis identified BPIV-3c. Sequencing resulted in 1,860,581 raw reads. After filtering 1,033,799 reads remained. The mean coverage of the 15,504 nt long scaffold was 4161.37 nt. Phylogentic analysis clearly placed the sequence of the Turkish BPIV-3 isolate in the genotype C group.

Conclusion
In summary an investigation into a case of respiratory disease in calves in the Ordu Province identified a first BPIV-3c isolate from Turkey. The overall epidemiology of BPIV-3 in Turkey needs to be investigated to be able to assess if vaccines should be introduced.

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Molecular investigation of the relationship between vector tick and host in lumpy skin disease

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Background
Lumpy skin disease (LSD) is an acute disease among cattle characterised by high fever, nodules on the skin, enlargement of superficial lymph nodes, salivation, lachrymation, rhinitis and keratitis. The disease is endemic in many African countries. Since 2000, it has spread to the Middle East, was confirmed in Turkey in 2013, and is now prevalent in many countries in the European Union. The virus is a member of the Capripoxvirus genus within the Chordopoxvirinae subfamily of the Poxviridae family. All cattle breeds and age groups are susceptible; however, the disease is more severe in young animals and cows in the peak of lactation. Hematophagous insects have been primarily associated with the transmission of lumpy skin disease virus (LSDV). Ticks have recently been implicated in the transmission of LSDV like other arboviral diseases. Currently, there is no more recorded data regarding the distribution of LSDV between vector tick and host. The aim of this study was to investigate the distribution of LSDV between vector tick and host (cattle, sheep and goat) in the provinces of northern Turkey.

Methods
In this study, the hard ticks and serum samples collected from ruminants (cattle, sheep and goat) in middle Black Sea region of Turkey (Samsun, Tokat Sivas). All studies animals were clinically healthy in the time of sampling. A total of 2508 ticks were collected between March and July of 2016 and 2017 from 509 sheep (530 pools), 93 cattle (97 pools) and 106 goat (118 pools) grazing in middle Black Sea region. In addition, a total of 708 blood samples were collected from same animal and all samples were analysed for the presence of LSDV DNA using a real-time polymerase chain reaction (rPCR).

Results
Nine tick species were identified and the most abundant were Haemaphysalis sulcata 28.9% (724/2508) and Rhipicephalus turanicus 28.7% (719/2508). No LSDV genomic DNA was found in tick and blood samples.

Conclusion
Turkey has experienced outbreaks of LSDV infections in cattle every year since 2013. From 2013 to 2015, an extensive epidemic occurred, starting in the southeast and then spreading over the whole country, resulting in many outbreaks. Although experimental transmission of LSDV between hosts may occur via ixodid tick vectors, we did not detect any evidence in our field studies. To understand LSDV transmission in nature better, additional studies focusing on major vectors are needed.

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A serological investigation of lumpy skin disease and Crimean-Congo Hemorrhagic Fever in domestic water buffalo (*Bubalus bubalis* Linneaus, 1758) in Samsun Province of Northern Turkey

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Background
Lumpy skin disease (LSD) is endemic in many African countries. Since 2000, it has spread to the Middle East, was confirmed in Turkey in 2013, and is now prevalent in many countries in the European Union. The virus is a member of the Capripoxvirus genus within the Chordopoxvirinae subfamily of the Poxviridae family. Crimean-Congo hemorrhagic fever virus (CCHFV) which is the Orthonairovirus strain of Nairoviridae. Currently, the presence of CCHF virus has been reported in many countries of Asia, Africa, the Middle East and Europe. The objective of this study was to investigate the Lumpy Skin Diseases (LSD) and Crimean Congo Hemorrhagic Fever (CCHF) infections as serologically in domestic water buffaloes (*Bubalus bubalis* Linneaus, 1758) in Samsun, Bafra province, Turkey. Also, another aim of this study was to investigate the efficiency of commercial capri pox vaccine against Lumpy skin disease virus (LSDV) in water buffaloes in northern Turkey.

Methods
A total of 272 buffalo's sera (33 males and 239 females) from different age groups were randomly sampled. In this study, sampled 132 buffaloes were vaccinated with capripoxvirus to protect against LSD. The study was conducted during the period from January 2017 to March 2018 to determine the antibody response against commercially available heterologous capripox (SPV-Bk: 107.5 TCID50/dose) vaccine. The efficiency of commercially available capripox vaccine against Lumpy skin disease virus (LSDV) and CCHF and LSD seroprevalences was evaluated on the basis of the antibody response surveyed by enzyme-linked immunosorbent assay (ELISA).

Results
In this study, a total of 272 buffaloes were tested by ELISA. All animals were negative for antibodies against CCHFV. In addition, all unvaccinated animals (n: 140) were negative for LSDV. However, as a result of ELISA test, out of vaccinated 132 buffaloes, ten (7.57%) were found to be seropositive for LSDV.

Conclusion
The results indicate that most of vaccinated buffaloes did not developed antibodies after the vaccination for LSDV. The results of our study dictate clearly that the control of commercial vaccine and vaccination strategy have to be evaluated by official Veterinary authorities immediately to combat the LSDV infection, since it was first reported 5 years ago in Turkey, yet threatening severely the population of large ruminants concerned.

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Serological evidence of the first and newly isolated genotype C bovine parainfluenzavirus type-3 in archived ruminant sera in Turkey

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Background
Bovine parainfluenza 3 viruses (BPIV-3) is one of the most important respiratory pathogens, leading cause of serious respiratory system illness in cattle as single or associated with other pathogens taking part in bovine respiratory disease complex (BRDC). The objective of this study to investigate whether antibodies against the genotype C bovine BPIV-3 (BPV-3c) which first isolated in Turkey, in randomly selected serum samples among archived ruminant sera that collected from in Northern Anatolia region of Turkey between 2013–2014.

Methods
A total of 772 sera consisting of 442 cattle, 330 sheep, and 124 goats were screened by using standard virus neutralization test.

Results
Overall seropositivity rate was 21.09%. Seropositivity rates for cattle, sheep and goat were determined 21.04%, 20.00%, and 24.19%, respectively. Antibody titers were ranging from 1/4 to 1/512 for both cattle and sheep whereas it was between 1/4 and 1/256 for goat.

Conclusion
The results indicated that BPIV-3c was in circulation in the past even though the first and newly isolated in Turkey.

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First molecular characterization of bovine papular stomatitis virus in Turkey

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Background
Bovine papular stomatitis virus (BPSV) is a parapoxvirus belongs to the family Poxviridae associated with papular and erosive lesions (painful reddish papules, ulcers, and scabby proliferative) on the muzzle, lips, and oral mucosa of cattle and also zoonotic disease. Although affects all age groups of a ruminant, the infection is frequently transmitted to human beings.

Methods
The present report describes BPSV infection affecting two calves in Erzurum province, Eastern Turkey and molecular characterization of the detected virus. Viral DNA was extracted from lesions and oral swabs. Polymerase chain reaction was performed using a set of pan-parapoxvirus primers for the partial B2L gene of BPSV and obtained approximately 590-bp PCR product.

Results
The sequencing results of BPSV study strains showed that nucleotides similarities of 99.6–95.6% with GenBank BPSV strains, 84.7–83.6% with Pseudocowpox virus strains and 83.6–82.1% with Orf virus strains. A phylogenetic tree based on the partial B2L sequence was constructed, showing that the virus clustered with BPSV isolates.

Conclusion
Although clinical cases of parapoxviruses (BPSV, Pseudocowpox, and Orf virus) have been previously reported, the present study reveals first-time molecular characterization of the BPSV in Turkey. And awareness of parapox infections, which are zoonotic, is increased as they are all molecularly characterized in our country.

Keywords
Bovine papular stomatitis virus, molecular characterization, Turkey

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Sacrifice Feast Disease (Ovine gammaherpesvirus-2) in cattle in Erzurum, Turkey

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Background
Ovine Herpesvirus-2 (OvHV-2) is a gamma-herpesvirus that belongs to genus Macavirus, and endemic in sheep throughout the world. The virus infects sheep sub-clinically but when it transmitted to cattle, induces malignant catarrhal fever (MCF) which is frequently fatal lymphoproliferative disease. It is important to keep cattle and sheep together in the pathogenesis of the disease. Muslim countries (such as Turkey), the Feast of Sacrifice is celebrated every year. During the festival time, keep all animals in the same regions together for 4 days. It is always possible to occur MCF disease after the festival.

Methods
Two months after the Feast of Sacrifice celebration in 2017, MCF suspects cattle (2 years old, male) was brought to our hospital in Erzurum, Turkey. We investigated the MCF presence by PCR in blood and ocular swabs samples using ovine herpesvirus-2 primers for the partial tegument protein gene and approximately 380-bp positive amplicon was characterized.

Results
A phylogenetic tree based on the partial tegument protein gene sequence was constructed, showing that the virus clustered 2 branches in all OvHV-2 strains. The result revealed that the study strain more similar on nucleotide level with India, Egypt, Iraq strains than Germany, Canada, Brazil, Norway, South Africa and previously reported Turkish strains.

Conclusion
This study was revealed for the first time OvHV-2 molecular characterization in cattle in Erzurum province (Eastern Anatolia) of Turkey. The new question is: Will this disease be triggered after every sacrifice feast in our country?

Keywords
Ovine Herpesvirus-2, molecular characterization, Turkey

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Detection and molecular characterization of feline alphaherpesvirus 1 in Erzurum, Turkey

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Background
Feline Respiratory Diseases (FRD) are common clinical and animal welfare problem in cats throughout the world. Feline alphaherpesvirus 1 (FeHV-1) and Feline calicivirus (FCV) are the main pathogens of respiratory diseases in cats.

Methods
In this study, oropharyngeal and conjunctival swabs and blood sample were taken in a cat with clinical signs of FRD. Polymerase chain reaction (PCR) and Reverse transcription (RT)-PCR were used in this case to determine the FeHV-1 and FCV infection.

Results
The result of PCR analysis revealed that FCV was negative; however, FeHV-1 was positive. Clinical findings of this cat included stomatitis, gingivitis, corneal ulcer, corneal edema and corneal vascularization. Laboratory diagnosis was made molecular level. This study was consistent with studies previously reported from Turkey.

Conclusion
This is the first molecular study on FeHV-1 in Erzurum province of Turkey. We think that FeHV-1 prevalence in infected cats will continue to increase year by year and in different geographical areas of Turkey.

Keywords
Feline Herpesvirus-1, molecular characterization, Turkey

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Molecular diagnosis of respiratory viruses in Turkish cattle

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Background
Bovine respiratory infections are responsible for major economic losses both in beef and dairy production worldwide. The most common viral agents implicated in the respiratory complex of cattle include Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza-3 (BPIV-3), Bovine Viral Diarrhea Virus (BVDV) and Bovine Herpesvirus-1 (BoHV-1). Though viral respiratory system diseases in cattle are common, the prevalence and incidence of the viral agents may vary in territories as well as regions.

Methods and Results
In this study, we aimed to determine the presence of bovine respiratory viruses (BRSV, BPIV-3, BVDV and BoHV-1) by polymerase chain reaction (PCR) in nasal swab (n=133) and lung samples (n=60) collected from total of 196 cattle in Turkey. Gene-specific primers were used for identification of BRSV (fusion gene), BPIV-3 (hemagglutinin-neuraminidase gene), BVDV (5' UTR) and BoHV-1 (gI gene). As a result of RT-PCR, viral RNA of BRSV, BPIV-3 and BVDV were identified detected in 5 (2.6%; 5/196), 1 (0.5%; 1/196) and 5 (2.6%; 5/196) samples, respectively. In addition, 2 (1.0%; 2/196) samples were positive for BoHV-1. Multiple infections were not detected in the same animal.

Conclusion
Requested viruses are circulating in Turkish cattle populations. Continuing genetic analysis on the detected strains may improve the knowledge on the epidemiology of these infections in Turkey.

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Isolation and characterization of BoHV-1.2 in Turkish cattle

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Background
Bovine herpes virus type 1 (BoHV-1) is an important viral agent for cattle production. Despite many countries have started eradication program there is no official control program applied in Turkey. Although presence of BoVH-1 is previously reported, there is no early study describing genetic characterization of BoHV-1 isolates from Turkey.

Methods and Results
Serum and whole blood samples from a total of 36 male cattle were submitted for possible lumpy skin disease (LSD) diagnosis. Additional nasal swab sample from one animal having respiratory signs was also submitted. Samples screened by PCR for the presence of LSD virus produced negative results. Further screening for bovine viral diarrhea virus by a commercial Ag-ELISA system was also resulted negative. Then the samples were inoculated onto MDBK and SFT-A cell cultures for 3 blind passages. One whole blood sample (sample ID: 8587) and the swab sample (ID: 8640) produced cytopathogenic effect in both cell culture system. The positive results obtained for these two isolates in BoHV-1 ELISA were confirmed by PCR. Thus, detected two isolates were recorded as BoHV-1 field strains from Turkey. Additionally, strains 8640 and 8587 were further identified by serum neutralization assay using positive serum produced by hiperimmunisation of rabbit with BoHV-1 Cooper strain. Isolate 8640 was selected for sequencing. Molecular characterization described this isolate to be in the cluster of BoHV-1.2.

Conclusion
According to available search on the web, this is the first data from Turkey to characterize BoHV-1 field isolates after cell culture propagation, and also the first report for the occurrence of BoHV-1.2 infections in Turkish cattle. Further field studies may help understanding BoHV-1 epidemiology in Turkish cattle population in order to develop national strategies for control of the infection.

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Possible causative agent in calf diarrhea: Aichivirus B

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Background
Kobuviruses are small round viruses with a non-enveloped capsid and a single strand positive-sense RNA of about 7.4 kb and form a large genus within the family Picornaviridae. The members of this genus infect a wide range of mammals including humans and frequently cause asymptomatic or mild infection with diarrhea. In this study, we aimed to investigate the presence and molecular characterization of Aichivirus B (formerly Bovine Kobuviruses) in calves with or without diarrhea in Turkey.

Methods
In this study, fecal samples from 71 calves were tested for bovine Aichivirus B. For this purpose, viral RNA was extracted by using Trizol LS Reagent and then RT-PCR targeting 3D (RdRp) gene region was carried out. After that, all positive samples were subjected to RT-PCR by using the primers targeting the VP1 region. The expected sized amplicons were sequenced. The partial VP1 region sequences were compared with cognate sequences available in GenBank by using the MEGA 6.0 software. The data was also interpreted with previous results obtained from other studies, which were on the other enteropathogens such as BRV, BCoV, and Cryptosporidium by us, conducted with same samples.

Results
Of the 51 samples from diarrheic calves 30 were found to be positive for Aichivirus B by RT-PCR. All the non-diarrheic calves samples were negative for Aichivirus B. Only two samples were able to be amplified for VP1 region. According to the sequence and phylogenetic analyzes of VP1 region, they were clustered in lineage 1. When the results of this study and our previous studies were examined together, it was observed that the Aichivirus B was present in diarrheic calves often with other pathogens such as rotavirus and cryptosporidium.

Conclusion
As a conclusion, Aichivirus B could be an important pathogen in neonatal calf diarrhea cases along with or without other possible agents which were not questioned in this study. Further studies on the detection and the molecular characterization of circulating Aichivirus B are likely to provide a more detailed data of the molecular epidemiology of Aichivirus B infection and its pathogenic importance in new-born calf diarrhea.

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Inactivation of foot-and-mouth disease virus in tissue samples to ensure safe transport from infected premises to diagnostic laboratories

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Background
Foot-and-mouth disease (FMD) is a major concern to livestock industries around the world. During an outbreak in an FMD-free country, provincial/state labs will handle samples, including epithelium from suspect lesions, which may contain high concentrations of infectious FMD virus (FMDV). Such samples present a biosafety risk, therefore treatment to inactivate virus prior to transport is important. The objectives of this project were to test the ability of RNA preservation reagents to inactivate FMDV in epithelium samples and ensure suitability of the FMDV RNA for RT-qPCR, sequencing, and recovery of infectious virus by transfection.

Methods
Lesion material was recovered from cattle infected with FMDV A IRN/22/2015 or O ALG/3/2014 (2 per serotype). Pieces of lesion epithelium of approximately 20–25 mm^2 were placed in 3 ml of either RNAlater, RNAShield or phosphate-buffered saline and incubated at RT for 2, 6, 24 or 48 h. After incubation, tissues were homogenised and the supernatant stored at –80˚C, with an aliquot used for virus isolation (VI) and RNA extraction on the same day. VI-positive samples were titrated, and extracted viral RNA was quantified by RT-qPCR, used for sequencing and transfected into LFBK-$\alpha V\beta 6$ cells to recover virus.

Results
RNAlater did not reduce serotype A virus titres after 2 or 6 h, however a 4 log10 reduction was seen after 24 h, and no infectious virus was recovered after 48 h incubation. While serotype O virus was detected following VI after 2, 6 and 24 h, titration yielded no infectious virus, likely as a result of freeze-thawing on the integrity of this strain. This was confirmed by titration of the 48 h sample fresh and following one freeze-thaw cycle. RNA loads were slightly reduced, particularly after 24 and 48 h. RNAshield was toxic to cells at high concentrations but was effective at inactivating both serotypes. A significant reduction of detectable viral RNA was observed in samples after 2 or 6 h incubation, but not following longer incubation periods. Transfection of FMDV RNA and recovery of infectious virus was possible for both serotypes regardless of reagent used or inactivation period. Samples incubated in any of the three reagents for 24 h allowed strain identification by sequencing.

Conclusion
Of the two reagents tested, RNAShield appears a better choice for inactivation of FMDV in tissue samples, however at least 24 h incubation is recommended before processing to ensure virus inactivation and preservation of the majority of viral RNA.

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Discovery of diverse astroviruses in neglected ruminant species

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Background
Astroviruses (AstV) are +(ss)RNA viruses with a genome length of 6.8 kb which includes three open reading frames (ORF). Enterotropic astroviruses are best known for causing diarrhoea in humans and are also found in many other mammals; in those, the relevance in gastroenteritis remains unclear. Recently described neurotropic astroviruses showed associations with encephalitis in humans as well as in other mammals. In our lab we identified neurotropic astroviruses in cattle, bovine astrovirus CH13 (BoAstV-CH13) and -CH15 (BoAstV-CH15), as well as in a sheep, named ovine astrovirus CH16 (OvAstV-CH16). OvAstV-CH16 shows high similarity to BoAstV-CH15. The aim of this ongoing study is to investigate the potential shedding of these neurotropic astroviruses in stool of ruminants other than cattle and to discover and analyze unknown astroviruses.

Methods
We extracted RNA out of 172 stool samples from sheep, goats, alpacas and deer to screen them for astroviruses. For the examination of the neurotropic astroviruses OvAstV-CH16/BoAstV-CH15 and BoAstV-CH13, we used quantitative real-time RT-PCR assays. To discover unknown astroviruses we used a heminested RT-PCR with degenerated pan-astrovirus primers, which is known to detect a 450nt long sequence at the 3'end of ORF1b of various astroviruses. Positive tested samples were Sanger-sequenced and, after confirmation of genetic similarity to known astroviruses, submitted to next generation sequencing (NGS). After quality trimming, host sequences will be – if the host genome is available – removed and the remaining data will undergo de novo assembly. The generated sequences will then be compared to nucleotide and amino acid databases, virus properties identified and phylogenetic analyses performed.

Results
All tested samples were negative for BoAstV-CH13 as well as for BoAstV-CH15/OvAstV-CH16. On the other hand 22 of 172 samples were tested positive in the heminested RT-PCR. Sanger-sequencing of the amplicons showed similarity to a broad panel of different astroviruses from diverse species, which we aim to investigate further using NGS.

Conclusion
We have found a diverse range of astroviruses in fecal samples of sheep, goats, alpacas and deer. To gain new insights into the fecal sheeding potential of these viruses in ruminants we will sequence the stool samples that scored positively by RT-PCR using NGS, aiming to recover full-length viral genomes and perform a phylogenetic comparison of the generated sequences with other known astroviruses. This study enables the so far first description of astroviruses in goats and alpacas and also the investigation of new genetic variants of multiple astroviruses in different ruminants.

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Genetic characteristics of BVDV strains occurring in Poland in 2016–2018

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Background
Bovine viral diarrhea virus (BVDV) belongs to the Flaviviridae family and the Pestivirus genus. BVD virus is widespread; it is considered one of the main causes of economic losses in cattle breeding. BVDV infection has been detected in many other species of domestic and wild animals. The clinical consequences of infection are widely manifested. The BVDV genome consists of RNA single strand with positive polarity and a length of about 12.3 kb with one open reading frame. The rate of pestivirus mutations is similar to those observed in other RNA viruses, which promotes the formation of further subtypes of the virus. Two species have been described so far: BVDV-1 and BVDV-2 containing many subtypes. Phylogenetic analyzes of pestiviruses are carried out most often using the sequences of 5'UTR, Npro, and E2 regions. In previous years, the dominant subtypes circulating in Poland were 1b and 1d, less frequently 1f and 1g. BVDV-2 was detected on one farm only in 2013. Vaccines used in Poland are based on subtypes 1a and recently also on 1b and BVDV-2. The subtype 1a has not been identified in Poland so far.

Methods
The test material was bovine serum collected in the years 2016–2018. Extraction of total RNA was performed by modified method of Chomczyński. Transcriptor One-Step RT-PCR Kit (Roche) was used for the RT-PCR reaction. Amplification was performed using specific primers within the 5'UTR region. Additionally, selected strains were amplified in the Npro and E2 regions. Phylogenetic analyzes were performed using the BioEdit, MEGA5.2 and DnaSP6 programs.

Results
BVDV was detected in 24 herds in Poland. Four subtypes of BVDV-1 have been identified: 1f in 9 herds, 1b in 8, 1g in 4 and 1d in three herds. No BVDV-2 or atypical pestiviruses were found. Only single subtypes were present in positive herds. In five herds vaccinated with the killed vaccine field viruses of different subtypes (1b and 1d) we identified. Strain 1a used in killed vaccine shows 83–90% identity within the 5'UTR region and approximately 71% in E2 region when compared with Polish field isolates. The 1b vaccine strain representing another vaccine has high homology only with homologous subtypes.

Conclusion
BVDV evolves in Poland and different subtypes are predominant compared to previous studies. Vaccine usage analysis indicates that effective cross-protection against non-homologous subtypes is hard to obtain.

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Molecular characterization of Italian strains of bovine leukaemia virus

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Background
Enzootic Bovine Leukosis (EBL) is an infectious disease now eradicated in many European countries but still present in Italy. While being underestimated, its economic impact is strong in territories. Phylogenetic analysis of the virus responsible for Enzootic Bovine Leucosis (BLV), conducted on the env gene region, has shown that isolates can be grouped into at least ten genetic groups. This classification is based on sequence data available in the databases including sequences from America, Asia and Australia and is therefore indicative of BLV strains circulating in the bovine populations of those continents. There is inadequate study of the genetic characterization and classification of BLVs isolated from infected animals from Europe. For this reason a phylogenetic study was implemented to analyse the sequences of BLV strains isolated in Italy.

Methods
Proviral DNA was extracted from buffy coat or infected organs of 27 samples collected between 2012 and 2016; it was used for the genotyping of a portion of env using a nested PCR retrieved from literature. PCR products were purified and sequenced. Our sequences have been aligned with others present in GenBank (400 bp). The phylogenetic trees were constructed using two algorithms available in the MEGA v.6 package: Maximum-Likelihood, with bootstrap analysis conducted on 1000 replicates with Kimura 2-parameter model of nucleotide substitution with gamma distribution (K2+G), and Neighbor-Joining with bootstrap analysis conducted on 10000 replicates, using a Kimura 2-parameter model; the tree was fixed on the G5 genotype in either case. Also a Bayesian (BI) inference analysis, with GTR+G substitution model, had been conducted.

Results
Our results indicate that the samples analyzed clustered into known genotypes: G2, G4, G6, G7, G8; only G7 had previously been described in Italy.

Conclusion
This study throws the basis for a more extensive analysis that aims to cover a larger time span and to investigate the evolutionary relations between isolates.

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Prevalence and molecular epidemiology of bovine norovirus in Swedish dairy calves

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Background
Bovine norovirus (BNoV) has been detected in feces from diarrheic calves and shown experimentally to cause diarrhea in young calves (1). The objectives of this study were to determine the prevalence in young dairy calves and compare sequences of the detected strains.

Methods
Five milk-fed calves per herd were sampled in 76 herds. Dairy herds in 5 geographic regions were sampled in 2005–2007 (study 1) and in 2 of the regions a different selection of herds were sampled in 2009–2010 (study 2). If available, calves up to 30 days old were sampled. Samples were analyzed by RT-PCR with primers J11U-F and CBECU-R (2), and specific amplicons were confirmed by sequencing. Thirty-six partial sequences of the polymerase gene (563 nt) were phylogenetically compared.

Results
Fifty of 250 calves (20%) sampled in 24 of the 50 herds (48%) in study 1 were positive for BNoV. Thirty-one of 130 calves (24%) sampled in 13 of the 26 herds (50%) in study 2 were BNoV-positive. Median age of the calves sampled in study 1 was 14 days, in study 2 21 days.
Most of the Swedish strains clustered with genogroup GIII.2 (Newbury2-like), while 9 strains clustered with genogroup GIII.1 (Jena-like). Nucleotide homology of the Swedish strains within cluster was 85–100% (GIII.2) and 80–96% (GIII.1) and nt homology with the reference strain within cluster was 88–91% (N2) and 84–89% (Jena). Temporal and geographic clustering was seen for some strains.

Conclusion
BNoV infection is common in milk-fed Swedish dairy calves and is a likely contributor to the neonatal calf diarrhea complex. BNoV strains belonging to both genogroups were detected, but Newbury2-like strains dominated.

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Carriage of bovine respiratory syncytial virus and bovine coronavirus in human nasal cavities and skin after exposure to virus

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Background
Bovine coronavirus (BCoV) and bovine respiratory syncytial virus (BRSV) are endemic pathogens in cattle populations worldwide. The relative contribution of different modes of transmission are unknown, and the role of humans as passive vectors are not determined. The aim was, therefore, to study the time span for detection of viral RNA and infective virus particles in nasal human mucosa and skin following exposure.

Methods
Virus presence in the human nasal cavity was investigated during two separate experimental infections of calves and samples were collected from personnel after contact with the calves. Viral RNA was quantified by RT-qPCR for BCoV and by droplet digital RT-PCR (RT-ddPCR) for BRSV and samples with highest RNA load were tested for virus infectivity in cell culture.
In order to study infectivity of BCoV on skin, human skin was experimentally contaminated. Personnel (n=8) applied a BCoV suspension on five marked areas on the dorsum of one hand, and the areas were swabbed after 15, 30, 60, 120 and 240 min, respectively. The infectious titre was estimated by use of a TCID50 assay.

Results
Human nasal cavity: For BCoV, 46% (n=80) of the swabs collected after 30 min of exposure were positive in RT-qPCR. After two hours, 15% were positive, after four hours, 5% were positive and all were negative after six hours. Infective BCoV were not detected. For BRSV, 35% (n=26) of the nasal swabs were positive by RT-ddPCR 30 minutes after exposure, but infective virus was not found.
BCoV on human skin: TCID50-results showed that the infectivity declined by time, however, after four hours the mean virus titer was reduced by only one log.

Conclusion
The results showed that both BCoV and BRSV RNA can be carried in the nostrils of humans after exposure to virus shedding calves, but the study could not confirm infectivity. For BCoV, virus on human skin remained infective for several hours.

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Concentration of viral RNA in serum of BVDV infected cattle

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Background
Bovine viral diarrhoea is one of the most economically important diseases of cattle worldwide. BVD virus causes either acute transient or persistent infection. Persistently infected (PI) animals develop after in utero exposure of foetuses to ncp biotype of BVDV before it is immuno-competent. This leads to a lack of recognition of viral antigens by the host immune system and continual production of virus to high titres throughout the life of affected animals. PI animals are the major source of the infectious virus for their herdmates and must be removed from herds to control BVDV infection.

Methods
A total of seventy seven serum samples from BVDV infected animals were tested to quantify BVDV RNA. Viral RNA was extracted from 140 µl of serum samples using QIAamp Viral RNA kit (Qiagen). A low concentration of reference virus (Canine coronavirus, CCoV) was artificially added to the serum samples before RNA extraction. Mean Cq value for reference virus, determined in eighty spiked serum samples, was calculated. Only serum samples with Cq value for reference virus within the range of mean Cq±1SD were chosen to quantify BVDV RNA.
Sequences of primers and probe were selected from 5'UTR of the BVDV genome. The reverse transcription and PCR was performed in a single tube using QuantiTect Probe RT-PCR kit (Qiagen).

Results
BVDV RNA copy numbers from $2.2\,10^6$ to 7.4×10^8 per ml were detected in serum samples of PI animals and 6.6×10^4, 1.8×10^7 and 3.3×10^7 in three acutely infected animals.

Conclusion
BVDV serum concentration of transiently infected (TI) animals can reach a similar level as in PI animals and one cannot accurately distinguish between TI and PI animals just from a single blood sample. The minimum viral loads of all PI animals in our study did not decrease below 10^6 viruses in one millilitre of serum samples.

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Blocking enzyme-linked immunosorbent assay for the detection of the antibodies specific to Akabane virus

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Background
Akabane virus (AKAV) is an arthropod-borne virus belonging to the Simbu group of the genus Bunyavirus in the family Bunyaviridae. The virus is transmitted by mosquitoes or biting midges such as Culicoides spp., or both and cause diseases of abortion, stillbirths and congenital abnormalities in cattle, sheep and goats. Theses outbreaks have been reported in South Korea, Japan, Australia and Middle East. In this study, blocking enzyme-linked immunosorbent assay (b-ELISA) using a recombinant nucleocapsid (N) protein and monoclonal antibody (MAb) was developed and validated for its specificity.

Materials and Methods
The antigen used for b-ELISA was prepared from the culture fluid of Vero cells infected with AKAV, Korean isolates, 93FMX strain. The purified AKAV was appropriately diluted and aliquots of 50 ul were dispensed into all wells of an ELISA plate. Three Mabs to AKAV was prepared in the APQA, and used for this experiment. The subtypes of the Mabs were determined by using a mouse monoclonal subtyping kit. Six Mabs were used to determine the subtype and blocking characterization.

Results
Antigen binding of the peroxidase conjugated Mabs was blocked efficiently by the 5B56 Mab.
The test was validated by using 207 sera in the b-ELISA compared to a commercial kit based on the b-ELISA using the inactivated virus antigen. The sensitivity of ELISA was 92.3%. Significant differences were observed for the data obtained by the two detection methods.

Conclusion
These results suggest that the b-ELISA is acceptable as a specific method for detecting antibodies to AKAV and is a potential alternative to the imported commercial kit, SN test

Keywords
AKAV, ELISA

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Diagnostics of Peste-des-petits ruminants (PPR) virus by conventional RT-PCR in Georgia

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Background
Peste-des-petits ruminants (PPR) is a widespread, virulent and devastating disease of small ruminants with significant economic, food security and livelihood impacts. There is only one serotype, but there are 4 lineages which are distinguishable by nucleic acid sequencing. In January, 2016 PPR was detected in Georgia. Georgia has vaccinated small ruminants (sheep and goat). There are no laboratory methods for serology to identify PPR vaccinated and native infected animals. The goal of the study was to implement PPR Conventional RT-PCR method. For the improvement of sero-survey design and ensuring early detection and eradication of the disease laboratories must be able to identify vaccinated or field strain at the occurrence of any case of PPR.

Materials and Methods
The conventional RT-PCR method with target specific reagents was used for the detection of Peste-des-petits ruminants virus that gave us opportunity to prepare samples for sequence to identify PPR strain. For this research we used field samples (n=20) blood, oral, nasal swabs, lymph node, spleen, lung and modified-live attenuated vaccine (LAV). For RNA extraction the viral RNA isolation kit from Qiagen (cat # 52906) and the one-step RT-PCR Kit from Qiagen were used and work on 5µl of the extracted RNA. To determine band size a 100bp ladder marker (invitrogen 10787018) was used. Primers used for PPR: NP3gtctcggaaatcgcctcacagact; NP4-cctcctcctggtcctccagaatct; PCR cycle conditions 50°C 30 min, 95°C 15 min, 94°C 30 sec, 60°C 30 sec (40 Cycle), 72°C 1 min; Hold 4°c; PCR product was observed on 1.5% agarose gel.

Results
For this research we used field samples and modified-live attenuated vaccine (LAV) diluted (dilution 10-fold) that was successfully used for vaccination. Results were as expected, and the band size of field as well as vaccine strain were 350 bp. For all positive results of the final PCR product will be directly used for sequencing that will give an opportunity to conduct laboratory-based molecular and genetic study of Peste-des-petits ruminants.

Conclusion
Conventional RT-PCR will be widely implemented in lab that will allow direct sequencing and the genotyping of the strains. The targeted study will enable us to diagnose the PPR and identify which strain is circulating, the native virus or vaccine strain. The results will help in terms of epidemiological surveillance to improve surveillance system on PPR in Georgia.

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Sheep and goat trade in Zambia and its implications on disease transmission

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Background

The importance of small ruminants (sheep and goats) has received increased recognition in recent years, due to their vital role in securing food and livelihood also to the poorest and most marginalised members of society. Because of this, infectious diseases of small ruminants, for example Peste-des-petits ruminants (PPR) and Contagious Caprine Pleuropneumoniae (CCPP), pose serious threats to food security for millions of people around the world, especially to resource constrained smallholders in countries such as Zambia. Trade of animals and animal products is often regarded as a key factor in the spread of infectious pathogens over large distances. Also, there are numerous instances where trade is the suspected reason behind pathogens crossing borders and finding their ways into naïve populations.

Methods

In our research, we aimed to map the movement of sheep and goats in the process of both national and international trade in Zambia. We sought to do this by conducting semi-structured interviews with relevant key informants, for example small ruminant traders and market officials. The reason behind our choice of research topic is simple. As of to date, there have been no confirmed clinical cases of either PPR or CCPP in Zambia. However, both pathogens are considered endemic in neighbouring Tanzania, and outbreaks of PPR have taken place in bordering DRC. Also, for PPR, there are strong suspicions that the virus was first introduced into both Tanzania and DRC by international sheep and goat trade, clearly highlighting the risk that these activities imposes.

Results and Conclusion

Our preliminary results indicate that sheep and goats are gathered in rural villages, mainly in Southern province, and transported to Chibolya city market in Lusaka. Here, small ruminants from various regions are congregated under one roof, creating an environment where the risk for disease transmission is high. According to our findings, the majority of the goats and sheep are slaughtered for human consumption, but many are also brought back home and introduced into the buyers' herd. This clearly constitutes a major risk for pathogen spread across vast regions within Zambia. In addition, international trade, most notably to Tanzania in the north-east and the DRC in the north, is common, which also poses non-negligible risks. There are measures in place to mitigate these risks, but since these international trade routes often are informal, the chance of these measures being bypassed is high.

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First case of TSE in moose in Finland

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Background

In 2016, CWD was found for the first time in Europe from free-ranging reindeer in Norway. This started an intense CWD surveillance. Nineteen wild reindeer (Rangifer tarandus tarandus) cases of CWD have been detected in Norway by April 2018. In addition, 3 cases of atypical CWD in moose (Alces alces) and one case in red deer (Cervus elaphus) were found.
No CWD was detected among the 2 438 cervids tested in 2003-2017 in Finland. A three-year monitoring program for CWD starting January 2018 (EU 2017/1972), and targeting on sick, fallen and road injured cervids will be carried out. It covers the main Finnish cervid species: semidomesticated reindeer (Rangifer tarandus tarandus), moose (Alces alces), white-tailed deer (Odocoileus virginianus), roe deer (Capreolus capreolus) and Finnish forest reindeer (Rangifer tarandus fennicus). In February 2018 a fallen moose was found in Kuhmo, Eastern Finland. The animal had shown neurological signs, and was examined for CWD.

Methods

The obex and the retropharyngeal lymph node were analyzed by IDEXX HerdCheck BSE-Scrapie Antigen Test Kit, by TeSeE Western blot (SHA31+P4), Bio-Rad and by APHA (Animal & Plant Health Agency, UK) BioRad Hybrid Western Blot (SHA31+P4+SAF84). In ELISA the lymph node was negative, but the brainstem gave an initial positive result. Obex was reanalysed from homogenized suspension and from a new sample. OD values were high 3,9204–3,9990 (maximum value).

Results

The positive results of brainstem in ELISA and WB, with the confirmatory results from APHA establish the clinical diagnosis of TSE. At the same time the results prove that TSE in Finnish moose is not compatible with any of the CWD cases identified in North America. The negative result of lymph node and advanced age of the moose, 15 years, suggests that the case could be sporadic. Further studies will clarify whether the Finnish and Norwegian moose cases are similar or whether a distinct type of TSE has been found.

Conclusion

The results show that the first case of cervid TSE in Finland has been found. So far the number of atypical CWD cases, 1 Norwegian red deer, 3 Norwegian and 1 Finnish moose, is too small to assess whether the disease is of sporadic or contagious nature. The geographic distance between the Norwegian and the Finnish moose cases, some 900 km, does not favour the latter. The three-year monitoring programme is expected to produce data, which clarifies the TSE status of cervids in Finland.

Poster Session 4.1
Viral infections of Carnivores, Equines & Ruminants

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Case report: Multiple cutaneous nodules in a dairy cow (LSD differential diagnosis)

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Background
The nodular cutaneous lesions in dairy cow recognize several causes for which, the differentiation, requires diagnostic investigations. The recent spread of Lumpy skin disease (LSD) in several European countries, such as Albania, Greece and Macedonia, has led greater attention to cases not easily differentiable from a clinical point of view from the aforementioned disease. Severe LSD is highly characteristic, but milder forms can be confused with: bovine herpes mammillitis, bovine papular stomatitis, papillomavirus, pseudocowpox, vaccinia virus and cowpox virus infections, dermatophilosis, demodicosis, insect or tick bites, besnoitiosis, rinderpest, hypoderma bovis, photosensitisation, urticaria, cutaneous tuberculosis, onchocercosis. Milder forms of these diseases/syndromes can be distinguished from LSD by histopathology and other laboratory tests. The present case report describes the clinical presentation and the investigation performed in a dairy cow with multiple diffuse nodular skin lesions.

Methods
In February 2018, multiple diffuse nodular skin lesions were found in 1 out of 60 cattle in a dairy farm in Reggio Emilia, northern Italy. The cow was 5-year-old. The lesions were distributed mostly around the neck, shoulders, back, forelimbs, hindlimbs, and bilateral of midline portion. Lesions were characterized by firm, hypotrichous to alopecic nodules of 0.5-3 cm of diameter. The enlargement of the right precrural lymph node was observed. Clinical examination of the affected cow revealed slight emaciation and rectal temperature of 39°C. A representative cutaneous nodule was sampled by biopsy. The pathological material was submitted to cytological and histopathological evaluation, to virological investigation (cell culture) and tested for capripox virus (real time PCR) and for bovine papillomavirus (PCR). The precrural lymph node was sampled by fine needle aspiration for cytology. Blood sample was collected and tested for bovine leukaemia virus (BLV) antibodies using the agar gel immune diffusion method (AGID).

Results
The real time PCR for capripox virus resulted negative, as well as the virological examination on cell culture. PCR for bovine papillomavirus (BPV) resulted positive (99% of homology with BPV-1). Cytology revealed neoplastic lymphocytes both in cutaneous and lymph nodal samples. Histologically, epidermal and dermal infiltrations of neoplastic lymphoid cells were observed. No histological lesions compatible with BPV infection were observed. AGID result excluded the BLV infection.

Conclusion
The present case report describes a cutaneous lymphoma in a dairy cow, occurring independently of BLV infection and affecting only a single cow in the herd. The case described underlines the importance of a correct diagnostic approach in case of nodular lesions in cattle.

Poster Session 4.2
Viral infections of Swine, Birds, Fish & Bees

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Norovirus GII in faeces of healthy pigs in North-East Italy

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Background
Norovirus (NoV) has emerged as one of the major causative agents of non-bacterial, food- and water-borne gastroenteritis in humans. NoVs, belonging to Caliciviridae, are classified into 6 genogroups (G), from GI to GVI, which are further subdivided into 30 genotypes. NoVs identified in human gastroenteritis cases belong to GI, GII and GIV. NoVs have also been isolated from several animal species, including pigs. Detection of human GII.4 NoV in swine faecal and retail raw meat samples has raised public health concerns about the zoonotic potential of porcine NoVs and the role of swine in the epidemiology of this infection, as possible source of new viral recombinant strains that can be dangerous for human. Currently, there are no data on the prevalence of Norovirus in Italy in pigs: the only case described is related to a Norovirus GII.11, identified in swine faecal sample in absence of gastrointestinal clinical signs.

Methods
Faeces were collected at slaughterhouse in 2017 in two regions of North-East Italy. Forty-six samples originated from Veneto and thirty-three from Friuli Venezia Giulia regions, were analysed for presence of Calicivirus. A two-step RT-PCR targeting the RdRP gene with p290-p110 primer pairs was used. Sanger sequence of the partial RdRP gene was conducted on samples presenting enough amount of the target amplified DNA. For NoV positive samples, a new primer pair was designed for amplification and molecular characterization of VP1 capsidic region. Phylogenetic analysis was carried out using the Maximum Likelihood method and Kimura two-parameter substitution model using PhyML software.

Results
Fourteen samples collected in Veneto region were positive by RT-PCR for Calicivirus. Nucleotide sequences of about 300bp were obtained from only two samples. BLAST analysis showed nucleotide similarity between 89% and 92% with swine NoV GII detected in Europe. Phylogenetic analysis of the RdRp gene showed that Italian strains belong to the GII.11 and cluster with other swine NoVs from Europe, Asia and South America. Complete VP1 nucleotide sequence was obtained from only one sample: BLAST analysis showed nucleotide similarity of about 88% with swine NoVs GII detected in Asia.

Conclusion
This study identified GII.11 NoVs in the swine population of North-East Italy, similarly to a previous report in 2011. Serological studies aimed at investigate presence of antibodies against GII.4 in selected swine farms are ongoing. The real distribution and the role of NoVs in swine need to be further investigated by proper sampling approach and full genome analysis.

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Surveillance of swine influenza A in Spain: white pigs, free-range Iberian pigs and wild boars

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Background
Spain is recognized by the OIE as a priority region for the surveillance of swine influenza because it meets the main risk factors for the spread and emergence of the influenza A virus in swine: high populations of swine, high prevalence of SIV infection, potential for reassortment between virus circulating in pigs and birds and high rate of swine movements and exports. Up to 10% of Spanish domestic pig population belongs to Iberian breed which are mostly bred in a free-range system. The wild-boar is other member of Sus scrofa species which is susceptible to swine influenza viruses. Its population is very high in many regions of Spain and they frequently come in close contact with other domestic and wild animals, importantly with wild birds. In this study, we aim to investigate the epizootiology of influenza infection and the characteristics of circulating influenza viruses in industrial farms of white pigs, in free-ranging Iberian pigs and in wild boars.

Methods
Sampling area and period: west-central Spain, proximity to Important Bird Areas (IBA)/wetlands (according to BirdLife International), 2016–17.
Type of samples: sera, lung tissue, nasopharingeal swabs (NAS) from white pigs of intensive farms (fatteners, 3–9 w.o.), Iberian pigs (fatteners, 12–16 m.o.) and wild boars (hunted or captured, 1–4 y.o.)
Methods of analysis:
- Serology: bELISA (NP), Hemagglutination inhibition, Neuraminidase inhibition, ELISA and microarrays with recombinant HAs.
- Virus detection and characterization: rtRT-PCR (M) for virus detection, chicken embryo and MDCK inoculation for virus isolation, RT-PCR and HAI for isolate subtyping, genomic sequencing of viral cRNA by Illumina technology.

Results
- White pigs samples: 498 NAS/18 farms/14 towns: 9.1% animals RT-PCR+, 61.1% farms+, 13 virus isolated: 7 H1N1 (clade 1C.2.1), 5 H1N2 (clade 1C.2), 1 H3N2.
- Iberian pigs samples: 357 NAS and 577 sera/22 farms/15 towns: 0% animals RT-PCR+, 56.8% animals sero+, 68.2% farms sero+, all sw-subtypes present.
- Wild boars samples: 533 NAS and 518 sera/20 locations: 0% animals RT-PCR+, 10.8% animals sero+, 25% locations sero+, all sw-subtypes present except H3N2.

Conclusion
- High genetic and antigenic homology among Spanish swine influenza isolates and circulating European viruses.
- Influenza epizootiology of wild boars and free-range Iberian pigs appears to be different from that of the intensive white swine.
- In spite of sharing habitat very closely, we have not found evidence of transmission of avian IVs from wild birds to wild boars or Iberian pigs.

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Expansion of the area of distribution of ASF in the Russian Federation in 2017

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Background
Since the registration of the first outbreak of ASF in Russia in 2007, the virus has spread not only in European regions of the country, but also in a number of Siberian regions in 2017.
The distinctive feature of ASF virus spread in Russia was noticed by outbreaks in regions of Siberia in 2017, where pig farming is at a very high level of development (Irkutsk, Omsk, Tyumen, Chelyabinsk regions, Krasnoyarsk Territory). Epizootological investigations of outbreaks showed that the most likely cause of spreading was the illegal trade of both live animals and infected swine products. In November 2017, the disease was first recorded in wild boar population in Kaliningrad region on the border areas with the Republic of Poland (unfavorable for ASF since 2014).
Due to the aggressive and unpredictable pattern of ASF virus spread, it is necessary to conduct a phylogenetic analysis of circulating isolates, which will not only allow objectively assess the molecular evolution of the viral population, but also form approaches in tracking new genetic variants.

Methods
At the reference laboratory of ASFV FGBI "ARRIAH" a phylogenetic analysis was conducted on six isolates of ASF virus from 2017 (Irkutsk 03/17, Krasnoyarsk 10/17, Krasnodar 07/17, Omsk 10/17, Kaliningrad 12/17, Belgorod 10/17) using molecular-genetic markers. The following genes were selected as markers: the complete sequences of the genes O61R, CP204L, E183L, A179L, E248, and DP96, fragments of B626L, and EP402L genes, intergenic regions MGF 5059R / 10R, and I73R / I329L and finally the central variable region (CVR) within B602L gene.

Results
Phylogenetic analysis divided the studied isolates into three groups. Isolates Irkutsk 03/17 and Krasnoyarsk 10/17 belonged to the first group scoring 100% match in all 12 genomic markers with reference isolate Georgia 2007/1. The second group consisted of isolates Krasnodar 07/17 and Omsk 10/17. Isolates Kaliningrad 12/17 and Belgorod 10/17 belonged to the third group.

Conclusion
Thus, the long circulation of the ASF virus in domestic pigs and wild boars populations on the territory of the Russian Federation since 2007 has contributed to the emergence of new genetically modified variants of the virus, whose virulence remains unclear.
In the future using genomic markers will help in identifying new isolates and grouping them according to their molecular characteristics. An increase in the number of markers will increase the accuracy of this analysis, which in turn will allow tracing molecular evolutionary changes and possible ways of ASF virus spread.

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Ripening procedures influence the African swine fever virus survival in typical Italian pork products

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Background
Incursions of African swine fever (ASF) into disease-free regions have significant consequences, resulting in ban of international trade and costly control measures. Spread of ASF virus (ASFV) occurs via direct contact between pigs, via indirect contact with virus-contaminated fomites or via ingestion of products from infected pigs.
One of the factors that affect whether or not a pig becomes infected upon ingestion of pork-derived material, is how long the pathogen may survive within the product under the condition to which it is exposed. In this respect, the products which may be cured prior to generation of waste are worthy of particular attention. The dry curing process is known to reduce the load of a number of animal pathogens, nevertheless studies evaluating the viability of porcine viruses in contaminated meat products have not been extensively performed.

Methods
In this work, we present the effect of the dry curing process on the inactivation of ASFV in three different Italian dry-cured meat products prepared from experimentally infected pigs slaughtered at the peak of viremia. The meat products were processed using commercial methods and industrial procedures currently being followed in Italy. Samples collected at predetermined intervals during processing were analyzed for virus survival by virus isolation and animal inoculation.

Results
Virus survival was demonstrated by virus isolation in samples of salami, pork belly and loin up to 18, 39 and 47 days of curing, respectively.
Pools of samples were given orally to pairs of piglets. The conclusive proof of virus inactivation was obtained after 26 (salami) and 137 (pork belly and loin) days of processing, when piglet infection was not followed by disease symptoms and animals did not develop ASFV antibodies after 21 DPI.
ASFV was detected by piglet inoculation for up to 18, 60, and 83 days of curing in Italian salami, pork belly, and loin, respectively.

Conclusion
The data obtained from the test conducted on the salami, support the conclusion that the curing time of such product is sufficient to inactivate the virus. In conclusion, this study will improve knowledge of ASFV survival, giving valuable data to develop criteria for importation and commercialization of these dry-cured meat products from infected countries. This could also provide the opportunity to review some criteria set out in the European legislation (Directive 2002/99/EC).

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Neurotropic viruses in pigs – focusing on congenital tremor and splay legs

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Background
The ability of neurotropic viruses to cause devastating congenital disease in new-borns has been documented in a wide range of animals. This project will contribute to an increased understanding of viruses with this ability by focusing on two congenital diseases affecting neonatal piglets – congenital tremor and splay legs. Both diseases are characterized by neurological symptoms due to hypomyelination of the central nervous system. Congenital tremor (CT) was first described in 1922 and its assumed infectious origin has eluded researchers ever since. The disease is characterized by severe tremor, resulting in difficulties to nurse and increased piglet mortality. When searching for viruses in brain-tissue from affected piglets several different viruses have been identified – atypical porcine pestivirus, porcine astrovirus, porcine cirocvirus -II and aichivirus-C. The finding of a wide range of different virus has led to the hypothesis that CT could be caused by a variety of viruses with the ability to cross the placenta.
Splay legs are characterized by a temporally limited function in the hind legs, resulting in restricted ability to stand and walk. When experimental infections with atypical pestivirus were done it caused both CT and splayed legs in the same litter. Thus, in the present study the two diseases were simultaneously investigated.

Method
In this project tissue deriving from the central nervous system of infected piglets will be analyzed. The samples will be analyzed using viral metagenomic methods with a sequencing depth of 1 Gb per sample. This will allow the detection of even very low abundance of viruses. The downstream analyze will include identification, isolation and characterization of viruses in combination with experimental infections of pregnant sows to elucidate the role of identified viruses in congenital disease.

Results
We have sampled 33 piglets, 13 with splay legs, 14 with CT and 6 healthy controls. The piglets' origin from farms in the middle and south of Sweden. The samples are being prepared for laboratory analyzes and histopathologic investigation.

Conclusion
By investigating the causative agent of CT and splay legs, not only the virus causing the diseases will be identified, but also the role of viruses in congenital disease will be elucidated. In conclusion this will be done by:
- Establish the virome of CNS, & blood in diseased and healthy pigs.
- Characterize viruses of importance for congenital disease in piglets.
- Discover viruses' ability to infect and cause neurological disorders in neonatal piglets.

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Whole-genome characterization of African swine fever viruses from Sardinia collected between 1978 and 2012

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Background
African swine fever (ASF) is an acute, highly contagious disease of pigs, which can induce high mortality rates. The disease is enzootic in many African countries and on the Italian island of Sardinia, where it has been present since 1978. The etiological agent, ASF virus (ASFV), is an enveloped DNA virus with a double-stranded genome of 170-190 kbp, flanked by inverted terminal repeats (ITRs). Previous genetic analyses of ASFV isolates from Sardinia have revealed that they all belong to the p72 genotype I, with only minor variations within the B602L and the EP402R genes. However, these studies were largely limited to a few selected genes. To better distinguish between these closely related isolates and more fully investigate the evolution of ASFV in Sardinia, near-full length or complete genome sequences are required. We have assembled and annotated the complete genome sequences of nine ASFV isolates collected in Sardinia between 1978 and 2012.

Methods
A total of nine samples were selected and DNA was extracted both with and without prior nuclease treatment as earlier described (Granberg et al. 2013). Initial sequencing was performed on an Illumina MiSeq using the Nextera XT kit for library construction. The raw data were reference-mapped and de novo assembled using Bowtie2 and SPADES, respectively. In order to generate long-read sequence data, that potentially could reduce the assembly complexity, one sample was also sequenced on the Pacific Biosciences (PacBio) RSII platform (SciLifeLab). The PacBio data was processed and de novo assembled using the SMRT Analysis system v2.3.0. All obtained contigs were further analyzed and corrected using CodonCode Aligner. Unclear regions were resolved by PCR and Sanger sequencing.

Results
Complete or near-full length genomic sequences were obtained for all the investigated isolates. The genome sequence of the ASFV isolate 47/Ss/08 has already been deposited in GenBank under the accession number KX354450. The isolates were genetically very similar with an identity at the nucleotide level of 99.5 to 99.9%. The observed differences included both smaller deletions, insertions and point mutations. Most of these differences occurred in a time-dependent manner and the ITRs were the regions with the greatest divergence.

Conclusion
As a whole, the results from our studies confirmed a remarkable genetic stability of the ASFV/p72 genotype I viruses circulating in Sardinia. However, the biological significance of the observed changes in annotated genes is still not completely understood.

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Evaluating the induced-African swine fever virus (ASFV) clinical infection by the interpretation of the laboratorial diagnostic results.

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Background
African Swine Fever (ASF) is a complex and serious infectious disease of swine. The spread of ASF into the wild boar population in Europe has highlighted the need for targeted surveillance and early-warning actions. Samples obtained from hunted/dead animals or animal debris are tested to determine the presence of the disease. However, one of the most pressing problems of the current epidemic is the lack of accurate information about the ASF clinical course in the hosts once the disease is detected. The laboratory diagnosis may provide useful information regarding timing of infection and disease evolution. Thus, the main goal of this study has been to set up fixed criteria that could determine the timing of the ASF clinical infection based on laboratory diagnostic results.

Methods
A total of 1,103 samples (497 bloods and 606 tissues) were collected from animals experimentally infected with P72 genotype II ASFVs of different virulence ranged from highly virulent to attenuated strains. Each sample was analyzed in parallel by real time PCR and by indirect immunoperoxidase test (IPT) to determine the presence of the virus and/or specific antibodies. Combining both virus and antibody laboratory results, cut-off points were set up to make a categorization of the course of infection.

Results
Bloods taken from acutely infected pigs were mainly PCR positives (66%) but negatives in IPT. Only 34% resulted virus and antibody positive with antibody titers ≤1:5,120 in the 87% of the bloods. In contrast, in chronically infected pigs, 67% of the bloods were only IPT positive against the 31% which resulted positive by both tests. High antibody titers (≥1:5.120) were obtained in the 93.5% of them. Interestingly, in bloods from subacute infected pigs, the highest percentage (87%) was positive for both tests with titers ≥1:5.120 in the majority of them (91%). Similar results were obtained in tissues. Thus, the overall analysis of the results allowed us to differentiate among acute to subacute-chronic infections. With regards to the antibody titer the cut-off was fixed in 1:5120 when samples were PCR and antibody positives.

Conclusion
The parameters established within this study can be extrapolated to field samples that allow the identification of chronic infections in affected animals, mainly in the wild boar population. This can be used as a new tool to gather useful epidemiological information about the evolution of the disease in endemic areas.

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African swine fever prevalence in Lithuania, 2014–2017

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Background
African swine fever (ASF) is a highly contagious hemorrhagic viral disease of domestic pigs and other members of Suidea family. In recent years, ASF has emerged in several European countries, namely Russia, Ukraine, Belarus, Latvia, Estonia, Poland, and, most recently, ASF cases have been reported in Czech Republic, Moldova and Romania. First case of ASF in Lithuania has been reported in 2014 and linked to infected wild boar migration from neighboring countries and close contact with wild boar carcasses. Since then, gradual increase in ASF prevalence has been reported every consecutive year, although a thorough overview of an epidemiological situation in the country has not yet been published.

Methods
A National surveillance program was introduced in Lithuania after detection of the first cases of ASF in wild boars in January 2014. Throughout the 2014–2017 period, hunted wild boar (Sus scrofa) (n=91511), wild boar carcass (n=3685) and domestic pig (n=80687) samples were collected and tested. Serum samples were tested using commercially available ELISA kits for antibody detection. In addition, for direct viral DNA detection, all samples were tested using real-time PCR with primers targeting conservative VP72 gene region. Prevalence and statistical difference analyses using Fisher's exact test were carried out to outline the epidemiological situation.

Results
ASF virus prevalence in hunted wild boars using PCR analysis increased from 0.83% [0.69–0.98] to 2.27% [2.05–2.48] from 2014 to 2016 respectively. However, there was a dramatic jump in the number of ASF positive wild boars cases in 2017 resulting in prevalence of 12.39% [11.91–12.86]. Prevalence of ASF virus in pigs ranged from 0.24% [0.17–0.32] in 2015 to 2.74% [2.33–3.15] in 2017. Correlation between the pig density and number of recorded pig ASF cases in affected regions was found only in 2017 ($R=0.78$, $p<0.05$).

Conclusion
An overview of ASF situation in Lithuania has been reported for the first time. A gradual increase in wild boar cases and a sudden peak in 2017 signify the importance of further implementation of disease control methods in local and international wild boar populations. In addition, rigorous biosecurity measures should be ensured in industrial size farms and a number of household pigs minimized to avoid possible contamination routs.

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African swine fever situation and diagnostic methods in Lithuania

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Background
African swine fever (ASF) is a serious disease of key importance occuring in wild boars and domestic pigs. As there is still no vaccine or treatment available, monitoring and controlling is the only way to control the disease. On 24th January 2014 Lithuania notified two primary cases of African swine fever (ASF) in wild boars. The animals were tested positive for ASF virus (ASFV) genome by real time PCR at the National Reference Laboratory for ASF in Lithuania (NRL), results were confirmed by the European Union Reference Laboratory for African swine fever (CISA-INIA). Intensive wild and domestic animal monitoring program was started. During the period of 2014–2017 ASF was confirmed in two large commercial pig holding with the highest biosecurity. Pigs were killed and destroyed. Since 2014 ASF outbreak territory from east and south has expanded to the middle of Lithuania.

Methods
Diagnosis by PCR is one of the highly recommended diagnostic methods by World Organization for Animal health (OIE) for diagnosis of ASF. Total DNA was extracted from 140–500 µL wide range of sample: tissue, blood-EDTA, serum using "Viral RNA Extraction Kit" (Qiagen) and "RNeasy Mini Kit" (Qiagen) following the manufacturer's procedure the final elution was done with 80–125 µL elution buffer. The aim of the present study was to compare singleplex real-time PCR assays to a duplex assay allowing the identification of ASF and internal control in a single PCR tube and to compare primers, which target the p72 gene (ASF 250 bp and ASF 75 bp) effectivity.

Results
In order for the PCR to be effective, it is important to choose the appropriate reaction buffer solution primers and probe concentration. For experiments were selected two different reaction buffers, selecting the appropriate primer concentration has been analyzed using primer concentration 600–900 nM and probe 100–400 nM. The lowest Ct value is obtained at 800 nM ASF primer concentration. Standard deviation between the experiments is the smallest, so you can expect the best repetitive results. From experiment results with different combination of probe concentration for the detection determine that optimum probe concentration is 200 nM. For experiments were used reference samples (INIA, Spain), and positive samples from infected animals in Lithuania. Results show 100% sensitivity and specificity.

Conclusion
Basis on this selected to perform experiment using Multiplex real-time PCR assays prove to be less time consuming and cost efficient and therefore have a high potential to be applied in routine analysis. It is important to have effective and fast method that allows virus detection at the beginning of disease for wild boar population and in outbreaks for domestic pigs. Described specific duplex real-time assay proved to be specific, sensitive and reproducible. This makes it a promising method that can complement the current methods. Using a combination of specific DNA extraction methods, an IPC, qPCR is applicable on many kinds of domestic pig and wild boar samples.

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Swine influenza virus seroprevalence in Umbria region (Centre of Italy) and identification of circulating substypes

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Background
Swine Influenza Virus (SIV) is endemic in most countries of the world and recognized as "door opener" for other infectious agents. Its antigenic variability makes the virus easily adaptable to new hosts and able to cross-species transmission.
The aims of our work were the evaluation of SIV seroprevalence and the identification of the circulating SIV subtypes within Umbria Region pig farms.

Methods
Considering the objective to estimate the SIV territorial herd seroprevalence, during the 2016 a statistically representative number of pig farms present in Umbria region were checked. The number of farms was calculated assuming a hypothetical prevalence value of 50% (s.e. 10%). In order to exclude the presence of seropositive animals within the herd, a prevalence value of 10% was hypothesized. The serum samples were randomly selected from the sera collected for Swine Vesicular Disease National Control Plan by the Public Veterinary Service.
The WIN EPI software was used for the statistical evaluation. The relative risk assessment was estimated by the ODDS RATIO calculation.
To identify the circulating subtypes, we collaborated with some practitioner vets who reported us all the SIV suspected clinical forms. Nasal swabs and/or lungs were collected and assayed by bio-molecular methods from "clinical suspects", necropsied animals, slaughtered animals with lung lesions and from imported weaners at the arrival.

Results
In the pig farms of Umbria Region, the ELISA tests registered a seroprevalence of 32.2% (C.I. 22,84% – 41,53% – C.L. 95%). The statistical elaboration highlighted "fattening unit" as more exposed pig farm typology to SIV. In fact, in this reality, the risk of infection resulted more than four times higher respect open-cycle breeding farms and three times high compared to complete-cycle breeding farms. Only the subtype H1N1 avian like was detected. None of the tested samples was positive for the pandemic A/H1N1 variant, nor for the type D influenza virus.

Conclusion
Our SIV seroprevalence results show significant differences respect those registered in the Italian Northern Regions and in another our survey carried out in Centre of Italy, more than ten years ago. The recent numerical reduction of breeding farms, in Umbria region, and the always more increasing Biosecurity measure implementation, could justify the very low seroprevalence. On the other hand, the practice of introduction of big groups of pigs in finishing units with partially "all in/all out" policy applied could explain "fattening" as the more risky typology.

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Comparison of pathogenicity and intestinal immunity outcomes in pigs infected with French S-InDel or US S-non-InDel strains of porcine epidemic diarrhea virus

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Background
In 2013, a severe PED epidemic struck the United States of America and two types of virus strains were isolated: S-InDel and S-non-InDel strains (insertion/deletion in S gene) with different pathological outcomes in the field. The aim of this study was to compare the pathogenicity and intestinal immune response induced during the infection by a PEDV S-InDel strain isolated in France in 2014 and a PEDV S-non-InDel strain isolated in the USA the same year.

Methods
This study has been carried out in Anses' animal facilities according to the regulations on animal experimentation. Two groups of 9 weaned pigs were orally inoculated with 5 ml of an inoculum containing 108 copies of viral genome/ml of each strain. In addition, 6 weaned pigs were used as controls. Pigs were slaughtered at 3, 7 and 14 days post-inoculation (DPI). Duodenum, jejunum, ileum, Peyer's patches, mesenteric lymph nodes (MLN) and colon were collected. The immune response was studied using transcriptomic analyses. Blood samples were also collected for seral immunoglobulin detection. Histopathological lesions of the intestine and PEDV interaction with intestinal mucosae using immunostaining assay were analyzed. PEDV genome load was quantified by RT-qPCR in feces and organs.

Results
At 3DPI, destruction of the villus epithelium and of the chorion in the jejunum, in the ileum and in the Peyer's patches was observed for the two strains. A stimulation of the MLN was also observed at the same date. No lesion was observed at 7 and 14DPI in microscopy. For the two strains, at 3 DPI, immunostaining assay revealed the presence of PEDV in the epithelial cells of the villus in jejunum, in ileum and the Peyer's patches and in the afferent lymph and the follicles in the MLN. The presence of PEDV in the colon appeared at 7DPI and at 14DPI for the S-InDel and the S-non-InDel strains, respectively. The transcriptomic analysis revealed that both PEDV strains impact signaling pathways of the inflammation (IL-8 and TNFα) and of the IgA immune response (MAdCAM-1, α4, BAFF, APRIL, TGF-β, SMAD2, NF-κB, CCL28/CCR10, CCL25/CCR9): under-expression of MAdCAM-1, α4, BAFF, APRIL, TGF-β, SMAD2, NF-κB, CCR10, CCL25/CCR9 and IL-8 and over-expression of CCL28 and TNF-α.

Conclusion
As soon as 3DPI, the two strains of PEDV caused damage of the villus epithelium. These two kinds of strains had similar impacts, trends in quite inhibition, on the establishment of the IgA intestinal immune response.

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Characterization of a PCV2d-2 isolate by experimental infection of pigs

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Background
Porcine circovirus type 2 (PCV2) is a major pig pathogen which has demonstrated genetic shifts since its recognition, with reported virulence differences between genotypes. The objective of this study was to characterize a PCV2d-2 isolate by means of monitoring viraemia and excretion as well as the serological response patterns, upon experimental infection of conventional pigs.

Methods
Ten week-old pigs, still carrying maternally derived antibodies to PCV2, were infected with the virus by the nasal route and were monitored at weekly intervals for 4 weeks for the presence of viraemia, fecal virus excretion and humoral immune responses. After the last sampling, at 14 weeks of age the animals were slaughtered, and their mediastinal and mesenteric lymph nodes were tested for viral load.

Results
The gradual depletion of maternally derived antibodies, present in the piglets at weaning (3 weeks of age) was demonstrated by three ELISAs and by a virus neutralization assay at the time of challenge at 10 weeks of age. Following infection PCV2 specific IgM antibodies were first detected at 14 days post challenge (dpch), while IgG class antibodies were demonstrated from 21 dpch. The test results of the applied ELISA kits correlated weakly/moderately with those of the virus neutralization assay.
Both viraemia and virus shedding could already be detected at 7 dpch, in 36 and 50 percent of the pigs, respectively. The ratio of shedders reached 100 percent by 14 dpch and remained at this level, while viraemia was demonstrated in 86, 100, and 100 percent of the pigs at 14, 21, and 28 dpch, respectively. Both the mediastinal and mesenterial lymph nodes contained high amount of PCV2, i.e. 7,6 and 8,5 log10 copies/mg tissue, respectively, which showed negative correlation with the virus neutralization (VN) and calculated ELISA pre-challenge titres.

Conclusion
Based on the results of viraemia, virus excretion and virus load in the lymph nodes this PCV2d-2 isolate could be considered as a suitable candidate as challenge virus for subsequent vaccine efficacy studies.

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The detection, prevalence and disease association of novel porcine parvoviruses in European samples

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Background
The Parvoviridae is host to non pathogenetic and acutely pathogenic viruses across many host species. The discovery of novel parvoviruses suggests that these structurally simple ssDNA viruses can undergo rapid evolution with substantial genetic divergence to generate new species. Porcine parvovirus 1 (PPV1) is endemic in pig populations and causes reproductive failure. The novel parvovirus ungulate tetraparvovirus 3 (porcine parvovirus 2, PPV2) was detected in pig sera in Myanmar 2001. In China, researchers isolated PPV2 from pigs with clinical symptoms of "high fever disease". It still remains unclear if there is a correlation between disease association and the presence of this novel virus. This study presents data regarding the prevalence and disease association of three novel parvoviruses in pigs; ungulate tetraparvovirus 3 (PPV2), ungulate tetraparvovirus 2 (PPV3) and ungulate copiparvovirus 2 (PPV4).

Method
The detection and prevalence of PPV2, PPV3 and PPV4 was conducted on archival diagnostic clinical tissue, faeces and sera pig samples from 1996 to 2012 (n=700). Viral nucleic acids were extracted. In house primers were applied to detect the novel PPV viruses by PCR and confirmed by Sanger sequencing. Detailed veterinary pathology data was scored for each sample and analysis of variance was used to identify possible association between the presence of these viruses and disease.

Results
PPV2, PPV3 and PPV4 were multi-systemic and detectable in all tissue types such as lymph nodes, spleen, lung and small intestine with the highest prevalence being PPV2 detected in 19.3% of samples. The viruses had a lower prevalence in serum samples suggesting a short viraemia.
PPV2 had the highest prevalence in lung tissue (33%) and a significant association with respiratory symptoms and scored pathogenesis. Significant associations were observed with detected PPV2 and PPV3 with peritonitis, and all novel PPV's with enteric ulcers. PPV2 and PPV3 also had an association with porcine dermatitis and nephropathy syndrome (PDNS). Novel PPVs showed no evidence of vertical transmission and were first detected in the weaner age group. The prevalence of novel viruses increased in growers however they were detected at the highest levels in the finisher age group.

Conclusion
Presence of the viruses in a wide range of sample types suggests a systemic tropism. The role of these novel parvoviruses as primary pathogens is unclear but statistically significant association with a number of pathologies merits further surveillance and continued research.

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Phylogenetic analysis of alphacoronaviruses from five different species of bats in Denmark

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Background
Bat populations worldwide harbour numerous viruses, some of which are known to be or potentially pathogenic to other animal species or humans. Therefore, it is important to monitor the populations and characterize these viruses. In this study, we investigated bat populations in Denmark for the presence of coronaviruses (CoVs). Previously, we have reported results from bat sampling in 2013–14 (Rasmussen et al. 2015); these sampling activities have continued biannually into 2017. This larger dataset of partial CoV sequences provided the basis for phylogenetic analyses and comparisons to sequences obtained from bats in other European countries.

Methods
Faecal samples were collected from species-identified bats captured in different geographical regions of Denmark between 2013 and 2017 with a focus on the important hibernacula in Mønsted limestone mines. The samples were screened for CoVs using a combination of 3 pan-CoV RT-PCR assays. Amplicons from positive samples were sequenced using Sanger sequencing and analysed using CLC Main Workbench. The nucleotide (nt) sequences from the 3 different assays were 130, 208 and 395 nt in length after primer trimming and they were aligned with reference sequences. Phylogenies were constructed using the Jukes-Cantor Neighbor-Joining method with 1000 bootstrap replicates and a minimum bootstrap value of 50 for phylogenies based on the 208 and 395 nt long sequences and a minimum value of 20 for the phylogeny based on the 130 nt long sequences. Nucleotide sequences were translated to the predicted amino acid sequences and compared.

Results
In total, 271 faecal samples were collected from 10 different species of bats, with the majority of samples collected from M. daubentonii. CoVs were detected in samples from 5 different species and the overall prevalence of CoVs in the samples was 21.4% (95% confidence interval [17–26%]). All generated coronavirus sequences fell into the genus Alphacoronavirus. Phylogenetic analysis revealed a species-specific clustering with the samples from M. daubentonii showing a close resemblance to coronavirus sequences obtained from the same species of bat in Germany and the United Kingdom. Likewise, the predicted amino acid sequences were distinctly different for each species.

Conclusion
This study presents the first phylogenetic analysis of CoV in Danish bats. Our results show that several distinct alpha-CoVs are present in the Danish bat population and that they are apparently species-specific.

Reference
Rasmussen TB, Chriél M, Baagøe HJ, Fjederholt E, Kooi EA, Belsham GJ, Bøtner A (2015) Bat Coronaviruses circulating in Danish bats. ESVV/EPIZONE, Montpellier

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Impact of host genetics on the interaction of PCV2 with peripheral blood monocytes

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Background
Porcine circovirus type 2 (PCV2) is associated with post weaning multisystemic wasting syndrome (PMWS) and other PCV2-associated diseases in pigs. It has been demonstrated that the severity of these diseases varies among breeds in the field. This difference in genetic susceptibility might partly be due to the power of monocytes to clear the virus and infected cells. However, this uptake process and its outcome were still unknown. Therefore, this study aimed to understand the fate of PCV2 in blood monocytes and the role of the genetic host background on the outcome.

Methods
Blood monocytes were isolated by plastic adherence of peripheral blood mononuclear cells. The uptake of PCV2 by monocytes were studied by visualization of virus particles with confocal microscopy and by chemical inhibition of endocytic pathways, followed by evaluation of the level of PCV2 entry. After PCV2 uptake, the fate of the capsid protein and the viral genomes were examined by doing time-course experiments, followed by quantification of intracellular viral capsids with image J and viral genomes with qPCR. Then, monocytes from four different pig breeds (the hybrid Piétrain × Topigs 20; purebreds Piétrain, Landrace and Large White) were isolated and inoculated with PCV2, followed by evaluation and comparison of virus uptake and disintegration.

Results
PCV2 uptake in monocytes was not affected by amiloride and methyl-β-cyclodextrin. In contrast, it was decreased by (i) chlorpromazine (84±7% reduction), (ii) cytochalasin D (82±11% reduction) and (iii) dynasore (50±24% reduction). After the uptake, a disassembly of the virions was observed up till 12 hours post inoculation, after which a low level of antigens remained present. The viral genomes persisted in cells with minor fluctuations. The monocytes from the four pig breeds were able to take up and then disintegrate PCV2 to a certain level. However, those from the Piétrain and hybrid pigs showed a higher level of PCV2 uptake and disintegration, compared with those from Landrace and Large White pigs.

Conclusion
Blood monocytes take up PCV2 via actin- and dynamin-dependent, clathrin-mediated endocytosis. The internalized viral capsid is disintegrated to a certain low residual level. The viral genomes are digested less efficiently with still a lot of DNA fragments constantly present in cells. Monocytes from the Piétrain and hybrid pigs are more capable to take up large amounts of PCV2 than those from Landrace and Large White pigs.

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Characterisation of the virome of specific pathogen-free and conventional pigs

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Introduction
Viral infections are of major importance within pig production, causing not only severe diseases and occasionally death but also subclinical infections can have large economic consequences due to retarded growth. It is becoming evident that a number of factors, including multiple microorganisms, often act together to create e.g. a respiratory disease which is referred to as the porcine respiratory disease complex (PRDC). The use of viral metagenomics and high-throughput sequencing has allowed us to identify all present viruses in a particular sample/environment simultaneously as well as to identify novel/previously undescribed viruses. In this study, we have used this technology to characterise the virome of conventional as well as specific pathogen-free (SPF) pigs.

Methods
Tonsil samples from eight conventional pigs from a herd with PRDC were used as well as tonsil samples from eight SPF pigs. Prior to RNA and DNA extraction the tissue homogenates were treated with nucleases. The nucleic acid was then randomly amplified before sequenced on the Ion 5SXL sequencing platform. Diamond was used for annotation of the sequences and for a number of the identified viruses further genetic characterisation was performed.

Results
In all pigs the presence of a large number of vertebrate viruses, both DNA and RNA viruses were detected. In total, viruses from eight families were identified and in a majority of these families not only one but several different genus were found. Members of the Adenoviridae and Picornaviridae family could be detected in all or in nearly all pigs, while other families such as Circoviridae and Caliciviridae were less common. Many of the viruses identified have not previously been characterised in Sweden. We also discovered parvoviral reads showing very high sequence divergence to any previously described member of this family.

Conclusion
This study showed a high co-infection rate in both SPF and pigs with respiratory diseases. Certain viruses seem to be present in most pigs while others in just a few. A number of these viruses have, through this study, been genetically characterised. However, to understand their potential role in health and disease further biological studies are needed. There was thus no specific virus that could explain the respiratory disease of these pigs.

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Transmission of fowl adenoviruses (FAdV) to broiler chickens in Sweden: horizontal spread predominates

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Background
Inclusion body hepatitis (IBH), caused by fowl adenovirus (FAdV), is one of the most important diseases in broiler chickens in Sweden. An increasing number of outbreaks were seen in 2014 and a research project was initiated to increase the knowledge about major FAdV genotypes circulating in Sweden and their routes of transmission. One major objective was to determine the relative importance of vertical and horizontal routes of transmission to broiler flocks.

Methods
Parent flocks were serologically screened (Indirect-ELISA, BioChek, The Netherlands) from 16 to 36 weeks of age. The numbers of parent flocks were 87 at 16 weeks and 52 at 24 and 36 weeks, due to merging of flocks. The total number of blood samples was 1900 (May 2015 to August 2016). 949 dead-in-shell embryos, laid by parent hens aged 26–27 and 36–37 weeks, were collected from 35 flocks. Each embryo was sampled (liver, ileocaecal tonsils) and analyzed by PCR for detection of the hexon gene (890 bp) of FAdV. To compare viruses from parents and their offspring, 124 samples (liver, ileocaecal tonsils) from four additional 24–26 weeks old parent flocks were analyzed by PCR. 20 embryos from each flock were also sampled and analyzed. 40 blood samples from the parent flocks, obtained at 16 weeks of age, were analyzed serologically.

Results
92% of the parent flocks had seroconverted at 16 weeks of age and in 43% of the seropositive flocks all 10 blood samples were positive for FAdV-antibodies. At 24 weeks of age 96% of the flocks were seropositive and at 36 weeks of age all flocks had seroconverted. In the four additional parent flocks, all four flocks had seroconverted at 16 weeks of age and in three out of four flocks all ten samples were seropositive. FAdV was not detected in any of the organ samples from parent birds or embryos.

Conclusion
Vertical transmission of FAdV could not be demonstrated in our study. Hence, the results suggest that vertical transmission was not a major route of transmission and could not alone explain the outbreaks of IBH in broiler flocks in the investigated population. When the collection of hatching eggs started around 25 weeks of age, the parent birds had already developed immunity which most likely prevented vertical transmission. We conclude that horizontal transmission from residual virus in the barns or other virus sources was the predominant source of infection of broiler flocks.

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Target enrichment NGS approach for generation of ASFV full-genome sequences

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Background
African swine fever, one of the most dangerous diseases of swine, is caused by African swine fever virus (ASFV), the only member of the genus Asfivirus and the family Asfarviridae. The viral genome, consisting of one linear molecule of double stranded DNA, has a length of 170–194 kbp, depending on the isolate. Due to the large genome size and the occurrence of 151–167 ORFs as well as inverted terminal repeat regions at both ends of the genome, whole-genome sequencing is challenging. Therefore, only 21 ASFV whole-genome sequences, including only one from Eastern Europe (Georgia 2007/1) are available, representing just a small part of the existing virus variants. Especially in Eastern Europe, where the epidemiological situation develops quickly with the introduction of ASFV into the Czech Republic, Moldova, Romania and recently Hungary, more full-genome sequences are needed to understand viral evolution, virus variation including possible attenuation as well as molecular epidemiology.

Methods
For the generation of ASFV whole-genome sequences, we used the myBaits® Custom Target Capture Kit (Arbor Biosciences) for in-solution capture of ASFV specific DNA from prepared NGS-libraries. After sequencing on an IonTorrent S5 and Illumina MiSeq instrument, we compared the results to data generated by a shotgun NGS-approach without prior enrichment.

Results
Using target enrichment, we were able to generate a complete ASFV-Armenia08 genome sequence from cell-culture supernatant; 95% of 1.3 million reads mapped to the published ASFV-Georgia07 sequence. Furthermore, an ASFV-Moldova whole-genome sequence from organ tissue was sequenced, and 85% of the 400 thousand reads could be allocated to ASFV-Georgia07. In comparison to NGS-based sequencing without prior enrichment, the proportion of ASFV-reads varied from 0.09% to 0.29%, depending on the input material.

Conclusion
By using target enrichment, we were able to generate high quality ASFV whole-genome sequences from different samples while drastically reducing the necessary number of reads. Therefore, target specific enrichment for NGS will be a very valuable tool in cost effectively generating ASFV sequences in the future.

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First detection of atypical porcine pestivirus in piglets with congenital tremor in Denmark

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Background
Atypical porcine pestivirus (APPV) is a newly recognized member of the Flaviviridae family. This novel porcine pestivirus was first described in the U.S. in 2015 and subsequent reports have shown that the virus is widely distributed in domestic pigs in Europe (Postel et al. 2017). APPV has been shown to be associated with congenital tremor (CT) type A-II in new-born piglets. In April 2018, samples from a Danish swine herd with CT symptoms in piglets were submitted to the laboratory. Up to 50% of the litters from gilts were affected and the symptoms had been observed within the herd for a few months.

Methods
Blood and brain samples from two different litters with CT symptoms were received. The samples were analysed using a combination of pan-pestivirus and APPV-specific RT-qPCRs targeting sequences within the NS3 and NS5B coding regions of the viral RNA genome. Partial nucleotide sequences of the amplicons obtained from positive samples were determined by Sanger sequencing to investigate their relationship to other APPV strains.

Results
Blood and brain samples from CT-affected piglets from both litters tested positive for APPV using RT-qPCRs with Ct values of approx. 30. Nucleotide sequencing of a part of the NS5B coding region showed high similarity (100% within 106 nt) to a German APPV strain from 2016.

Conclusion
APPV was found in samples from a herd with CT in new-born piglets from gilts. This is the first detection of APPV in Danish pigs.

Reference
Postel et al. (2017) High Abundance and Genetic Variability of Atypical Porcine Pestivirus in Pigs from Europe and Asia. Emerg Infect Dis. 23(12):2104–2107.

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Performance of five commercial ELISA kits for detection of antibodies binding classical swine fever virus glycoprotein E2

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Background
Classical swine fever (CSF) is a severe notifiable pig disease. Diagnostics are an important tool to diagnose the disease as soon as possible, enabling quick measures against it. Commercial enzyme-linked immunosorbent assay (ELISA) kits are used to test for presence of antibodies binding the classical swine fever-virus (CSFV) antigen glycoprotein E2. These tests are executed for screening or suspicion of CSF in domestic pigs or wild boar. This study is performed to investigate performance of five commercially available CSFV-E2 ELISA kits and decide which are the best to use for diagnostic purposes.

Methods
Each test was executed according to the manufacturers' specifications. Diagnostic sensitivity (percentage correct positive results) was studied using 400 serum samples from previously experimentally infected animals, from days 14, 21 or 28 post infection. Tests should at least give positive results from 21 days post infection. Diagnostic specificity (percentage correct negative results) was studied using 1545 serum samples from a CSF-free pig population. Furthermore, 60 bovine virus diarrhoea or border disease antibody positive samples were used to study analytical specificity (selectivity). Receiver operating characteristic (ROC) curves were constructed and Youden indexes calculated to find the optimal cut-off values (threshold positive – negative result). Also the best combination of kits to use one test for screening (first) and another test for confirmation (second) of positive samples from the screening test, before using the more labour intensive virus neutralisation test (VNT; gold standard), was studied. This was carried out by evaluating the sensitivity and specificity of the different combinations of kits.

Results
For 21 plus 28 days post infection diagnostic sensitivity ranged from 0.74 to 0.87, and specificity from 0.98 to 1 (based on cut-off values indicated by manufacturers). Selectivity ranged from 0.78 to 0.95. Youden indexes and ROC curves showed some options to improve sensitivity by changing cut-off values. Some combinations of kits showed good performance as screening and confirmation test.

Conclusion
Taking results together some kits performed better than others, and certain combinations of kits as screening and confirmation test could decrease the number of VNTs for final confirmation. This is one of the largest evaluations of these CSFV-E2 ELISA kits, valuable for manufacturers of the tests and significant for diagnostic laboratories.

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Pigs vaccinated with BA71ΔCD2 and surviving the homologous challenge with BA71, are capable to efficiently resist further challenge with Kenya06, while prime-boosting with BA71ΔCD2 do not

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Background
African swine fever virus (ASFV) is a highly pathogenic virus that affects pigs, causing a hemorrhagic fever that reaches up 100% mortality. To date, 23 different genotypes have been described circulating in the sub-Saharan countries. The lack of treatment or an effective vaccine against ASF complicates its control, causing great economic losses in the affected countries.
We have previously demonstrated that immunisation with the live attenuated virus (LAV) BA71ΔCD2, protect 100% of pigs against ASFV genotypes I and II, including Georgia07, the currently Europe circulating isolate (Monteagudo et al, 2017).

Methods
Twelve pigs were immunised with one dose of BA71ΔCD2. Three weeks later were subsequently separated in two groups of six pigs. The first group were boosted with the same dose of BA71ΔCD2, and the second one, were infected with the homologous virulent strain, BA71. At the same time, three non-vaccinated pigs were used as a control group.
Three weeks later, all animals were challenged with lethal dose of Kenya06.
Pigs were bled, and nasal swabs were taken during all experiment at different time points.

Results
Here we extend these studies, demonstrating that the protection afforded against the Kenya06 virulent strain (genotype IX) was rather limited, perhaps reflecting the large phylogenetical distance that exists between genotype IX and genotypes I and II.
Interestingly, pigs surviving the homologous challenge (vaccinated with BA71ΔCD2 and challenged with BA71), were capable to efficiently resist further challenge with Kenya06, while prime-boosting with BA71ΔCD2 did not.
The cross-protection afforded seemed to correlate with the induction of CD8 T-cells capable to specifically recognize every single ASFV strain tested in vitro.

Conclusion
These results open new avenues to understand the mechanisms involved in cross-protection and new expectations for designing more efficient cross-protective vaccines in the future. Furthermore, our results suggest that vaccination in endemic areas with multiple viruses circulating might benefit directly and indirectly from vaccination with ASFV vaccines such as BA71ΔCD2.

Keywords
African swine fever, vaccine, cross-protection, CD8+ T-cells

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Full-length genome sequence of quail deltacoronavirus is more similar to porcine counterparts

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Background
Coronaviruses (CoVs) identified in avian hosts generally belong to two genera, Gamma- and Deltacoronavirus within Coronaviridae family. The main representative of Gammacoronavirus is infectious bronchitis virus (IBV). Coronaviruses identified in 2009 in birds of Passeriformes formed a novel genus, Deltacoronavirus. Currently, the Deltacoronavirus genus comprises the eight species, including 7 birds' and one pigs' coronaviruses but they were also identified in Asian carnivores.
The quails (Coturnix coturnix japonica) seem to be susceptible to infection with two genera of avian coronaviruses, gamma- as well as deltacoronaviruses as their concomitant presence were recently reported in Italy and Brasil. However, only short sequences (fragments of replicase and S1 genes or 3'UTR) of identified viruses are public available. In this study, the full-length genome sequence of quail deltacoronavirus (dCoV) identified in Polish quail commercial farm was determined.

Methods
In February 2015, acute enteritis was reported on a quail farm located in south-eastern part of Poland, in Dolnośląskie province. Liver and kidney samples of dead quails were used for "next generation sequencing". The RNA virus libraries were prepared with NEBNext Ultra RNA Library Prep Kit for Illumina (New England BioLabs GmbH, Germany). They were then validated and quantified using Bioanalyser and real time PCR. Sequencing was done in paired-end technology using MiSeq Reagent Kit V2 (Illumina Inc, San Diego, USA).

Results
The full-length genome consisted of 25918 nucleotides and begins from 5'UTR followed by two large ORFs which encode polyproteins 1a (pp1a) and 1b (pp1b), then ORFs S, E, M, NS6, N, NS7a and NS7b ended with UTR3'. The phylogenetic analysis of the complete sequence showed that it formed separate branch distinct from all of 25 full-length genome sequences analyzed in this study. The complete genome showed the highest identity (82%) with the sequences of porcine dCoV strain USA/Nebraska145/2015 and the lowest (52.1%) to that of Hong Kong wigeon dCoV HKU20 strain. The sequences of the individual genes possess various degree of similarity with different dCoV strains. Most of genes were more homologous (81–88%) to porcine dCoV strains except the S1 coding region of S gene which was more similar to avian (munia) dCoV but with the S2 coding region again more homologous to mammalian dCoVs.

Conclusion
No significant recombination events have been identified, so it appears that this virus has evolved through the accumulation of mutations in the quail organism.

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Detection and genetic characterization of rotaviruses in Polish turkey flocks

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Background
Rotaviruses (RVs) are segmented, double-stranded RNA viruses, which cause a severe enteritis in human, animals and in avian species worldwide. In poultry, RVs infections result in diarrhea, weight loss and sometimes increased mortality causing huge economical consequences. In Poland, despite strict biosecurity measures implemented, enteritis in turkey flocks are commonly reported. The aims of this study were the identification and molecular characterization of avian rotaviruses (AvRVs) in commercial turkey flocks in Poland.

Methods
Samples of intestinal tissues or cloacal swabs (n=147) from turkeys of 1–10 weeks of age, collected between 2015–2018 were screened by real-time RT-PCR targeting VP6-encoding gene to detect AvRV groups A and D (RVA and RVD) (Otto et al., 2012). Positive samples were further examined, i.e. their VP4, VP6, VP7, NSP4 and NSP5 genes fragments were amplified and sequenced (Schumann et al., 2009). Obtained sequences were aligned and analyzed using MEGA v7.

Results and Conclusion
In the present study, 47 (31%) of the samples were positive for RVA. In four RVA-positive samples (5%) RVD was additionally detected. Overall, RVs were detected in turkeys of 1–3-week-old, in which clinical signs typical for enteritis were observed. All detected RVA strains had the following genotypes constellation: G22-P[35]-I4-H4. The NSP4 genes of all detected RVA strains grouped together with the reference RVA-P35-Tu-03V0001E10 (EU486957) strain and the VP7 genes with RVA-G22-Tu-03V0002E10 (EU486983) strain. VP6 and NSP5 genes sequences of most identified Polish RVAs were assigned with I4 and H4 genotypes, respectively. However, VP6 gene sequences of two RVA strains revealed only 73.2% similarity to I4 reference strain RVA/Pigeon-tc/JPN/PO-13 evident using the Genotyping Tool for Rotaviruses (RotaC v2.11b). According to guidelines for rotavirus classification (80% cut-off value for VP6) such value might indicate the presence of a new VP6 genotype, which however should be verified by the Rotavirus Classification Working Group.

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Pathogenicity of H9N2 avian influenza virus for turkeys, quail and ducks

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Background
Avian influenza (AI) is currently one of the most important threats in the poultry production. The H9N2 subtype of AI virus is of high epizootic importance due to its endemicity in Asia and North Africa. There have been a number of outbreaks caused by H9N2 AIV in recent years in Europe. However, the virus causing them was not derived from the lineages circulating in Asia and Africa, but originated from wild birds. The aim of this study was to assess its pathogenicity potential for various poultry species.

Methods
Ten turkeys, ducks and quail were inoculated oculonasally with a dose of 10^6 EID50 of LPAIV H9N2 subtype. Three naïve birds were introduced at 1 day post infection (dpi) to serve as the control of transmission efficiency. The control group consisted of five mock-inoculated birds. Birds were monitored for clinical signs and mortality for 14 days. At selected time points oropharyngeal and cloacal swabs were collected and tested for viral RNA in real time RT-PCR. At the end of experiments, blood samples were collected and tested in hemagglutination inhibition (HI) test. Statistical comparisons were performed with U-Mann-Whitney test.

Results
No clinical signs or mortality were recorded in quail and ducks. Turkeys showed slight apathy lasting until the end of experiment. One turkey died at 9 dpi. The longest duration of virus shedding (until 14 dpi) was observed in turkeys, both from trachea and cloaca. Quail shed the virus until 6 dpi, mainly from the respiratory tract. Both turkeys and quail showed higher level of virus shedding from respiratory tract than from cloaca. In contrast, cloacal swabs collected from ducks contained larger amounts of viral RNA than oropharyngeal swabs. In ducks, shedding was detected until 7 dpi. Efficient transmission to contact birds was observed in all species. Seroconversion was also demonstrated in infected and contact birds, with turkeys showing the highest antibody titres.

Conclusion
The study demonstrated low pathogenicity of European H9N2 AIV for turkeys, quail and ducks. Efficient replication of virus with no or only mild clinical signs and efficient transmission to naïve birds indicates that all these bird species can be a silent reservoir for the virus. Additionally, comparison of results with those from previous experiments on turkeys co-infected with bacterial respiratory pathogens demonstrated that the virus alone has less potential to cause an overt clinical disease.

The study was funded by National Science Centre in Poland (grant no. 2015/17/N/NZ6/03515).

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The identification and characterization of high impact avian viruses circulating in wild and domestic birds in Trinidad and Tobago, West Indies

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Background
Avian viruses pose a significant veterinary and public health threat within Trinidad and Tobago (T&T) and the wider Caribbean region. They are major contributors to mortality and poor health in poultry, leading to significant economic impact worldwide. In wild bird populations, avian viruses threaten many species and can potentially spread to domestic and commercial birds. To date, there is limited information on the circulation of viruses in avian populations of T&T and there is little knowledge of the risks posed by these viruses.

Objectives and Methods
The purpose of this research was to identify, where possible, the extent of circulation and specific characteristics of various high priority avian viruses including; Avian Influenza virus (AIV), Infectious bronchitis virus (IBV), Newcastle disease virus (NDV), Infectious laryngotracheitis virus (ILTV) Avian metapneumovirus (aMPV), Infectious bursal disease virus (IBDV), Chicken infectious anaemia virus (CIAV), Fowl adenovirus Gp1 (FADV) and Egg drop syndrome virus (EDSV). An initial broad based surveillance study was carried out for the detection of antibodies to the viruses by ELISA. Additionally, passive field surveillance targeting birds showing clinical signs of disease was carried out by PCR and sequencing to identify and characterise the circulating viruses and to determine their serotype and genotype. Wild bird surveillance was also conducted to identify whether AIV was circulating, and to assess the possible risk of AIV transmission to domestic poultry.

Results
Antibodies were detected against IBV, NDV, ILTV, APV, IBDV, FADV and EDSV and viral nucleic acid was detected for IBV, APV, CIAV and FADV in domestic poultry in T&T. Further characterisation of FADV revealed that serotypes 8a, 8b, 9, and 11 were circulating in diseased birds, along with CIAV. Phylogenetic analysis of circulating IBV strains identified two lineages, one with high similarity to the vaccine strains and the second being identified as a unique lineage to T&T. AIV antibodies with high neutralising titres against a low pathogenic H5N3 strain were detected in sera from three wild birds, and AIV RNA was detected by PCR in a swab sample taken from another wild bird.

Conclusion
This research identified for the first time the presence of various high-impact avian viruses in domestic poultry and wild birds in T&T. The results significantly add to current knowledge on the identity and distribution of avian viruses, as well as on the risks posed by these viruses to the domestic poultry populations, in T&T and the wider Caribbean region.

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Avian influenza in Serbia: epidemiological situation during 2016–2017

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Background
Avian influenza (AI) is a disease of birds caused by Influenza A virus from the family Orthomyxoviridae, genus Influenzavirus A. Due to high morbidity and mortality AI causes devastating losses in wild bird population and poultry industry. Also, AI is a zoonotic disease and constant threat to human health.

Methods
During 2016/17 Europe was affected by a huge epizootic of AI and 28 countries reported outbreaks. The mostly reported subtype was HPAI H5N8 but H5N5 and other subtypes were reported too. The first case of AI was reported in Hungary in October 2016. Immediately after that outbreak the veterinary authorities in Serbia implemented the active and passive surveillance zone on the northern part of country and implemented the ban of import of live birds and poultry products from affected countries. The increasing of the biosecurity measures on poultry farms was strongly recommended.

Results
The first case of HPAI H5N8 virus in territory of Republic of Serbia was detected on 29th of November 2016 in 6 dead mute swans found in ponds near settlement Kovilj which is located in Vojvodina, the north province of Serbia. During the active monitoring surveillance of poultry in surrounding settlements, the virus was found in 3 domestic ducks and 2 geese in the backyards. There was no introduction of the virus in the big poultry farms. In total, during 2016/17 in Republic of Serbia, there were 23 outbreaks of the HPAI H5N8, 19 of which in wild birds and 5 in backyard poultry and one outbreak of HPAI H5N5 in swans. Nucleotide sequencing and the phylogenetic analysis of HA gene of H5N8 isolates showed high similarity with isolates from Croatia (A/mute swan/Croatia/70/2016) and Hungary (A/mute swan/Hungary/51049/2016). Since all cases of AI were recorded in the wild birds or in the backyards poultry that had common drinking water with wild birds, it can be concluded that introduction and transmission of AIV in Serbia was from the wild birds.

Conclusion
During the AI eradication, 816 animals in 27 contact households were euthanized. Although the epizootiological situation in the whole region was unfavorable, due to Government imposed measures such as strict movement control, import bans, trade control and increased biosecurity measures, there was no outbreak of HPAI on big poultry farms in Serbia and economical losses can be assessed as minimal.

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Infectious bronchitis virus – neglected troublemaker in poultry

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Background
Vaccination imposed, problem solved? Not so fast. Infectious bronchitis virus (IBV) proved us wrong, and continuous to do so. It is prone to mutations and recombinations that lead to new variants appearing all over the world. Infectious bronchitis is often missed to be considered as an underlying problem that predisposes poultry to bacterial infections. In recent years, it appears to be a possible predisposing factor that has triggered a development of new highly resistant E.coli (APEC) in poultry, subsequently causing considerable economic losses in Croatian flocks. Preliminary screening has revealed a presence of IBV circulating in breeding and laying flocks.

Methods
Tissue samples of kidneys and cecal tonsils from hens' carcasses were collected at the necropsy. Preliminary screening for positives was done using molecular methods. Total RNA isolated from tissue samples was analysed using RT-qPCR according to Collison et al. (2006). Positive samples were further subjected to Nested one-step RT-PCR and PCR, using primers as in Worthington et al. (2008). Amplicons were purified and subjected to sequencing. Acquired sequences were analysed in silico (BLAST, Ugene, MEGA7).

Results
Positive samples were found in both, kidneys' and cecal tonsils' samples originating from laying hens and breeders. BLAST analysis showed similarity with strains 7/93, 207 and Chinese strains GX NN130048 and CK/CHGD/XX12. Further multiple sequence alignment and phylogenetic analysis revealed that previously undetected strains are related, but also differ from vaccinal strains used according to the recommendation of Croatian Poultry Center.

Conclusion
Preliminary screening results confirm the necessity of further investigation of IB prevalence in farms affected by colibacillosis and other bacterial pathogens, and adjustment of the vaccination program to newly detected IBV variants. Complete characterisation of new variants is required. Therefore, further investigation is in progress.

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