

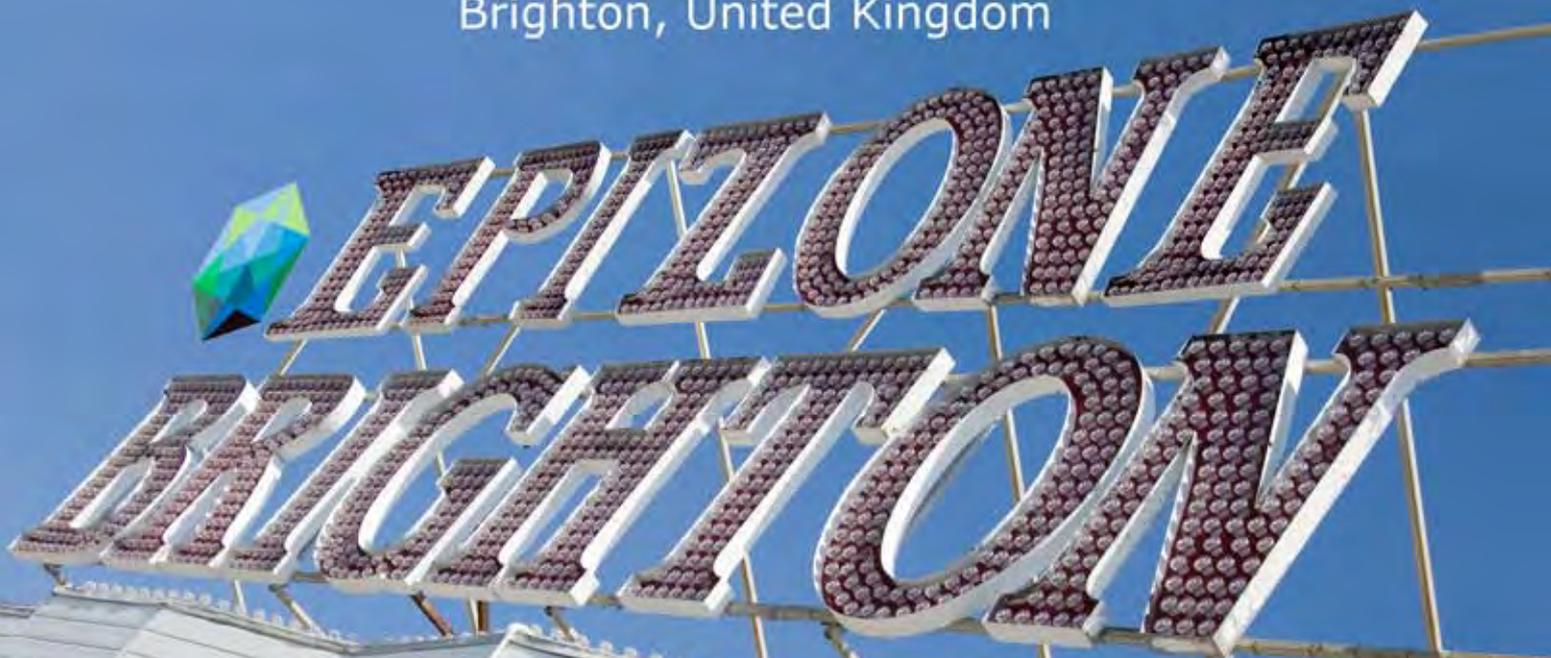


Abstracts

6th Annual Meeting EPIZONE "Schmallenberg Virus"

15th June 2012

The Thistle Hotel
Brighton, United Kingdom



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EPIZONE 6th Annual Meeting Satellite Symposium on the topic 'Schmallenberg virus'

15th June 2012, Thistle Hotel, Brighton

Schmallenberg virus (SBV) is a novel orthobunyavirus first identified in cattle in Germany in Autumn 2011.

The virus is most likely transmitted by midges (*Culicoides* spp.), and infections likely occurred in summer and autumn of 2011, but foetuses that were exposed to the virus in the womb were born later. SBV has been detected in several European countries. A risk assessment for public health was issued by the European Centre for Disease Prevention, saying it is unlikely that this new orthobunyavirus can cause disease in humans.

Still a lot of questions remain to be answered. Which vector species is transmitting the disease? Can animals infect each other directly? And of course, where did the virus come from? What can be done to control the virus and can we develop a vaccine? Scientists within Europe need to work together and exchange knowledge to control this new epizootic in Europe.

Schmallenberg virus in Germany, Detection, characterization and experimental infection in cattle

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In summer and autumn 2011, farmers and veterinarians in North Rhine-Westphalia (Germany), but also in the Netherlands, reported about mild clinical signs in dairy cattle including fever, reduction of milk production and diarrhea. Cattle in several farms showed a similar clinical picture, which disappeared after some days. Most cases in Germany were reported in September and October, and blood and swab samples taken from diseased cattle were tested by real-time RT-PCR for all common bovine viruses like bluetongue virus, epizootic hemorrhagic disease virus, pestiviruses, foot-and-mouth disease virus, bovine ephemeral fever virus or Rift Valley fever virus. However, all samples tested negative and none of the known pathogens could be connected to those cases.

Therefore, a metagenomic approach was chosen to analyze a pool of three selected samples from dairy cattle from a farm near the city of Schmallenberg which had fever and showed a decrease in milk production of more than 30%. The samples were analyzed following a protocol for RNA and DNA preparation and subsequent next generation sequencing in a *Genome Sequencer FLX* instrument (454/Roche) in November 2011. Using a newly developed software routine, all reads were compared to sequence databases, resulting in 7 reads showing a high homology to viruses of the genus *Orthobunyavirus*.

This sequence information was used to develop a specific real-time RT-System which allowed investigation of all collected blood samples from affected cattle farms. Subsequently, the first 5 positive farms could be detected, confirming the presence of “Schmallenberg virus” genome in the blood of several cattle. Subsequently, the virus could be isolated on cell culture and first animal trials in calves were conducted. In addition, the full-length sequence was determined, and it could be shown that the new virus is most related to viruses of the so-called Simbu serogroup.

Since December 2011, more than 1400 cases of SBV-PCR-positive malformed lambs or calves were reported in Germany. Epidemiological data about the distribution of cases, the sero-prevalence in Germany as well as the results from different infection experiments with cattle will be presented and discussed.

OIE experience with the Schmallenberg virus outbreak and the importance of a research network

Dr Elisabeth Erlacher-Vindel, Deputy Head, Scientific and Technical Department, World Organisation for Animal Health,(OIE), 12 Rue de Prony, 75017 Paris, France (e.erlacher-vindel@oie.int).

The World Organisation for Animal Health (OIE) is an intergovernmental organisation with a mandate from its 178 Members to improve animal health worldwide. It is responsible for ensuring transparency of the animal disease situation worldwide, including diseases transmissible to humans, as well as safeguarding the sanitary safety of world trade of animals and animal products and ensuring food safety.

The OIE works with the permanent support of over 265 Reference Laboratories and Collaborating Centres and 11 regional offices worldwide, which play a key role in veterinary scientific research and information.

Collection and publication of veterinary scientific information, notably animal disease prevention and control methods, is one of the main objectives mentioned in the OIE's 5th Strategic Plan.

Following the emergence of Schmallenberg virus in Western Europe, OIE Member Countries asked OIE to provide information and guidance. The OIE decided to convene a meeting of experts at very short notice to review existing knowledge of the new virus and provide information to its Members and stakeholders.

The existing research network made it possible to provide a consolidated expert opinion on Schmallenberg virus very rapidly and to share experiences and available knowledge in a very transparent manner.

Good Veterinary Governance is key to managing emerging diseases, and appropriate decisions have to be taken on a scientific basis. To provide such scientific knowledge, research networks are needed to coordinate scientific projects and provide consolidated expert opinions at an early stage of the event, without unnecessary delay and waste of resources.

Schmallenberg virus is a good example that demonstrates the efficiency of an existing research network, and the EPIZONE symposium will further illustrate what can be achieved when "scientific preparedness" is a reality.

Orthobunyaviruses and in particular Simbu serogroup viruses in Europe

Richard M. Elliott, University of St Andrews.

The *Orthobunyavirus* genus within the *Bunyaviridae* family contains more than 170 named viruses that can be conveniently divided into 18 serogroups. The largest of these is the Simbu serogroup with 25 viruses isolated from South America, Africa, Asia and Australia that includes a number of human (Oropouche virus) and animal (e.g. Akabane, Sathuperi, and Shamonda viruses) pathogens. Schmallerberg virus is most closely related to these animal-infecting viruses and thus represents the first occurrence of a Simbu serogroup virus in Europe. The relationships between these different viruses will be discussed. In addition, the establishment of a reverse genetic system for Schmallerberg virus (based on the system first described for the prototype Bunyamwera virus) will be reported. Exploitation of reverse genetics to create recombinant attenuated viruses with vaccine potential will be evaluated.

Schmallenberg virus from policy point of view

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M of Economic Affairs, Agriculture and Innovation¹

Key words: Schmallenberg, policy

Schmallenberg Virus (SBV) is an emerging virus detected in November 2011 in Germany. By now the virus has been detected in 8 countries in Europe. There is still much unknown about the virus and infected countries are collecting as many data as possible to get a good insight in the epidemiological situation. Much research is initiated on the pathogenesis of the disease, virus characterization, epidemiology and vaccine development.

An introduction of a new virus in a country or region is always a concern for policy makers. The first question that needs to be answered is whether the virus may also pose a risk for human health. In this case an early risk assessment judged the risk for human health as very low. From a disease control point of view for the national authorities it is furthermore very important to quickly get a good insight in the epidemiological situation. The Friedrich Loeffler Institute that discovered the virus made diagnostic techniques available so all countries had the possibility to test for the virus. To get better insight in the epidemiological situation The Netherlands and some surrounding countries chose to make the disease notifiable and reported positive results to the European Commission and to the OIE. In response to these notifications many third countries have put in place export restrictions which is a big financial burden for exporting countries like The Netherlands, Germany and France. The OIE has made a scientifically based overview of the risks of transmission of the virus by trade of animals or products, based on the currently available knowledge. However one could argue that import restrictions are not proportionate since the economical impact of the disease in the affected countries is not very high.

The introduction of SBV shows that, although the economical impact of a virus is low and it is not a zoonotic disease, there are many policy issues to be dealt with. The policy issues are national, on EU level but also related to trade with third countries.

Experiences with viruses from the Simbu serogroup in Australia and future prospects

P.D. Kirkland, Virology Laboratory, EMAI, Camden, NSW, Australia.

In Australia there are 7 vector borne viruses from the Simbu serogroup, 5 of which infect livestock. There are many aspects of the biology of these viruses that will provide an insight into the epidemiology and pathogenesis of Schmallenberg virus.

The Australian Simbu viruses are spread by *Culicoides* species, mainly *C. brevitarsis*. The geographical distribution and seasonal occurrence of a virus is determined by the abundance and activity of the vector. Therefore, virus transmission is usually limited to the summer and autumn months. Unlike bluetongue viruses, the Simbu viruses are transmitted with very high efficiency. Only small midge populations are required to spread a virus in the livestock population. As a consequence there can be a very high annual incidence, often approaching 100%. The mammalian host is partly determined by vector preferences with cattle the main host species but infections occur in small ruminants and equidae.

In a cattle population, a high level of immunity is maintained and disease in locally bred animals is rare. Disease usually occurs following changes to the usual pattern of distribution and activity of the insect. Under climatically favourable conditions, if the insect range expands, and there are pregnant animals present, disease is likely to occur. Under short-term adverse climatic conditions, the midge distribution may contract. When a normal weather pattern returns, and the usual midge distribution resumes, susceptible animals will be infected. Introduction of susceptible stock into an endemic area can result in disease.

The pathogenic Simbu viruses found in Australia almost affect developing foetus. Only Akabane is a significant pathogen and Aino virus has been infrequently associated with disease. Akabane virus induced disease in sheep in Australia is rare, firstly because few sheep are raised in regions where the main vector is present and secondly because few sheep are pregnant at the time of vector activity. The incidence, severity and range of congenital defects following infection of the bovine foetus is mostly due to the stage of gestation at which infection occurs. Hydranencephaly (HE) is the outcome of infection in the 3rd and 4th months of gestation while arthrogryposis (AG) mostly occurs following infection in the 5th and 6th months. Infection late in gestation can be manifest as encephalitis at birth, with affected calves showing a flaccid paralysis of the limbs. The incidence of defects is greater earlier in gestation but can vary with the strain of virus. An incidence of deformed calves of about 25% is common under field conditions but can be as high as 50%. Experimentally in sheep, the incidence of defects has ranged from 15-85% with different virus strains. The deformities seen in sheep or goats does not show the distinct progression from AG to HE as seen in cattle and affected progeny can show a wide range of abnormalities, including lesions in other organs such as lung and thymus.

Owing to the very high efficiency of transmission of these viruses, it is more likely that an agent such as Schmallenberg will become established in many regions. However, it is possible that the virus will only become endemic in more temperate regions, with incursions into regions that are colder, either due to latitude or at higher altitude, under favourable conditions. Both short and longer term climatic changes may play a role.

Schmallenberg Virus in France

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Anses¹; INRA²

Key words: Schmallenberg virus, molecular and serological diagnosis

On January 25th, the first case of infection by Schmallenberg virus (SBV) was reported in France. Diagnosis was established from the brain of a newborn lamb showing congenital malformation by using the SBV -specific real time RT-PCR assay developed by FLI which targets segment L of the virus. In March 2012, more than 1000 outbreaks were reported in cattle, sheep and goats.

This communication will describe the measures and actions put in place before and after the report of the first cases in France. The surveillance program will be shortly described.

The diagnostic tools used, developed and validated (molecular and serological assays) will be presented. A network of 57 regional labs has been set up for SBV detection by RT-PCR using commercial kits. The serological methods such as SNT and ELISA (with native and recombinant antigens) have allowed to precise the seroprevalence in some herds. Sequence comparisons between French and German strains will be also related.

Epidemiology of Schmallenberg Virus Infections in Germany

Conraths, Franz¹; Staubach, Christoph¹; Sonnenburg, Jana¹; Fröhlich, Andreas¹; Kramer, Matthias¹; Gall, Yvonne¹; Probst, Carolina¹; Höreth-Böntgen, Detlef¹; Teske, Kathrin¹; Kämer, Doris¹; Beer, Martin¹

FLI¹

Key words: Schmallenberg virus, epidemiology, Germany

After causing mild disease in adult dairy cattle in late summer and autumn 2011, Schmallenberg virus (SBV) infections emerged as a major cause of congenital malformations in sheep, cattle and goats from December 2012 onwards. In Germany, the majority of outbreaks reported so far occurred in sheep holdings, followed by cattle farms and goat holdings. A single case was detected in a European bison. There is serological evidence for SBV infections in roe deer and red deer in an area with a high outbreak density in domestic ruminants. Since mid-February 2012, the number of affected calves increased while the number of reported SBV cases in sheep lambs started to decrease.

The spatial distribution of reported SBV cases within Germany shows considerable variation. While affected farms have been reported from all federal states, the area with highest case density includes North Rhine-Westphalia, Lower Saxony, Hesse and Schleswig-Holstein. There seems to be a West-East- and a North-South-gradient in the density of outbreaks. First epidemiological analyses show that the spatial density of outbreaks in sheep holdings is statistically significantly associated with the population density of sheep. Backward calculations of the likely time of transplacental infection of SBV-infected lambs suffering from congenital malformations of the AHS type suggest that the majority of transplacental infections took place since mid-September 2012. The seasonal peak of the transplacental SBV-infections coincided with the peaks of the BTV-8 infections observed in 2006 and 2007 as well as with the maximum of BTV-8-infected biting midges detected in 2007. These results are in accord with the proposed role of *Culicoides* spp. in the transmission of SBV.

Role of *Culicoides* biting midges in the transmission of Schmallenberg virus

Simon Carpenter

Culicoides biting midges are among the smallest haematophagous flies commonly found on livestock and have a practically worldwide distribution. In this talk I will outline the lifecycle of *Culicoides* with reference to arbovirus transmission and then discuss their unique biology through comparisons drawn with other blood-feeding Diptera such as mosquitoes and sandflies. These studies will highlight aspects of the epidemiology of *Culicoides*-borne arboviruses that are poorly understood and additionally draw conclusions regarding barriers to virus spread from patterns of bluetongue virus (BTV) movement in southern and northern Europe. I will then specifically examine the reasons why arbovirus transmission appears to be limited to selected species within the genus. This will include a discussion of characterised and inferred barriers to arbovirus dissemination within *Culicoides* of both laboratory and field origin, which are thought to be inheritable and environmental factors modifying such barriers. I will also discuss results of recent studies that have examined the infection and dissemination of Schmallenberg virus in colony lines of *Culicoides* and mosquitoes as models vectors for future research. These results are then compared with those produced for BTV in the laboratory with the same colony lines. Finally, I will consider the future of vector competence research on *Culicoides* and arboviruses worldwide. This will include a discussion of the recently initiated *Culicoides* genome project, based at the Pirbright laboratory, which will enable detailed analyses of genetic components of vector competence and opportunities for worldwide collaboration.

Evidence for *Culicoides obsoletus* group as vector for Schmallenberg virus in Denmark

Rasmussen, Lasse Dam¹; Kristensen, Birgit¹; Kirkeby, Carsten¹; Rasmussen, Thomas Bruun¹; Belsham, Graham J¹; Bodker, René¹; Botner, Anette¹

VET-DTU¹

Key words: Schmallenberg virus RNA, RT-PCR, biting midges, vector, *Culicoides* spp

Schmallenberg virus (SBV) was first identified in Germany in late 2011 by the Friedrich Loeffler Institute and has now been found in several European countries including Holland, France, Belgium, U.K. and Spain. The disease, which affects sheep, cattle and goats, was first recognized due to transient clinical symptoms including fever, diarrhea and loss of milk production. However, a more significant consequence of infection in pregnant animals is the production of severe congenital malformations in newborn animals, especially lambs. The virus is a member of the Orthobunyavirus genus within the Bunyaviridae family and is closely related to Shamonda and Akabane viruses. These viruses are transmitted by insect vectors (including biting midges (*Culicoides* sp.) and mosquitoes). To determine whether these insects may act as vectors for SBV, biting midges (*Culicoides* spp.) caught in October 2011, in the south-west of Denmark (close to the German border), were sorted into pools and tested for the presence of Schmallenberg virus RNA by RT-qPCR. From 18 pools of 5 midges from the *C. obsoletus* group, 2 pools were both found positive in two separate assays, targeting the L- and S- segments of the SBV RNA. However, 4 pools of *C. punctatus* s.str were negative. The sequence of 80bp (excluding the primer sequences) from the amplicons (ca. 145bp) was identical to that published for the expected region of the SBV L-segment. The levels of SBV RNA detected in the biting midges were much higher than could be accounted for due to the residue of a blood meal and no ruminant actin mRNA could be detected either. These results strongly suggest that SBV has replicated within specimens of the *C. obsoletus* group and indicates that these biting midges can act as vectors for this virus. To date (end of March), no cases of disease due to SBV have been detected in sheep, cattle or goats in Denmark.

RT-PCR screening for Schmallenberg virus in *Culicoides* spp. caught in Belgium in 2011.

De Regge, Nick¹; Deblauwe, Isra²; Vantieghem, Pieter²; De Deken, Reginald²; Smeets, François³; van den Berg, Thierry¹; Cay, Ann Brigitte¹

VAR-CODA¹; Institute of Tropical Medicine²; Université de Liège³

Key words: SBV, *Culicoides*, Belgium, RT-PCR, vector

Since Schmallenberg virus (SBV) was first identified by researchers from the FLI (Germany) in November 2011, its presence has in the meanwhile been confirmed in the Netherlands, Belgium, United Kingdom, France, Luxembourg, Italy and Spain. The rapid and large expansion of this virus, together with the knowledge that related viruses belonging to the same Simbu serogroup of Orthobunyaviruses are generally spread by midges and mosquitoes, led to the assumption that also SBV is probably spread by these vectors. In order to examine the potential role of midges in the spread of SBV, midges caught at several locations in Belgium by UV light traps were analyzed by RT-PCR (protocol for L and S segments detection were kindly provided by FLI, Germany). Till now, midges caught in September and October 2011 were screened after they were morphologically identified at species level and pools consisting of ≤ 20 heads of *Culicoides* were prepared. Initial experiments confirmed that the RT-PCR detecting the S segment was more sensitive than the PCR detecting the L segment, and was consequently used for further screening. At the time of writing, 214 pools were already screened and 34 pools originating from 8 different locations (Betekom, Berlaar, Eindhout, Verlaine, Bonnelles, Sart-Tillman, Nandrin and Bettincourt) were found positive for SBV. These positive pools consisted of heads of parous females of *C. obsoletus* s.s., *C. obsoletus* complex, *C. dewulfi*, *C. pulicaris* and *C. chiopterus*. None of the pools containing nulliparous midges tested positive. These results strongly indicate a role of at least 4 different species of biting midges in the transmission and spread of SBV. This indication is strengthened by the fact that pools consisted exclusively of heads, suggesting that midges act as real amplification vectors and were not simply SBV positive after a blood meal on viraemic animals. More pools of *Culicoides* will be tested to identify other possible SBV vectors among *Culicoides* species and *Culicoides* caught earlier during the vector season will be tested to shed light on the time of introduction of SBV in Belgium.

Comparative analysis to determine the most appropriate tissue for Schmallenberg virus detection by RT-PCR in aborted lambs and calves

De Regge, Nick¹; Van Loo, Hans²; Bertels, Guido²; Saulmont, Marc³; Vandebussche, Frank¹; van den Berg, Thierry¹; Cay, Ann Brigitte¹

VAR-CODA¹; Diergezondheid Vlaanderen²; ARSIA³

Key words: SBV, diagnosis, RT-PCR

In November 2011, scientists from the Friedrich-Loeffler-Institute (FLI) in Germany identified a new virus in the blood of dairy cows that showed clinical signs such as fever, milk drop and diarrhea. The newly identified virus was named Schmallenberg virus (SBV) and belongs to the Simbu serogroup of the genus orthobunyaviruses. Besides the clinical signs in adult cattle, the virus is transmitted to the fetus when infection occurs during early pregnancy and causes congenital malformations in lambs and calves. Malformations that are often noticed at autopsies are torticollis, arthrogryposis, scoliosis, brachygnathia inferior and brain abnormalities like hydranencephaly and hypoplasia of the cerebrum and/or cerebellum. Since lambs and calves that are suspected to be infected with SBV on the basis of gross lesions need to be confirmed by diagnostic tests, we determined the most suitable tissue for SBV detection by RT-PCR.

Cerebrum, cerebellum, brain stem, spinal cord, thymus, spleen and lymph nodes from suspected lambs were tested by a RT-PCR detecting the L segment of the virus (protocol kindly provided by FLI, Germany) to determine the viral load. As could be expected from the neurological symptoms and lesions, brain material seemed to be the most appropriate matrix to detect SBV by RT-PCR. SBV was detected in the brain stem of all animals that were found positive in at least one tissue tested, followed by cerebellum (83%) and cerebrum (81%), showing that brain stem is the most suitable tissue for SBV detection in aborted lambs. In a considerable amount of lambs that were found positive for SBV in brain samples, SBV could also be detected in lymphoid tissues like spleen (31%), lymph nodes (43%) or thymus (27%), suggestive for a possible lymphotropic tropism of this virus.

For aborted calves, a similar analysis was carried out and the viral load in cerebrum, cerebellum, brain stem, spleen and meconium were also compared but using the more recent RT-PCR detecting the S segment of the virus (protocol also kindly provided by FLI, Germany). Using this more sensitive protocol, the brain stem was found positive in 90% of the calves that were found positive in at least one tissue tested, followed by cerebrum (65%) and cerebellum (48%). Theoretically, a pool consisting of brain stem and cerebrum would lead to a sensitivity of 96 % compared to when all tissues would be tested. Meconium could be found positive in 33% of the cases and spleen only in 5%.

This study shows that SBV can be found in several different organs in aborted lambs and calves infected by the virus. Brain stem however seems to be the preferred matrix for SBV detection by RT-PCR if only one organ has to be considered for testing.

Biological and genetic characterizations of two isolates of Schmallenberg virus (SBV) propagated from naturally infected sheep fetuses

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University of Namur¹

Key words: virus isolation, titration, plaque assay, molecular epidemiology, genetic drift

During the lambing period of January 2012, severe atrogryposis and nervous system defects were observed in 28 lambs delivered by 99 ewes whose breeding period started at mid-August 2011. From RNA samples extracted from brain and blood of the 28 deformed lambs, 10 were tested positive for SBV infection through real-time quantitative reverse transcription PCR (RT-qPCR) developed by the Friedrich Loeffler Institute (Hoffmann et al., 2012), with cycle threshold (Ct) values of 15 to 40. The Ct values obtained on the nervous system of two animals showed high levels of viral RNA copies in these samples respectively tested with Ct of 15.3 and 19.6.

Two viruses were isolated from brain tissues prepared from the two highly positive animals. Pieces of 500 mg of homogenized tissue were suspended in cell culture medium. Serial dilutions (1/2, 1/4 and 1/8) of the tissues suspensions were incubated on Baby Hamster Kidney cells (BHK-21) for virus isolation. The inocula were removed after 3 hours, and replaced with Glasgow Minimum Essential Medium. At the second passage, cell cultures inoculated with SBV-RT-qPCR positive brain tissues showed several plaques compatible with viral cytopathic effect. The amplification of SBV was confirmed by testing in RT-qPCR the culture supernatants collected at two consecutive days. Viral stocks were prepared from passages 3 of the two viral isolates designated SBV-Namur1 (-Na1) and SBV-Na2.

The biological characterization of the two isolates included (i) viral titration, (ii) plaque assay and (iii) neutralization assays. (i) The viral titers were 2 and 3 log₁₀ lower than the virus titer of the cell adapted SBV-FLI strain. (ii) The plaque assay, adapted from a protocol used for murine norovirus (Mathijs et al., 2010) showed that the plaque sizes and morphologies induced by SBV-Na1 and -Na2 were different from those induced by the SBV-FLI strain. (iii) Virus infectivity of SBV-Na1 and -Na2 was neutralized both by the reference positive serum (provided by the FLI) and by serum obtained from ewes tested positive in SBV serum neutralization assay.

The genetic characterization was based on the PCR amplification and sequencing of the whole S segment and five overlapping fragments encompassing the whole M segment. Sequencing of the S fragment showed three mutations shared by the two isolates, allowing discrimination of the SBV-Na1 and -Na2 viruses from the original German SBV isolate. These mutations were identified before and after the cell culture amplification. Sequencing of the amplicons obtained in the M segment will be completed in the next weeks.

In conclusion, two SBV isolates were obtained from naturally infected sheep fetuses through a direct isolation assay on BHK cells. The preliminary biological and genetic characterization indicated that the two isolates are different from the original German SBV isolate. The level of genetic drift (both between the two isolates and in comparison with other cattle and sheep SBV isolates) will be further examined through whole genome analysis.

Natural infection of a sheep flock with Schmallenberg virus: clinical, serological and virological features

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University of Namur¹

Key words: sheep, transplacental infection, incidence, PCR, seroneutralisation

The first manifestations of newborn or stillborn lambs that were affected by Schmallenberg virus (SBV) were reported at 23 of December 2011 in the Northern part of Belgium. The sheep flock of the University of Namur was one the first SBV-affected flocks located in the Southern part of Belgium; the first stillbirth of a SBV-affected lamb occurring on 7th January 2012. The present investigation provides information about the clinical manifestations, the serological and virological findings of in utero SBV-infected lambs born in January 2012.

Pregnant ewes (n=99) and their lambs (n= 163) born in January 2012 were investigated. Serum samples were prepared from all ewes. In case of normal lambing and birth of clinically healthy lambs, blood was sampled immediately after birth (T0) as well as 36h after colostrum intake (T36) in randomly selected healthy lambs. In case of SBV-lambing, blood was sampled in all clinically healthy siblings at T0 and T36 and whenever possible, at T0 in lambs showing clinical signs of SBV infection. SBV-affected lambs underwent necropsy within 36h and central nervous system (CNS) tissue was sampled. Virological diagnosis of SBV was performed on CNS tissue by using RTq-PCR and seroneutralisation (SN) for SBV was performed on serum sampled at T0 and T36. RTq-PCR results were considered positive if Ct was lower than 40. Results of SN were expressed as positive or negative.

Among the 99 ewes, 76 gave birth to clinically healthy lambs (n=124) and 23 gave birth to 28 SBV-affected lambs and 11 clinically healthy siblings. Birth of SBV-affected lambs was more frequent in primiparous ewes (9 SBV lambings vs 11 normal lambings) than in multiparous ewes (14 SBV lambings vs 65 normal lambings). Clinical manifestations of SBV-affected lambs included stillborn lambs presenting severe arthrogryposis (n=4), lambs with severe arthrogryposis and dying at birth by asphyxia because of complete muscle paralysis (n=18), and alive lambs showing arthrogryposis of at least one limb (n=6) and that were euthanased shortly after birth. RTq-PCR was positive for 10 SBV-affected lambs. SN revealed positive results in 9/11 SBV-affected lambs at T0. Among clinically healthy siblings of SBV-affected lambs, 6/7 showed SBV-antibodies prior colostrum intake and all were tested positive at T36. Clinically healthy lambs born without SBV-affected siblings (27 lambs tested) did not show SBV antibodies at T0 but were positive at T36, indicating that their ewes had undergone seroconversion. All 99 ewes showed positive results for SN.

This investigation of this sentinel sheep flock revealed that SBV infection had occurred in 100% of the ewes that lambed in January 2012. The incidence of transplacental infection equaled 23% and was higher in primiparous ewes. Transplacental infection led to 17% of lambs showing clinical signs of in utero SBV-infection which could be detected by RTq-PCR in CNS tissue in 37% of the cases. SN assay revealed positive results in 82% of SBV-affected lambs at birth and in 86% of their clinically healthy siblings, whereas gestations leading to exclusively clinically healthy lambs did not suggest the existence of asymptomatic in utero infection by SBV.

The authors acknowledge Benoît Bolkaerts, Christine Baricalla, Marianne Raes, Nicolas Noël and Amélie Limbourg for their valuable technical assistance during lambings, sample collection and sample analysis. Martin Beer and the Friedrich Loeffler Institute are acknowledged for providing material and protocol allowing virological and serological diagnosis of SBV.

“Schmallenberg” virus from sheep: Molecular characterization of virus present in the brain of a malformed lamb

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⁴

Key words: “Schmallenberg” virus, molecular characterization, brain, malformed lamb

A novel orthobunyavirus, “Schmallenberg virus”, was detected in cattle (BSBV) in the end of the summer in Germany (1), and in late autumn in sheep (OSBV) in the Netherlands. To compare the sequences of the genomic segments (L, M and S) of OSBV present in the brain of a malformed, stillborn lam to that of BSBV originating from blood of cattle, OSBV was sequenced. Using primers derived from the published BSBV sequence (1) overlapping cDNA fragments were amplified directly from total RNA isolated from brain tissue of the affected lamb and analyzed by Sanger sequencing. In addition, OSBV was isolated and cultured in Vero cells. After grown for 5 passages on these cells virus was concentrated from the culture medium and enriched for negative sense viral RNA segments. A cDNA library prepared from this RNA was sequenced using the MiSeq genome analyzer from Illumina. Full-length genome sequences of the L, M and S segments of native and tissue culture OSBV were assembled and compared to the published sequences of BSBV originating from cattle. The results of these comparisons will be presented.

(1) Bernd Hoffmann, Matthias Scheuch, Dirk Höper, Ralf Jungblut, Mark Holsteg, Horst Schirrmeyer, Michael Eschbaumer, Katja V. Goller, Kerstin Wernike, Melina Fischer, Anke Breithaupt, Thomas C. Mettenleiter, and Martin Beer (2012). Novel Orthobunyavirus in Cattle, Europe, 2011. EID Volume 18, Number 3 March 2012.

"Schmallenberg" virus: the EFSA reports on analysis of the epidemiological data and overall assessment of the impact on animal health, animal production and animal welfare together with a characterisation of the pathogen

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Following a request from the European Commission, the European Food Safety Authority (EFSA) issued a technical report in February 2012 on likely epidemiological scenarios in Europe in relation to the recently detected virus provisionally named "Schmallenberg" virus (SBV) (Simbu serogroup, Bunyaviridae family, genus *Orthobunyavirus*), found in ruminants. The report also included guidance on data to be collected in Member States, with harmonised case definitions and reporting guidelines for a minimum dataset at herd/flock level and an extended dataset at animal level. Three reports containing analyses of epidemiological data collected by affected countries based on that guidance and an overall impact assessment have been published by 14 June 2012.

As of the reporting deadline of 15 May 2012, eight Member States (Belgium, France, Germany, Italy, Luxembourg, the Netherlands, Spain and United Kingdom) had confirmed cases of SBV¹. The total number of SBV confirmed herds in Europe as of the 15 May 2012 was 3745. No confirmed acute cases had been reported in adult animals in 2012 to that date.

The maximum proportion of reported sheep holdings with SBV confirmed was 4% per country and 7.6% per region while for cattle less than 1.3 % of holdings were reported as SBV confirmed at both country and regional level. The data collected indicates that the impact of SBV is greater in sheep than in cattle holdings. The impact on animal welfare and animal production as well as the within herd impact were not assessed due to lack of data.

In order to assess the impact of SBV (spatial and temporal spread, proportion of affected holdings and potential projection of arthrogryposis hydranencephaly syndrome (AHS) cases) three models were used. The geographical spread model predicts that SBV is most likely to re-emerge between mid-April and the end of May 2012 with the most likely affected areas being at the south and east regions of the previously-affected areas. The model of geographical and seasonal within holding transmission using bluetongue virus (BTV) parameters suggests that virus transmission and spread become possible at temperatures around 15°C with a temperature optimum between 18-19°C with most of Europe having a suitable climate for within holding vector borne transmission. The projection model shows that further cases of AHS are likely to be very rare in lambs for the year 2012 after April and that further cases in calves could be observed until July. Assuming SBV survival in winter, the models suggest that in unaffected regions or regions with low prevalence with suitable temperatures for within herd transmission by vectors and high density of susceptible species (cattle and sheep), SBV infection is likely to spread in 2012.

EFSA recommends to continue serological investigations in affected regions and regions neighbouring affected areas, to investigate impact within herds and at animal level and to monitor the putative vector population. The possible origins of the virus should be investigated as more information becomes available on the virus characteristics and infection epidemiology.

¹ Since then, Denmark has confirmed the presence of SBV through laboratory testing.

Evaluation of the zoonotic potential of Schmallenberg virus.

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Key words: sbv vnt zoonoses

The recent emergence of Schmallenbergvirus (SBV) as a cause of malformations in ruminants has triggered questions of possible risks to human health. In a rapid risk assessment, we concluded that the potential for human infection is low but can not be excluded. Therefore, as a precautionary measure, the Center for Infectious Disease Control in the Netherlands monitors since December 2011 the occurrence of cases of febrile illness within 2 weeks of direct exposure during delivery of ruminants from affected farms. These events triggered a laboratory response to address possible human infections with SBV. The laboratory response included the implementation and validation of a SBV reverse-transcription polymerase chain reaction and virus neutralization test (VNT).

To address the zoonotic potential of SBV, an integrated serological study to assess possible evidence for infections of humans was conducted using the recently developed VNT. These serological studies target populations expected to be at a high risk for exposure, either via the vectorial route or through direct contact by living/working at or in proximity of affected farms. The following serum collections were analysed::

- 1) sera collected in the period 15 July- 15 October 2011 in municipalities with known affected SBV farms were compared with sera collected in the same period and municipalities in 2010.
- 2) paired serum samples collected from veterinary students collected in 2006/2008 and 2011;
- 3) sera collected in March/April 2012 from farmers, workers and residents of affected SBV ruminant farms were compared with sera collected from farmers, workers and residents of ruminant farms in 2009;
- 4) sera collected from veterinarians who assisted at SBV affected farms since summer 2011 were compared with sera collected from veterinarians assisting at ruminant farms in 2009.

The sampling and testing of farmers, workers (including veterinarians) and residents of SBV affected farms in March/ April 2012 (studies 3 and 4) was part of a larger epidemiological study including questionnaires addressing putative risk factors for SBV exposure/infection. The laboratory results and, if zoonotic transmission occurs, preliminary data on human risk factors will be presented.

Seroprevalence of antibodies to Schmallenberg virus in dairy cattle, winter 2011-2012, The Netherlands

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CVI¹; NVWA²; GD Deventer³

Key words: Orthobunyavirus, Schmallenberg virus, seroprevalence, cattle

Introduction

Since the autumn of 2011, infections with Schmallenberg virus (SBV) have been associated with congenital malformations in calves, lambs and goat-kids in at least five European countries. Currently there is limited knowledge specifically related to SBV. In the Netherlands, there is an obligatory reporting of suspect cases (occurrence of malformations of the arthrogryposis hydranencephaly syndrome) followed by confirmatory testing of brain tissue samples by RT-PCR. It is likely that observed suspect cases underestimate the true rate of infection. Therefore there is a definite need for serodiagnostic studies to detect past exposure to SBV in ruminant populations in the affected countries.

Material and Methods

A seroprevalence study was executed to detect past exposure to SBV in dairy cattle in the Netherlands. Furthermore, in order to get some preliminary insight into the within-herd seroprevalence of infected herds (based on PCR test results), in two sheep flocks and two cattle herds a considerable number of animals were blood-sampled.

A stratified random sampling design was set up, with the 12 provinces in the Netherlands as a stratification level. The sampling frame comprised of dairy cattle that were blood sampled in the period November 2011 – January 2012 a) for monitoring testing of antibodies to Bluetongue virus, and b) in the framework of a surveillance investigation in 125 dairy farms to exclude introduction of Brucella and Foot and Mouth Disease. Because we presumed a high intra-class correlation with respect to serological status of animals within herds (based on preliminary test results from a few infected herds), on average two dairy cattle (minimum: 1, maximum: 4) from the same dairy herd were allowed in the sampling list to prevent occurrence of too many cattle from the same herd. This selection procedure resulted in a total of 1,123 samples of dairy cattle from 489 dairy herds to be tested. The mean age of cows tested was 23 months (range: 12 – 79 months). Sera from 1,123 randomly selected dairy cattle were tested for antibodies to SBV using a virus neutralisation test.

Results

SBV-seroprevalence in dairy cattle was 73% (N=1123; 95% confidence interval of seroprevalence (CI): 70 – 75%). SBV-seroprevalence of dairy cattle in the central-eastern part of the Netherlands (N=462; seroprevalence: 83%, 95% CI: 79 – 86%), was significantly higher compared to that in the northern (N=465; seroprevalence: 67%, 95% CI: 63 – 71%) and southern part of the Netherlands (N=196; seroprevalence: 61%, 95% CI: 54 – 68%).

High (70-100%) within-herd seroprevalence was observed in two SBV-infected sheep and dairy herds in which a considerable number of animals was tested.

Implications

We showed a high seroprevalence of antibodies to SBV present in dairy cattle in the Netherlands in the winter of 2011-2012. This indicates widespread exposure to SBV in 2011, and exemplifies the considerable underestimation of the infection rate when one can only rely on observation of clinical suspect cases. Furthermore the SBV-seroprevalence in dairy cattle was significantly higher in the central-eastern part of the Netherlands compared to the northern and southern part of the Netherlands. This could be an indication that introduction of SBV into the Netherlands started somewhere in the eastern part of the Netherlands. Our preliminary results concerning SBV within-herd seroprevalence indicate that by the end of an outbreak season, most of the animals within an affected herd might be infected.

Detection en epidemiological findings of Schmallenberg virus in the Netherlands

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Key words: Schmallenberg virus, impact, risk factors, dairy cattle

The aim of the study is to describe the detection of the Schmallenberg virus (SBV) epidemic in the Netherlands and the epidemiological findings in infected dairy herds. In the Netherlands, a surveillance system is in place for detection of (emerging) infectious diseases at the Animal Health Service. This system consists, amongst others, of a telephone service that provides advice to veterinarians and farmers about animal health related problems that they encounter. The signals that are obtained from the field are discussed weekly in a team of veterinarians, epidemiologists and pathologists. At the end of August 2011, several reports were made – first in the eastern part of the country - of a severe drop in milk production, watery diarrhoea and sometimes fever in dairy cattle. After two weeks, reports were also received from other regions, however reporting ceased in October. Blood and manure samples of clinical cases were then obtained and tested, but no definite causal agent was found. On November 18th, the Friedrich Loeffler Institute (FLI) in Germany reported the isolation of SBV. In the first week of December, an increased number of congenital malformations in new-born lambs throughout the country were reported. Ovine congenital malformations are not uncommon, but usually only a few cases occur per farm. In this case many flocks and many lambs were affected throughout the country. A real-time PCR for SBV was carried out on brain tissue of 54 deformed lambs, on 50 serum samples of dairy cows which had shown clinical signs in August, and on 115 serum samples of healthy cows sampled in November. In 22 lambs and 18 cows the virus was detected. The healthy cows all tested negative. The first clinical cases in calves were reported in December and on January 23rd the first two calves were tested SBV positive. Up to mid-March, about 700 calves, 300 sheep and 30 goats are submitted for necropsy with clinical signs of SBV. To identify clinical symptoms following SBV infection as well as potential risk factors for introduction of SBV in dairy cattle herds, a case-control study was set up. Data will be collected in 150 dairy herds; 75 confirmed cases of SBV and 75 herds without any signs of SBV. Seventy cows will be sampled per herd and tested in an indirect ELISA. Risk factor and morbidity data will be collected by means of a questionnaire. All stillborn calves and calves that die within 3 days after birth will be necropsied. Calves of 32 dams in infected herds (16 PCR positive / 16 PCR negative) will be tested at birth (pre-colostrum) and in subsequent months with an ELISA and PCR. Census data from sampled herds regarding production, fertility and animal health will be analysed for 2008-2012 using national databases.

Preliminary findings of the impact of SBV on morbidity and mortality rates in adult cattle and calves after primary infection with SBV as well as in calves that have been exposed to SBV during foetal development will be presented. The relation between seroprevalence and the level of clinical symptoms at herd and animal level will be discussed, as well as possible herd- and cow-level risk factors for introduction and within-herd transmission in dairy herds. The study will provide information on the many aspects and impact of a primary SBV infection in dairy herds. Also, the sensitivity of the Dutch surveillance system for SBV and emerging diseases in general is discussed.

Development of a pan-Simbu real-time RT-PCR for the reliable detection of Simbu serogroup viruses

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FLI¹

Key words: Orthobunyavirus, Simbu serogroup, Schmallenberg virus, real-time RT-PCR

Schmallenberg virus (SBV), a novel Orthobunyavirus from the Simbu serogroup, was first identified in German dairy cattle in October 2011 where it caused fever, diarrhea and a decreased milk production. SBV was also detected in sheep and goats where it causes congenital malformations and stillbirths. Concerning this current occasion, we developed a pan-Simbu real-time RT-PCR system for the reliable detection of viruses from the Simbu serogroup and compared it with different diagnostic SBV real time RT-PCR assays. All PCR systems were tested with a panel of different Simbu serogroup viruses as well as several field samples from diseased cattle, sheep and goats originating from all over Germany. The pan-Simbu real-time RT-PCR system was able to detect all tested members of the Simbu serogroup as well as most of the field samples. Furthermore, in silico analyses indicate the capability for the detection of a broad Orthobunyavirus spectrum. For the diagnosis of SBV the SBV-S3 assay turned out to be most suitable with an analytical sensitivity for the SBV-S3 single assay determined as one copy per well and for two duplex assays including an internal amplification control (IC2-RNA, beta-actin) defined as ten copies per well for both duplex systems.

Schmallenberg virus outbreak in The Netherlands: Routine diagnostics and test results

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CVI¹

Key words: Schmallenberg virus, diagnostics, results

At the end of 2011, a new Orthobunya virus named Schmallenberg virus (SBV), was discovered in Germany. Soon thereafter in the Netherlands, the virus was associated with decreased milk production, watery diarrhoea and fever in dairy cows, and subsequently also with congenital malformations in calves, lambs and goat kids. By the 20th of December 2011 in the Netherlands malformations in new-borns of ruminants were made notifiable. After a notification by a farmer or veterinarian, a maximum of five malformed new-borns per farm were necropsied. The diagnosis of Schmallenberg virus disease was based on the pathologic findings and RT-PCR test results of brain tissue of the malformed new-borns. In addition blood samples from mothers of affected new-borns were collected and tested for antibodies against SBV using a virus neutralization test. Between 20th of December and March the 8th, in total 1165 brain tissue samples were tested in the RT-PCR: 577 originated from lambs, 444 from calves and 42 from goat kids. In the VNT 681 blood samples were tested: 329 originated from ewes, 329 from cows and 23 from goats. Results showed that 8% of the tested calf brains, 31% of the tested lamb brains and 12% of the tested goat kid brains were RT-PCR positive. The number of malformed lambs and RT-PCR positive lamb brains decreases over time while the number of malformed calves and RT-PCR positive calf brains increases. In the VNT 95% of the ewes, 93% of the cows and 23% of the goats tested positive. Combining the results of the RT-PCR and the VNT, 20% of all farms tested positive in both the RT-PCR (genetic material of SBV in brain tissue in malformed new-borns) and the VNT (antibodies against SBV in blood from mothers of affected new-borns). In goats the number of seropositives is far lower than in sheep and cattle. Less samples from goats were tested and the estimated seroprevalence is therefore less precise. Furthermore number of RT-PCR positive calves and goat kids is far lower than in sheep. Given that goats, in contrast to cattle and sheep, are often housed indoors, and the SBV is supposedly transmitted by *Culicoides* vectors, this lower number of test positive results would not be surprising. In addition, the difference in pregnancy length between cows and sheep might explain why it is more difficult to detect genetic material of SBV in brain tissue of calves and, assuming that infection of cows and sheep was around the same period, it might also explain the difference in number of malformations and RT-positives over time. Supposing that all malformations notified are truly caused by the Schmallenberg virus, on farm level, diagnostic sensitivity of the RT-PCR is much lower in comparison with the VNT. The results reported here are based on testing up to the first week of March 2012. Additional test results which will be available in June will be presented also.

POSTER S1: PRELIMINARY VALIDATION OF THE ID SCREEN® SCHMALLEMBERG VIRUS INDIRECT ELISA

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IDvet¹; Anses²; VAR-CODA³

Key words: Schmallenberg virus, serology, ELISA

Introduction:

Schmallenberg virus (SBV) is the name given to a vector-transmitted orthobunyavirus related to the Shamonda and Akabane viruses, initially reported in November 2011. The disease has caused foetal congenital malformations and stillbirths in cattle, sheep, and goats. The virus has been detected in Germany, the Netherlands, Belgium, France, Luxembourg, Italy and the United Kingdom.

Serological testing is essential for disease surveillance and epidemiological studies. While antibodies can be detected by virus neutralization and immunofluorescence, these techniques are time-consuming, difficult to implement for large numbers of samples, and do not offer standardized result interpretation.

Available as of March 2012, the ID Screen® Schmallenberg virus Indirect ELISA is the first ELISA developed for SBV diagnosis. The test allows for the detection of SBV antibodies in ruminant serum and plasma. It is a rapid, standardized assay which is automatable and therefore suited to high throughput testing.

This study presents validation data for this test. Data will be added for any second generation tests developed by IDvet between March and June 2012.

Method:

- The ID Screen® Schmallenberg virus Indirect ELISA was performed as per manufacturer's specifications.
- Specificity was studied using panels of sera collected prior to 2010.
- Sensitivity was evaluated on sera tested positive with the virus neutralization test (VNT).
- An experimental infection was performed in April 2012 in order to evaluate the seroconversion response in cattle, sheep and goats.

Results and Discussion:

Preliminary validation studies indicate excellent test specificity and high correlation with other serological techniques, making the the ID Screen® Schmallenberg virus Indirect ELISA an efficient tool for disease surveillance and epidemiological studies.

POSTER S2: DEVELOPMENT OF A REAL TIME RT-PCR DIAGNOSTIC TEST, ADIAVET™ SCHMALLEMBERG VIRUS, FOR DETECTION OF THE NEW ORTHOBUNYAVIRUS

Gracieux, Patrice¹; Versmisse, Yann¹; Leborgne, Maelle¹; Blanchard, Beatrice¹

Adiagene¹

Key words: Schmallenberg virus, diagnosis, real-time RT-PCR

The Schmallenberg virus (SBV) was isolated for the first time in Germany in 2011 by the FLI from blood of infected cows. The name is based on the geographic origin of the virus (village of the North Rhine-Westphalia). First phylogenetic analyses suggest that the novel virus is a Shamonda-like virus within the genus Orthobunyavirus and Simbu serogroup.

Clinical signs of SBV infection in adult ruminants are mainly mild or non-existent. The main clinical signs of SBV are congenital malformations in newborn animals.

The viruses of Simbu serogroup are transmitted by insects (Culicoides midges and mosquitoes). It is likely that SBV is also transmitted by these insects but this has not been confirmed yet.

Viral culture and PCR amplification are the only methods to detect the virus.

Adiagene developed a real time RT-PCR test according to the guidelines of the AFNOR XP U47-600-2 standard for the development and validation of a veterinary PCR kit to detect specifically the Schmallenberg virus.

The real-time ADIAVET™ SCHMALLEMBERG VIRUS ready-to-use RT-PCR kit provides a screening assay for the detection of Schmallenberg virus in blood (EDTA whole blood, serum or plasma) and tissue samples of cattle and smaller ruminants. Simultaneous detection of the Schmallenberg virus and an endogenous ruminant gene allows the validation of all the steps (extraction and amplification) of the analysis process for all the samples.

The specificity of the kit was evaluated against 97 organisms preferentially found in the same ecologic niche than SBV and/or close phylogenetically related. No cross-reaction was observed with others organisms tested including Akabane virus, an amplification can be observed with Shamonda virus. This data is in agreement with the phylogenetic analysis leading to consider SBV as a Shamonda-like virus.

The specificity of the kit was also evaluated on a panel of 21 qualified SBV RNA solutions provided by European laboratories. Synthetic SBV RNA was produced and quantified to assess the detection limit of the PCR generating a positive result in 95% of cases.

RNA extraction protocols were developed and validated to detect the virus in tissue (brain, spleen), blood and sera sampling from bovine, ovine or caprine. The analysis of the tissue samples needs a grinding step before the RNA purification. Two grinding processes have been validated (Mixer mill (Verder) and Ribolyser (MP Biomedical)). Easy RNA purification protocols have been developed based on silica column or magnetic bead technologies.

The detection limit of the method (RNA extraction and PCR amplification) generating 100% positive results was assessed by spiking SBV-free samples with a titrated viral culture. Each level of spiking was analysed in eight times in two experiments.

The diagnostic sensitivity was evaluated from 32 field samples with known SBV status according to the reference RT-PCR test given by the ANSES Maisons-Alfort (results obtained with FLI methods). The 20/32 SBV positive samples were found positive with ADIAVET™ SCHMALLEMBERG VIRUS kit and 12/32 qualified as negative were found negative.

The ADIAVET™ SCHMALLEMBERG VIRUS kit allows the specific detection of all Schmallenberg virus tested. The kit showed good performance to detect SBV from field samples. The French Agency for Food, Environment and Occupational Health Safety recommended to veterinary laboratories the use of ADIAVET™ SCHMALLEMBERG VIRUS kit.

POSTER S3: DEVELOPMENT OF A SCHMALLEMBERG VIRUS ANTIBODY ELISA

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IDEXX Switzerland AG¹; IDEXX Montpellier SA²; IDEXX Laboratories Westbrook³

Key words: Schmallenberg virus, antibody ELISA, Orthobunyavirus

Introduction

Schmallenberg virus was first identified in Germany in late 2011 as a new Orthobunyavirus genetically highly similar to viruses of the Simbu serogroup. The virus infects ruminants such as cattle, sheep and goats. Schmallenberg virus infections have been recently confirmed in several European countries including Germany¹, The Netherlands, Belgium, France, United Kingdom, Luxembourg, Italy and Spain. Clinical signs include reduced milk yields, fever, diarrhea, abortions and malformed newborns. Virus detection by real time RT-PCR or virus cultivation is not the method of choice for infection monitoring due to the very short viremic period. Therefore, there is an urgent need for an antibody ELISA to identify infected animals.

Materials & methods

Hundreds of samples originating from clinically affected herds, from virus positive animals and from areas with no Orthobunyavirus presence are being collected. Samples will be further characterized by using an inhouse immunofluorescence assay, PCR and epidemiological and clinical data. An antibody ELISA will be described. Microtiter plates will be coated with inactivated Schmallenberg-virus antigen. Binding of Schmallenbergvirus antibodies will be visualized by colour change in the wells of the microtiter plate. The diagnostic relevance of the result will be assessed by comparing the optical density (OD) of the samples with the OD of the positive control.

Results

Preliminary data will be presented. Specificity and sensitivity data will be calculated using characterized samples. The data will allow for setting a cut-off to identify Schmallenbergvirus antibody positive and negative samples.

Discussion & conclusions

The data will be discussed and conclusions will be proposed according to obtained antibody test performance.

Acknowledgements

We kindly acknowledge the collaboration with the FLI (Friedrich-Loeffler-Institut), Insel Riems, Germany.

We also would like to thank for the assistance by various institutes and veterinarians across Europe in helping to collect samples and additional data.

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POSTER S4: First report of Schmallenberg virus in Italy

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IZS-Ve¹; Istituto G. Caporale²

Key words: Schmallenberg virus, Italy, goat

In the last months of 2011 and at the beginning of 2012, Schmallenberg virus (SBV) has been reported in ruminants (cattle, sheep, goats and bison) in Germany, the Netherlands, Belgium, United Kingdom, France, Italy and Spain. Preliminary studies on its genome suggest that the virus is a member of the Simbu serogroup belonging to the Bunyaviridae family, genus Orthobunyavirus. Animals infected with SBV show mild clinical signs persisting for approximately a week and characterized by fever, loss of appetite, up to 50% reduction in milk yield and, sometimes, severe diarrhoea. SBV infection is also associated to foetal malformation and stillbirths in domestic ruminants. At the beginning of February, a female goat that died the day after parturition of a healthy kid was submitted for post-mortem examination. The post-mortem revealed the retention of a dystocic foetus showing congenital malformations, namely scoliosis, arthrogryposis and ankylosis of some of the limb joints. Samples of brain tissue and spleen were collected for virus detection. The goat belonged to a small flock with 1 calf and 6 goats located in Northern Italy, in the area of Treviso.

After RNA extraction, samples were submitted to two different one step real-time RT-PCR protocols, developed by the Friedrich Loeffler Institut (FLI), targeting the L1 and S3 genomic fragments, respectively. Brain tested positive to both protocols, whereas spleen tested negative. Spleen and brain samples were sent to the National Reference Centre for Exotic Diseases (ICT- CESME, Teramo) which confirmed the presence of SBV in the brain by qRT-PCR and partial sequencing of the viral genome.

In collaboration with the CESME an epidemiological investigation in the farm was carried out. Epidemiological information on the flock (animal species, number, age and movements) were provided by the farmer. Whole blood and serum samples were collected from all the animals for virological and serological investigation, respectively. All whole blood samples tested negative for SBV, whereas serological positive results were obtained by virus-neutralization (VN) and immunofluorescence (IF) assays performed in Teramo from four goats and the calf.

To date this is the first detection of the new Orthobunyavirus, Schmallenberg virus in Italy. The epidemiological investigations excluded the introduction of SBV in the farm from other EU infected countries. A local virus circulation, therefore, has been confirmed by CESME in the area and 6 *Culicoides* collections made from September 6th to November 25th 2011 in 3 farms around the SBV outbreak were positive by real time RT-PCR for SBV. A re-enforced passive clinical surveillance system has been implemented by Italian Veterinary Authority, focusing on clinical signs in adult animals and abortions, stillbirths and malformed ruminants. Several suspected cases have been submitted to CESME laboratory for confirmation. A clear definition for suspected and confirmed SBV case has been developed in agreement with those suggested by the European Food Safety Authority (EFSA).

POSTER S5: Emergence of Schmallenberg Virus : Development and validation of a PCR detection kit

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LSI¹

Key words: Schmallenberg, RealTime PCR, Outbreak

Between August and December 2011, outbreaks of disease in adult cattle, abortions and births of malformed animals in sheep, cattle and goats were reported in the Netherlands, Germany and Belgium. A new virus was identified as a cause of these problems and was named "Schmallenberg virus" after the place where it was first identified. This virus belongs to the Bunyaviridae family, genus orthobunyaviridae and is closely related to Akabane, Aino and Shamonda viruses. We present here the development of new real-time PCR kits for identification of Schmallenberg virus, by targeting the S segment, and the different validation steps leading to final authorisation for SBV diagnosis by the French National Reference Laboratory (ANSES Maisons-Alfort) and by the Friedrich-Loeffler-Institut (FLI) in Germany.

Systems for specific detection of Schmallenberg Virus were designed on the basis of the sequence deposited on Genbank. A first system was designed on the L segment. Then, according to the FLI recommendations, we realised a new design, based on the detection of the S segment. The LSI SBVS kit allows the simultaneous detection of SBV target and an endogenous IPC. Detectability of the both kits was compared with the FLI design using serial dilution of SBV RNAs provided by the Friedrich-Loeffler Institut (Germany). Analytical specificity and sensitivity of L and S segment kit were assessed using several field samples (brains), coming from France and Belgium. Specificity of prototype kits was evaluated on a panel of ruminant pathogens. Both prototype kits were sent to the FLI and the S segment prototype kit was sent to the French NRL for evaluation of their specificity, sensitivity, detectability and repeatability on field samples, in order to receive official validation for diagnosis in France and Europe.

The both systems showed good specificity, with detection of all positive samples and no detection of other ruminant pathogens. These kits have equivalent detectability with the FLI designs, with better sensitivity for the S segment relative to the L segment (for FLI and LSI design).

On 34 field samples (brains coming from stillbirths with malformations), we have better detectability with LSI systems, with 29 positives samples with LSI S segment PCR and 25 positives with FLI PCR. Characteristics of the both kits obtained at LSI were confirmed at the French NRL and the FLI. Kits showed good sensitivity, specificity and detectability.

The SBV-S segment kit commercialised by LSI received official authorisation for utilisation in the French network for diagnosis of Schmallenberg virus and was also validated by the FLI, for use in European market.

In conclusion, the initial validation of the TaqVet™ SBVS kit at LSI shows good results in sensitivity, specificity and detectability. A panel of 34 field samples show good results, for SBV target and for IPC. All characteristics obtained at LSI were confirmed at the French NRL and at the FLI, giving to this kit an official authorisation for use in SBV diagnosis in France and Europe.

Hoffmann, B. et. al., (2012) Novel Orthobunyavirus in cattle, Europe, 2011 – Center for Disease Control and Prevention. Vol 18, N°3, p1-6.

POSTER S6: Detection of Schmallenberg virus in pools of Obsoletus Complex stored during the Bluetongue Italian surveillance program

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Key words: Schmallenberg virus, Culicoides, Obsoletus Complex

It is now more than ten years that the Bluetongue (BT) entomological surveillance program is operative in Italy. This activity allowed to store in alcohol 70% Culicoides collections trapped on a weekly basis in farms from all over the country.

Last February Schmallenberg virus (SBV) was detected in a goat foetus and SBV antibodies were found in a cow with stillbirth problems. Both animals were from farms located in the Veneto region.

A SBV retrospective survey was carried out on 87 Culicoides collections caught from six BTV surveillance permanent traps located in the surrounding of the two SBV cases between June and November 2011. The selected sites included Veneto and Friuli Venezia Giulia regions. In particular two were in Treviso province (Istrana and Volpago del Montello), one in Belluno province (Feltre) and three in Pordenone province (Caneva, Montereale Valcellina and Sequals).

Old parous and, when present, engorged females were separately sorted out in pools according to species and tested by qRT-PCR for the presence of SBV.

Of a total of 175 pools examined, 6 were positive to SBV, precisely four collected on September 6th, October 21st, November 3rd and 25th, respectively, from Feltre (Belluno, Veneto), one collected on the 4th of October from Caneva (Pordenone, Friuli), and one collected on November 7th from Istrana (Treviso, Veneto).

All positive pools consisted of species of the Obsoletus Complex. Five of them were composed by parous females (ranging from 5 to 47) and one by a single engorged midge collected from Feltre on September 6th. *Culicoides obsoletus sensu strictu* was the most abundant species found when nulliparous females of the Obsoletus Complex collected in the selected sites were identified by multiplex PCR.

This study highlights and confirms the benefit of having a good entomological surveillance program in place. It allowed to both monitor the BTV circulation and, retrospectively, get critical information on SBV infection in Italy.

As a result of this retrospective study it was possible (i) to demonstrate that SBV has circulated in at least 3 Italian provinces since early September, (ii) to confirm that species of the Obsoletus Complex play a role in transmitting SBV and (iii) to evidence that *C. obsoletus sensu strictu* is likely to be the principal vector in the Italian SBV outbreaks.

Studies to determine the exact role of each species of the Obsoletus Complex in transmitting SBV are in progress.

POSTER S7: Preliminary insight into Schmallenberg virus infection impact in sheep flocks

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Key words: Schmallenberg, impact, sheep

Background

Schmallenberg (SBV) virus outbreak emergence is closely monitored in France. Farmers are urged to contact their veterinarian when encountering cases of ruminant neonates or fetuses stillborn, malformed or showing nervous disorders. A brain sample is collected on any suspected newborn cases until a first confirmation of the infection is obtained for the herd. SBV diagnostic is performed in state diagnostic laboratories, using real-time quantitative reverse transcription PCR (RT-qPCR). The first outbreak of SBV infection was confirmed in France, on the 25th January 2012. As of the 30th March 2012, 1,048 SBV positive premises have been identified, 958 sheep, 76 cattle and 14 goat herds.

Method

In the framework of the French National Surveillance Platform for Animal Health, a survey has been implemented in the cattle, sheep and goat SBV positive herds to get an estimate of the apparent attack rate as well as an estimation of the frequency of congenital deformities in newborns. A preliminary investigation is carried out as soon as possible after the first confirmation of the SBV infection. A second investigation will be performed in the same premises at the end of the breeding season to achieve a global impact assessment. Farmers are interviewed using a standardised questionnaire exploring general farm information and information on the females that have given birth and on the newborns. Data is entered into a web-based interface developed for the survey. We present the first results of the preliminary investigation survey in sheep flocks.

Results

As of the 29th March 2012, a preliminary investigation has been carried out in 384 SBV positive sheep flocks, in 38 districts. At the time of the preliminary investigation, a total of 42,219 ewes had already given birth to 68,237 lambs (accounting, on average, for 78% of the ewes in the flocks). Full data characterizing the ewes that had given birth was collected in 362 flocks (accounting for 40 635 ewes). On average in the SBV positive flocks, 34,470 (85%) ewes gave birth normally. Among the 6,165 (15%) ewes that had lambing problems, 4,465 (72%) had of full time birth but at least one of their lambs was deformed, born dead or died within 12 hours after birth and 1,700 (28%) had an abortion. Among the ewes that had lambing problems, 724 (12%) died within 15 days following the delivery.

Full data describing the lambs that were born at the time of the preliminary investigation was collected in 363 flocks accounting for 64 611 lambs. On average in the SBV positive flocks, 54,904 (85%) lambs were healthy; 8,457 (13 %) were born dead or died within 12 hours after birth, 1,250 (2%) showed deformities but were still alive 12 hours after birth (however the viability of these animals was expected to be low in 74% of the flocks).

A total of 6,513 (10%) lambs were deformed. Stiff joints were the most common deformity (observed in 96% of the flocks). Nervous disorders were more uncommon, absent in 63 % of the flocks.

Discussion

Preliminary results of this impact survey provide a first gross estimate of SBV virus infection impact in sheep flocks. The imputability of SBV virus infection in the occurrence of the lambing problems reported in ewes or in the death or deformities reported in lambs is not assessed.

This survey is continuing in sheep, cattle and goat SBV positive herds; the results of the preliminary investigation will be completed by a final assessment at the end of the breeding season.

POSTER S8: Preliminary results of Schmallenberg virus detection in ruminants in Turkey; preparing for the future

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Key words: Schmallenberg, Virus, Turkey, Cattle, Sheep

The aim of this study was to investigate the presence of Schmallenberg virus in sheep, goats and cattle in Turkey in the same period that the virus has been circulating in Europe. For this, archival samples from sheep, goats and cattle were analysed for the presence of Schmallenberg virus RNA by real-time RT-PCR using specific probe. Stored cDNA samples and RNA extracted from archival 96 aborted fetuses of sheep (46), goats (11) and cows (38) and samples from the blood of 40 lambs and 20 sheep collected in 2011 from Marmara (EU border), Aegean and Blacksea regions of Turkey were analysed. RNA was also extracted from stored samples. No Schmallenberg virus RNA was detected in any of the samples of sheep and cattle. The investigation is still going on and sequencing will be performed if this virus exists in Turkey.

Development of a vaccine that protects sheep and cattle against challenge with Schmallerberg virus, a novel Orthobunyavirus recently found in Europe.

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Decrease in milk production, watery diarrhea and fever were reported in several dairy herds in The Netherlands and Germany in the summer of 2011. Congenital malformations in young animals (lambs and calves) were observed in the following months. The responsible pathogen, a novel Orthobunyavirus, named Schmallerberg virus (SBV), was identified in Western Europe in November 2011. With the support of veterinary experts around the globe MSD Animal Health has developed in a fast track program a vaccine to be able to protect livestock in Europe against SBV. A challenge model has been developed. The efficacy and safety of the vaccine has been tested in young animals (calves and lambs) as well as in pregnant ewes. Animals were vaccinated twice and three weeks after the last vaccination vaccinates and controls were challenged with a virulent SBV strain. The efficacy of the vaccine was evaluated based on the level of virus neutralizing antibody titers induced after vaccination and the blockage of viraemia. SBV neutralizing antibody titers were measured after vaccination. All control animals became viraemic after challenge. Protection (blockage of viraemia, as tested by real time RT-PCR) against the challenge with the virulent SBV strain was seen in all animals.

Based on these data good protection against SBV is to be expected in the field and further development of this vaccine is ongoing.



EPIZONE European Research Group is the international network of veterinary research institutes working on epizootic animal diseases and plays a key role in research on prevention, detection and control of diseases of poultry, swine, fish, sheep, cattle, horses and wildlife in order to reduce both the risks and the harm to animal health in the EU and beyond.

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