EPIZONE

Programme and Abstracts

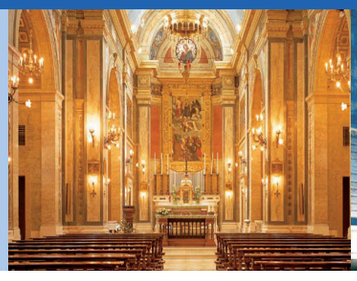
Second Annual meeting EPIZONE

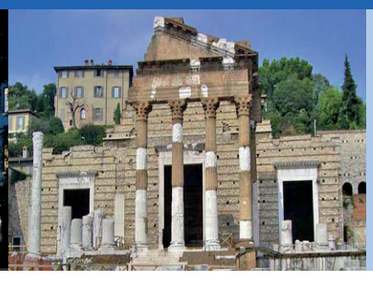
" Need for Speed"

4,5 and 6 June, 2008

Brescia, Italy

Hosted by ISZLER





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Content

EPIZONE

Welcome

Piet van Rijn

Welcome

Dear participants,

We welcome you all to the 2nd Annual Meeting of our EU funded Network of Excellence, EPIZONE
"Need for Speed"

The mission of EPIZONE is to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks through increased excellence by collaboration.

The title "Need for Speed" of this 2nd Annual Meeting relates to many different aspects of combating animal diseases. Due to many changes in the modern world, risk and impact of animal diseases has been enormously enlarged. Intensified animal movements, higher densities of animals, and global warming are only a few of the factors increasing risk and potential losses. Quick, adequate and socio-acceptable actions are needed. This starts with preparedness and alertness of the environmental situation, followed by a quick and specific diagnosis of suspicions, and effective control measures to reduce losses as much as possible.

During this meeting you will meet scientists from many different fields of research, but all share their interests in animal disease control. This will give you the chance to share ideas and thoughts, to learn from each other, to build new relationships and to strengthen the existing ones.
We hope to fulfill your liking with this program composed of plenary lectures by invited speakers, presentations and posters related to Diagnostics, Intervention strategies, Epidemiology and Surveillance and Risk Assessment. In addition to this program, time is scheduled to meet and discuss scientific issues with colleagues involved in animal disease control.

We are grateful to Professor Stefano Cinotti and all involved members of IZSLER for the generous hospitality, and the great and enthusiastic support in organizing this 2nd Annual Meeting of EPIZONE.

We wish you all a successful and happy meeting

Piet van Rijn, Coordinator EPIZONE
The scientific committee and the organizing committee

Scientific committee:
Dr W. van der Poel (chair) (CVI)
Dr M. Beer (FLI)
Dr L. Dixon (IAH)
Dr U. Hesterberg (VLA)
Dr L. Kelly (VLA)
Dr E. Erlacher-Vindel (OIE)
Dr A. Donaldson (Bio-Vet Solutions Ltd)
Professor M. Czub (University Calgary)
Dr C. Griot (IVI)
Professor M. Woolhouse (University Edinburgh)

Organizing committee:
Sylvia Bellini (IZSLER)
Margriet Vedder-Rootjes(WUR)
Petra van der Laag (CVI)
Jitty Oosterga-Land (CVI)

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Programme

Wednesday, June 4th 2008

Meeting room	No 15	No 14	No 8	No 13	No 18	No 21	No 22	No 17	No 16	No 7
10.00 - 13.00	Financial Officers		EAP		WP 5.4	WP 6.1		WP 6.3		
13.00 -14.00	**Lunch**									
14.00 - 15.00				WP 4.3	WP 5.3					
15.00 - 16.00				WP 4.3	WP 5.2					
16.00 – 17.00					Theme 5					
14.00 – 17.00	Young EPIZONE	WP 1.2	WP 4.1			WP 6.1	WP 6.2	WP 6.3	Theme 7	CF
18.00- 21.00	Welcome reception Registration Poster set up Presentation drop off									

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Thursday, June 5th 2008

7.30 – 8.30	Registration Poster set up Presentation drop off
8.30 - 9.30	*Piet van Rijn, Coordinator EPIZONE* 'Welcome'

9.30 – 10.30 KEYNOTES SURVEILLANCE AND EPIDEMIOLOGY

Main Hall

9.30 - 10.00	*Dr Karim Ben Jebara, Head OIE, Animal Health Information Department* 'The OIE's New World Animal Health Information System (WAHIS)'
10.00 - 10.30	*Dr Angus Cameron, AusVet Animal Health Service, Epidemiologist, Animal disease surveillance* 'Unleashing surveillance - faster, better, cheaper'

10.30 – 11.00 Coffee break

11.00 – 12.00 ORAL PRESENTATIONS SURVEILLANCE AND EPIDEMIOLOGY

	Main Hall **Moderators: Uta Hesterberg & Alex Donaldson**	**Hall 10** **Moderators: Søren Alexanderson & Koen Mintiens**
11.00 – 11.15	*Hesterberg, Uta* Avian Influenza surveillance in the EU - Results of epidemiological analysis of surveillance data from 2006 and 2007 and implications for targeting of future surveillance	*Özyörük, Fuat* Phylogenetic Surveillance of FMD Type O Epidemics in Turkey between 1996–2007
11.15 – 11.30	*Welby, Sarah* Evaluation of Belgian's Avian Influenza active surveillance programme in domesticated birds	*Kock, Petra* Animal Health Surveillance in the Netherlands

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11.30 – 11.45	*Greiser-Wilke, Irene* Development of the Classical Swine Fever (CSF) virus database in Hannover: from compilation of virus isolates to automated genotyping	*McElhinney, Lorraine* Molecular diversity of Rabies virus strains in Southern Europe
11.45 – 12.00	*Yin, Shuanghui* Genetic Typing of Classical Swine Fever Virus Isolates From China	*Ferrè, Nicola* INSPIRE: an opportunity to standardise, harmonise and integrate zoonotic data and services

12.00 – 13.00 POSTER SESSION / MEET & GREET

12.00 –13.00 WP poster-presentation and meet & greet / discussions with all Work Package leaders and Theme leaders

Meet & greet speakers

General poster session

13.00 - 14.00 Lunch

14.00 – 14.30 KEYNOTE DIAGNOSTICS

Main Hall

14.00 – 14.30 *Professor Ian Lipkin M.D., Columbia University, Department of Epidemiology, New York:*
'A staged strategy for efficient pathogen surveillance and discovery'

14.30 – 15.30 ORAL PRESENTATIONS DIAGNOSTICS

	Main Hall **Moderators: Tony Fooks & Martin Beer**	**Hall 10** **Moderators: Piet van Rijn & Christian Griot**
14.30 – 14.45	*Parida, Satya* Detection of FMDV persistent cattle by salivary IgA test	*Kirkland, Peter D* Equine Influenza in Australia – the need for speed
14.45 – 15.00	*King, Donald* Fulfilling the potential of molecular assays to detect and characterise FMDV: updated findings from the 2007 outbreaks in the United Kingdom	*Fooks, Anthony* Development of a novel serological assay for the detection of Rabies virus neutralising antibodies using lentiviral pseudotypes

15.00 – 15.15	*Volpe, Carmelo* An advanced field deployable "pen side" sample preparation and PCR diagnostic system	*Cetre-Sossah, Catherine* Development and evaluation of a real-time quantitative PCR assay for Culicoides imicola, one of the main vectors of Bluetongue (BT) and African Horse Sickness (AHS) in Africa and Europe
15.15 – 15.30	*Kühne, Sabine* EPIZONE Databases	*Banyard, Ashley / Barett, Tom* Developing a marker vaccine for rinderpest virus by epitope deletion

15.30 – 16.00 Coffee break

16.00 – 16.30 ORAL PRESENTATIONS DIAGNOSTICS

	Main room **Moderators: Tony Fooks & Martin Beer**	**Hall 10** **Moderators: Piet van Rijn & Christian Griot**
16.00 – 16.15	*Hoeper, Dirk* Rapid in depth diagnostics of viral full length genomes using the Genome Sequencer technology	*Dauphin, Gwenaelle* Improved but still limited HPAI testing capacities in developing countries
16.15 - 16.30	*Offord, Victoria / Watson, Michael* DetectiV: visualization, normalization and significance testing for pathogen-detection microarray data	*Kalthoff, Donata* Experimental infection of mute swans with highly pathogenic Avian Influenza virus H5N1

16.30 – 17.30 POSTER SESSION / MEET & GREET

16.30 - 17.30	WP poster-presentation and meet & greet / discussions with all Work Package leaders and Theme leaders
	Meet & greet speakers
	General poster session
	Drinks
16.45 – 17.30	MAB meeting with coordinator **(Room No 8)**

20.00 – 23.00 Organised dinner – dress code cocktail

Friday 6 June

8.30 – 9.00	Registration Poster set up Presentation drop off

9.00 – 9.30 KEYNOTE RISK ASSESMENT

Main Hall

9.00 - 9.30 *Professor Mark Woolhouse, Professor of Infectious Disease Epidemiology at the University of Edinburgh in Scotland.*
'From host-pathogen interaction to emergency response'

9.30 – 10.30

	ORAL PRESENTATIONS RISK ASSESSMENT	ORAL PRESENTATIONS INTERVENTION STRATEGIES
	Main Hall **Moderators: Paul Gale & Armin Elbers**	**Hall 10** **Moderators: Linda Dixon & Thomas Vahlenkamp**
9.30 – 9.45	*Volkova, Victoriya* FMD 2007: prioritizing post-movement ban surveillance in Scotland	*König, Patricia* NPRO deletion mutants as efficient and safe Bovine Viral Diarrhea (BVDV) vaccine candidates
9.45 – 10.00	*de Vos, Clazien J.* The residual FMD risk after declaring a region free from disease with an application to vaccinated pigs	*Szelechowski, Marion* Vectors derived from Canine Adenovirus type 2 (Cav2) for vaccination: biosafety assessments
10.00 – 10.15	*Schley, David* Quantifying the risk of localised movement bans for Foot-and-Mouth Disease	*Barcena, Juan* Generation and characterization of chimeric calicivirus like particles displaying immongenic epitopes
10.15 – 10.30	*Elbers, Armin* To report or not to report: opinions and attitudes of Dutch pig farmers and veterinary practitioners regarding clinical situations possibly caused by a notifiable pig disease	*Dixon, Linda* Functional analysis of the African Swine Fever virus virulence factor DP71L

10.30 – 11.00 Coffee break

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11.00 – 11.30	ORAL PRESENTATIONS MISCELLANEOUS	ORAL PRESENTATIONS INTERVENTION STRATEGIES
	Main Hall **Moderators: Paul Gale & Armin Elbers**	**Hall 10** **Moderators: Linda Dixon & Thomas Vahlenkamp**
11.00 – 11.15	*van der Poel, Wim H. M.* Wildlife surveillance for lyssaviruses in North-West Europe	*Rodriguez, Fernando* Partial protection against African Swine Fever virus in the absence of antibodies by using DNA vaccines
11.15 – 11.30	*Gale, Paul* Assessing the impact of climate change on vector-borne livestock diseases in the EU through elicitation of expert opinion	*Griot, Christian* Laboratory accidents and how to prevent them

11.30 – 12.30	POSTER SESSION / MEET & GREET
11.30 – 12.30	WP poster-presentation and meet & greet / discussions with all Work Package leaders and Theme leaders Meet and greet speakers General poster session
11.30 – 12.30	Meeting EAP with MAB **(Room No 8)**

12.30 – 13.30 Lunch

13.30 – 14.00	KEYNOTE INTERVENTION STRATEGIES
	Main Hall
13.30 - 14.00	*Dr Jean-Christophe Audonnet DVM, Senior Director, Vaccinomics Platform And Recombinant Vaccines Discovery Research, Merial* 'The Design of Vaccines against Emerging Diseases: understanding Options and Constraints for providing Realistic Solutions'

14.00 – 15.00	ORAL PRESENTATIONS INTERVENTION STRATEGIES	ORAL PRESENTATIONS RISK ASSESSMENT
	Main Hall **Moderators: Markus Czub & Marie-Frèdèrique LePotier**	**Hall 10** **Moderators: Elisabeth Erlacher-Vindel & Franz Conraths**
14.00 – 14.15	*Sevilla, Noemí* Interaction of Foot-and-Mouth Disease Virus (FMDV) with dendritic cells: role of IL-10.	*Harbit, Andrew* Release assessment for Avian Influenza into the EU through quantitative risk assessment modeling displayed on GIS maps

14.15 – 14.30	*Keil, Guenther M* Baculovirus-based vaccination induces immune responses against foot-and mouth-disease vius in mice and conveys protection from lethal rabbit haemorrhagic disease virus infection in rabbits.	*Sánchez-Vizcaíno Buendía, Fernando* A Quantitative Assessment of the Risk from Imported Live Poultry Infected with Highly Pathogenic Avian Influenza virus to Region of Valencia
14.30 – 14.45	*Li, Pinghua* Reverse genetics of foot-and-mouth disease virus	*Hagenaars, Thomas J* Understanding and predicting between-farm spread of highly transmissible animal diseases: the role of mathematical modelling
14.45 – 15.00	*Harmsen, Michiel M.* Development of a novel proteomics-based approach for analysis of stability of FMDV antigens	*Martinez, Marta* Association between number of wild birds sampled for identification of H5N1 Avian Influenza virus and probability of outbreak in the European Union

15.00 - 15.30 Coffee break

15.30 – 16.00	**ORAL PRESENTATIONS INTERVENTION STRATEGIES** **Main Hall** **Moderators: Markus Czub & Marie-Frederique LePotier**	**ORAL PRESENTATIONS MISCELLANEOUS** **Hall 10** **Moderators: Elisabeth Erlacher-Vindel & Franz Conraths**
15.30 – 15.45	*Vrancken, Robert* CSFV-replication successfully inhibited in pigs by imidazo[4,5-c]pyridines.	*Hoffmann, Bernd* Labelled Positive Control (LPC) - improved Safety in real-time PCR assays
15.45 – 16.00	*Renson, Patricia* Microarray analysis of CSFV virulence	*van Schaik, Gerdien, G* Monitoring of the EBL-free status in the Netherland

16.00 - 17.00 Farewell drink

16.30 – 17.30 Meeting EAP, MAB and EC **(Room No 7)**

Oral Presentations

ORAL: DetectiV: visualization, normalization and significance testing for pathogen-detection microarray data

Watson, Michael[1]; Offord, Victoria[2]

Institute for Animal Health[1]; Royal Veterinary College[2]

Key words: Virus, pathogen, microarray, statistics, bioinformatics

1.Introduction and Objectives

DNA microarrays offer the possibility of testing for the presence of thousands of micro-organisms in a single experiment. However, there is a lack of reliable bioinformatics tools for the analysis of such data. We have developed DetectiV, a package for the statistical software R. DetectiV offers powerful yet simple visualization, normalization and significance testing tools.

2.Material and Methods

DetectiV uses established visualisation, normalisation and significance testing techniques to identify the most likely infectious agent. We have applied DetectiV to two publicly available data sets, and demonstrate that DetectiV performs better than previously published software.

3.Results

We show that DetectiV correctly predicts the correct agent in 56 out 57 arrays from the Wang et al dataset, and predicts the correct agent in 12 out of 12 arrays from an IAH/VLA dataset. We also present some results on 'rogue' oligos, and attempt to present the relationship between percentage identity and intensity on the array

4.Discussion and Conclusions

We discuss the results of DetectiV in the context of the general problem of noisy data from microarrays. Whilst the results shown here are encouraging, it is unlikely that the analysis of such arrays will become automatic as errors are still present, and these datasets are likley to require human interpretation. DetectiV is a highly accurate tool for the analysis of pathogen detection microarray data, offering simple but powerful visualization, normalization and significance testing functions. DetectiV performs better than previously published software on a publicly available microarray dataset. DetectiV is available as a package for R, a platform-independent statistical software package, and requires little configuration or customization.

5.Acknowledgements

This work was supported by the Department of Environment, Food and Rural Affairs (DEFRA) project codes SE4102, SD0443, SE1120 and the Biotechnology and Biological Sciences Research Council (BBSRC). Some of the oligonucleotide probes were provided by Dr M Banks of Veterinary Laboratories Agency (VLA).

6.References

Watson M (2007) DetectiV: Visualisation, normalisation and significance testing for pathogen-detection microarray data. Genome Biology 2007, 8:R19

ORAL: ASSESSING THE IMPACT OF CLIMATE CHANGE ON VECTOR-BORNE LIVESTOCK DISEASES IN THE EU THROUGH ELICITATION OF EXPERT OPINION

Gale, Paul[1]; Brouwer, Adam[1]; Ramnial, Vick[1]; Kelly, Louise[2]; Kosmider, Rowena[1]; Snary, Emma[1]

VLA[1]; University of Strathclyde[2]

Key words: Climate change, qualitative risk assessment, vector, expert opinion

1.Introduction and Objectives

The objective of the work presented here was to assess the impact of climate change in the 2080s on the risks to the European Union (EU) of five vector-borne, livestock viruses: African horse sickness virus (AHSV), Crimean-Congo haemorrhagic fever virus (CCHFV), Rift Valley fever virus (RVFV), African swine fever virus (ASFV) and West Nile virus (WNV).

2.Material and Methods

Qualitative risk assessments were developed based on the OIE framework (OIE 2004). Estimates for the release, exposure and consequence assessments were gathered through elicitation of opinion from 18 experts at a workshop. Experts were asked to consider the risk overall and also via six specific routes namely, vectors, livestock, wildlife, meat products, persons and pets. The risks of incursion and of the virus becoming enzootic in the EU were calculated through each route using a matrix to multiplicatively combine the risks from the release, exposure and consequence assessments.

3.Results

It was predicted that climate change will increase the overall risk of AHSV, CCHFV and RVFV incursions into the EU with ASFV and WNV being less affected. Climate change was predicted to increase the risk of incursion through the vector route for all five viruses to some degree; the strongest effects being predicted for AHSV, CCHFV and WNV. In the case of WNV and ASFV, climate change was predicted not to affect the risks of incursion through the main routes namely wildlife and the import of meat products, respectively.

4.Discussion and Conclusions

Breaking down the risks into the specific routes provides further information to assist risk managers. The risk predictions made here through elicitation of expert opinion should be used in conjunction with consideration of the impact of climate change on vertebrate host reservoirs, arthropod vectors, farming practice and land use. The two approaches together provide a powerful tool to furthering our understanding of the potential impact of climate change on the emergence of vector-borne, livestock viruses in the EU.

5.Acknowledgements

This project is funded by the EU Network of Excellence, EPIZONE, and is part of work package 7.4. We thank the 18 experts who attended the workshop.

6.References

OIE (2004). Handbook on Import Risk Analysis for Animals and Animal Products. Volume 1, Introduction and qualitative risk analysis. World Organisation for Animal Health.

ORAL: Evaluation of Belgian's Avian Influenza active surveillance programme in domesticated birds

Welby, Sarah[1]; van den Berg, Thierry [1]; Marché, Sylvie[1]; Hooyberghs, Jozef[2]; Mintiens, Koen[1]

VAR (Vetrinary and Agrochemical Research Center)[1]; FASFC (Federal Agency for safety of the food chain) [2]

Key words: Avian Influenza-Surveillance programme-Sensitivity analyse-Target sampling

Introduction

After the devastating epidemic of 2003, which had severe repercussions in the poultry industry, Belgium remained free of Avian Influenza. The dramatic consequences of such outbreaks underline the need of detecting the pathogen before it actually spreads. Following the commission decision 2006/101, compulsory surveillance programmes in the different European Member States were implemented. The Belgian surveillance programme consists in active and passive surveillance in wild and domestic birds. The aim of this study was to evaluate the effectiveness of Belgian active surveillance programme for domestic birds in professional poultry holdings.

Material and Methods

The methods for analysis of complex surveillance systems as proposed by Martin et al (Martin, Cameron et al. 2007; Martin, Cameron et al. 2007) were used. For this purpose a scenario tree, identifying each step in the detection or infection process, was developed and implemented in @RISK 4.5 (Palisade Decision Tools corporation). Following their differential risk of infection or probability of detection, respective reference population proportions and surveillance system component population proportions were attributed to each node in the tree. A stochastic quantitative analyse combining all these multiple complex data sources was run to obtain sensitivity estimates for the detection of an infected bird.

Results Discussion Conclusion

As well as the total sensitivity of the Belgian active surveillance system in domesticated birds, the sensitivity of the different surveillance system component were obtained from the model. The detailed results will be presented at the meeting. Sensitivity analyse within the model was conducted to estimate the influence of the different input parameters on the outcome. The sensitivity analyse was a very effective tool to provided insight onto how to improve or enhance target sampling.

Acknowledgements

We wish to thank Tony Martin for his continuous support to the development of the model.

References

Martin, P. A., A. R. Cameron, et al. (2007). "Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees." Prev Vet Med 79(2-4): 71-97.

ORAL: RELEASE ASSESSMENT FOR AVIAN INFLUENZA INTO THE EU THROUGH QUANTITATIVE RISK ASSESSMENT MODELLING DISPLAYED ON GIS MAPS

Harbit, Andrew[1]; Gale, Paul[1]; Kosmider, Rowena[1]

Veterinary Laboratories Agency - Weybridge[1]

Key words: Avian Influenza, quantitative risk assessment, GIS

1.Introduction and Objectives

The objective of this work is to identify the most likely mechanism and pathway by which Avian Influenza may enter the European Union (EU), through a combination of existing knowledge, as risk probability estimates.

2.Material and methods

Release pathway routes including migratory wild birds, legal trade, illegal imports and intra-community trade in live poultry, poultry meat, poultry products, captive wild birds, pet birds and mechanical transmission are being used in a quantitative risk assessment model based on the OIE framework (OIE, 2004), in order to elucidate the likelihood of Avian Influenza incursion into different regions of the EU, and to later populate a scenario tree model, for exposure and consequence assessments. The model is being parameterised with trade data, existing knowledge on risk probability estimates and through recent experimental evidence.

3.Results

The model is currently being populated and the initial results will be presented at the conference.

4.Discussion and Conclusions

This current model builds on the previous qualitative risk assessment models developed for the introduction of AI into GB (Defra, 2007), EU Member State countries, and the BTO/VLA project developed to identify priority areas for surveillance of H5N1 in wild birds in GB (Snow et al, 2007).

5.Acknowledgements

This project is funded by the EU and Defra. It is part of Work Package 2 of a Sixth Framework programme project with the acronym “FLUTEST”. The full project title is; “Improved diagnosis and early warning systems for avian influenza outbreak management”

6. References

Defra (2007) Highly pathogenic avian influenza – H5N1: Recent developments in the EU and the likelihood of the introduction into Great Britain by wild birds. (Authors: Sabirovic, M., Hall, S., Wilesmith, J. and Coulson, N.) Released 12 July 2007

OIE (2004). Handbook on Import Risk Analysis for Animals and Animal Products. Volume 1, Introduction and qualitative risk analysis. World Organisation for Animal Health.

Snow, L.C., Newson, S.E., Musgrove, A.J., Cranswick, P.A., Crick, H. Q. P., Wilesmith, J. W. (2007) Risk-based surveillance for H5N1 avian influenza virus in wild birds in Great Britain. The Veterinary Record, 161, 775-781

ORAL: To report or not to report: opinions and attitudes of Dutch pig farmers and veterinary practitioners regarding clinical situations possibly caused by a notifiable pig disease

Elbers, Armin[1]; Gorgievski, Marjan[2]; van der Velden, Peter[3]

Central Veterinary Institute[1]; Institute of Psychology, Erasmus University [2]; Institute for Psychotrauma[3]

Key words: reporting, notifiable disease, farmers, veterinarians

Introduction

Delayed reporting of a suspect situation caused by notifiable animal diseases (NAD) will seriously hamper the eradication after introduction into a free country. NAD-diagnosis based solely on clinical signs is difficult because they are often unfamiliar to farmers and vets and because signs may vary considerably. Farmers and vets are sometimes reluctant to report early clinical signs because they may be caused by other pig diseases.

Methods

An electronic questionnaire (9 pages: 15 minutes to fill in) was sent via an e-mail newsletter to a pig farmer union and was posted for 3 weeks on the website of the Veterinary Association.

Results

75 pig farmers and 334 vets responded. When asked : if you think on your farm a problem might be caused by NAD, how certain do you want to be before you report to authorities, 20% vets and 36% farmers needed > 80% certainty before they would report; 40% farmers and 49% vets indicated that reporting a suspect situation, when retrospectively this was a false alarm, had a negative consequence for the financial situation of the farm; 5% farmers and 23% vets indicated that such an event would have a negative influence on the relationship between farmer and vet. Farmers (57%) and vets (13%) indicate that they would report faster when there is a strong relationship between farmer and vet. Farmers (27%) and vets (5%) indicated that the probability of a NAD at their farm or clients was negligible. Farmers (29%) and vets (31%) indicate that the fuss linked to reporting is often a reason for not reporting.

Conclusions

There is a gap between what authorities expect and what farmers and vets feel as their responsibility. Clear information (internet) is needed on when, how and what regarding reporting and more specific: photo/video of sick animals. The relationship between farmer and vet plays a role in the willingness to report a suspect situation, and that there is also an area of tension between farmer and vet if it comes to reporting (retrospectively) a false alarm: do I trust the competence of my vet ? I am loosing a client (farmer) if my reporting is a false alarm? The possibility to submit samples from selected animals to a reference laboratory in the case of non-specific clinical signs, to rule-out disease caused by a NAD, without government involvement and without isolation of the farm, is a solution put forward by farmers and vets.

ORAL: Genetic Typing of Classical Swine Fever Virus Isolates From China

Yin, Shuanghui[1]

Lanzhou Veterinary Research Institute [1]

Key words: classical swine fever virus; Genetic typing; Chinese isolates

1.Introduction and Objectives

The E2 genes of 73 CSFV originated from CSF suspected cases in different regions of China between 1984 and 2006 were genetically characterized and compared with reference CSF viruses. All Chinese viruses characterized were segregated into two major groups and subdivided into four subgroups. Most of isolates (61.6%) were belonged to group 2 and were further divided into three subgroups, subgroup 2.1, 2.2 and 2.3. Subgroup 2.1 was the largest subgroup which contained 46.6% of isolates, whilst subgroup 2.3 was the smallest subgroup which contained only one isolate (1.4%). The remaining 38.4% of isolates were classified into subgroup 1.1 within group 1. However, none group 3 and subgroup 1.2 and 1.3 viruses were found in this study.Records of CSF in China date back to 1920s. Owing to a nation-wide policy of twice-yearly vaccinations of pigs (spring and autumn), a general guideline of government, CSF is well controlled in China, with large-scale outbreaks rarely seen. However, sporadic outbreaks were still reported every year. The aim of this work was to analyze the phylogenetic relationship of CSFV isolates originating from China, based on the partial nucleotide sequences of the E2 glycoprotein gene.

2.Material and Methods

2.1. Viruses

2.2. RNA extraction, RT-PCR and sequencing

2.3. Phylogenetic analysis

3.Results

In the phylogenetic tree, the viruses were segregated into three major groups (Groups 1–3) and their subgroups. the Chinese isolates were divided into group 1 and 2. No viruses belonged to group 3.

Chinese field isolates included in this study were classified into subgroup 1.1 represented. Two different clusters of Chinese isolates were found in subgroup 1.1. None of Chinese field viruses were found in subgroup 1.2 and 1.3.

4.Discussion and Conclusions

In conclusion, our results provided important information on the molecular epidemiology characterization of E2 gene of CSFV is relatively stable during 1945 to 2006 in China and provide reliable fact that is HCLV strain vaccine is generally accepted to be very safe in pigs.

5.Acknowledgements

ORAL: Vectors derived from Canine Adenovirus type 2 (Cav2) for vaccination: biosafety assessments

Szelechowski, Marion[1]; Fournier, Annie[1]; Eloit, Marc[1]; Zientara, Stephan[1]; Klonjkowski, Bernard[1]

AFSSA[1]

Key words: viral vector, vaccination, adenovirus, virulence attenuation, semi-replicative

In order to respond to veterinary medicine needs and thanks to recent progress in molecular biology and virology, viral vectors have been developed and produced for vaccination. In comparison with other viral vectors, those derived from adenoviruses elicit particularly robust immune responses. More over, they are poorly pathogenic, easy to manipulate and produce, and able to transfer large transgenes in a wide variety of cells without integration within the host genome. As an alternative to the use of human viruses, we develop vectors from canine adenovirus type 2 (Cav2). Up to now, two kinds of vectors have been developed: one is replication competent (R+ Cav2), as it enables the whole cycle of the wild type virus, the other (R0 Cav2) has been deleted for the early genes in the E1 region of Cav2 genome, thereby blocking the viral cycle after entry into the host cells.

We will first present the ability of R+ and R0 Cav2 vectors to establish a specific and protective humoral immunity in mice, especially after per os administration, using the G glycoprotein of the rabies virus as a model antigen. However, for some species of veterinary interest the defective vector which is more appropriate for biosafety reasons requires, to attain the same efficiency, the inoculation of a substantially larger dose of vector, which makes it inappropriate for industrial vaccine development.

These observations led us to pursue the development of a new class of vectors derived from Cav2 which should be able to replicate its genome so by increasing the amount of the vaccine antigen, without producing new viral particles. This can be achieved by the deletion of defined viral genomic regions, in particular within the late expression region which is dispensable for the first stage of the replication cycle.

Therefore, a transcriptional map of the late expression section of Cav2 genome has been elaborated, thus enabling us to define the accurate targets to delete. We studied the specific elements of late Cav2 gene expression: leader and promoting sequences. We will show that the Cav2 Major Late Promoter is a strong promoter that is inducible by infection and that Cav2 leader sequences facilitate genetic expression.

Finally, we will show how fundamental investigations in virology can be useful for medical virology, as exemplified by the first promising results with the new semi-replicative Cav2 vectors.

ORAL: Functional analysis of the African swine fever virus virulence factor DP71L

Zhang, Fuquan[1]; Childs, Kay[2]; Abrams, Charles[3]; Poole, Emma[4]; Alcami, Antonio[5]; Goodbourn, Steve[2]; Dixon, Linda[3]

Institute for Animal Health[1]; St Georges[2]; IAH[3]; Cambridge University[4]; CBM[5]

Key words: African swine fever, virulence, interferon, protein phosphatase 1

1.Introduction and Objectives

African swine fever virus (ASFV) is a large double-stranded DNA virus that replicates in the cytoplasm of infected cells. The DP71L protein of ASFV is present in the genomes of all pathogenic ASFV isolates analyzed and encodes either a long form (184 amino acids) or short form (70 to 72 amino acids). Deletion of the short from reduces virulence of the virus for pigs. The DP71L gene is similar to a myeloid differentiation primary response gene, MyD116, a protein involved in DNA repair GADD34 and the neurovirulence-associated protein (ICP34.5) from Herpes simplex virus (HSV). The objectives of this work were to investigate the function of the DP71L protein.

2.Material and Methods

Plasmids expressing the long or short forms of the DP71L protein were co-transfected into cells with luciferase reporter constructs dependent on elements of type I IFN promoters. Induction of these promoters was measured by total luciferase activity in cell extracts.

Levels of protein phosphatase 1 activity in cell extracts was measured by release of 32P from a phosphorylated substrate protein. Confocal microscopy was use to determine the localisation of DP71 protein within cells.

3.Results

We showed that the DP71L protein binds to the catalytic subunit of protein phosphatase 1 (PP1c) and activates its phosphatase activity. Our hypothesis is that DP71L acts as a regulatory subunit of PP1 to activate and target the phosphatase t specific substartes. Our results show that this protein, when transiently expressed, can enhance translation in host cells in a dose dependent manner. This is probably through a mechanism used by ICP34.5, which targets PP1 to dephosphorylate the alpha subunit of eukaryotic translational initiation factor 2 (eIF-2α), because a selective inhibitor of eIF-2α phosphorylation by PP1, Salubrinal (Sal) can antagonise this effect of DP71L on translation. Our data also shows that DP71L can also suppress the induction of INF-β by dsRNA in a dose-dependent manner. The mechanism of this is under investigation.

5.Acknowledgements

We thank Lynnette Goatley

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ORAL: Quantifying the risk of localised movement bans for Foot-and-Mouth Disease

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Institute for Animal Health[1]

Key words: control zones, transmission, outbreak.

1. Introduction and Objectives

The maintenance of disease-free status from Foot-and-Mouth Disease is of significant socio-economic importance to European countries. The imposition of bans on the movement of susceptible livestock following the discovery of an outbreak is deemed necessary to prevent the spread of what is a highly contagious disease, but has a significant economic impact on the agricultural community in itself [1,2]. Here we consider the risk of applying movement restrictions only in localised zones around outbreaks in order to help evaluate how quickly nation-wide restrictions could be lifted after notification.

2. Material and Methods

The expected probability of primary and secondary transmission outside of restriction zones around infected premises was calculated, based on a model transmission kernel fitted to the later stages of the 2001 UK outbreak [3]. Historic values for the mean reproduction ratio R0 [4] were then applied to the situation of the 2007 UK Foot-and-Mouth Disease outbreak.

3. Results

We show that such a policy is possible provided the basic reproduction ratio of known infected premises can be estimated. The risk of spread from different sized zones can be quantified and the potential benefits of permitting animal movements in other parts of the country appraised.

4. Discussion and Conclusions

It is ultimately up to policy makers and stakeholders to determine what is an acceptable level of risk, involving a cost benefit analysis of the potential outcomes, but results indicate that it should be possible to contain an outbreak with a very limited number of cases, such as that seen in the UK in 2007, with only short initial nation-wide movement restrictions, provided strict bio-security procedures are followed within a regional restriction zone.

5. Acknowledgements

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ORAL: PARTIAL PROTECTION AGAINST AFRICAN SWINE FEVER VIRUS IN THE ABSENCE OF ANTIBODIES BY USING DNA VACCINES

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Key words: DNA vaccine, T-cell response, African Swine Fever Virus

1.Introduction and Objectives

African Swine Fever (ASF) is a highly infectious disease of obligatory declaration in the OIE. There is not a vaccine available against ASFV and the control of the disease is based on a rapid diagnostic and the sacrifice of the animals. The aim of the present work was to develop new vaccines against ASFV and trying to clarify the relevance of the cellular responses in protection. To do so, we designed DNA vaccine protocols aiming to enhance T-cell induction in the absence of antibodies.

2.Material and Methods

The ORFs encoding the ASFV p54, p30 and a soluble form of hemmaglutinin (sHA), were fused and cloned in a modified pCMV vector (Clontech) to obtain the pCMV-sHAPQ. Aiming to enhance the SLA I presentation and CTL induction, we constructed a new plasmid version: pCMV-UbsHAPQ, encoding sHAPQ as a fusion with ubiquitin in order to drive the antigen to the proteasome (1). 600ug of the corresponding DNA constructs were inoculated once or more times at 15 day intervals and animals (four each group) were bled prior vaccination and 30 days after the last dose. Sera were finally used to determine the induction of specific antibodies by ELISA and Western Blot. To measure the cellular responses induced, PBLs from immunized pigs were -ELISPOT. 100DI50 from the E75L3 virulent strain of ASFV wereγused in an IFN intramuscularly used to challenge the animals 30 days after the last vaccine dose.

3.Results and discussion

Pigs vaccinated only once with pCMV-sHAPQ plus OLR adjuvant or with up to four doses of pCMV-UbsHAPQ, induced cellular responses in the absence of antibodies. Surprisingly, one only shot of pCMV-sHAPQ plus OLR adjuvant conferred a similar level of protection than four shots of pCMV-UbsHAPQ with all pigs showing a delay in the onset of clinical signs and virus appearance in serum. In spite these animals survived one week more than pCMV-immunized animals, they finally died between days 13 and 15 post ASFV infection. We believe that inducing both neutralizing antibodies and cellular responses will be mandatory to obtain sterile protection against highly virulent strains of ASFV.

4.Acknowledgements

The authors acknowledge the Ramón y Cajal program and projects AGL2004-07857-C03-01, TRT2006-00035-C02-00 and CONSOLIDER-INGENIO 2010 from the Spanish Government for their support.

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ORAL: Laboratory accidents and how to prevent them

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IVI[1]

Key words: containment laboratories, biosafety, accindents

1. Introduction

Animal diseases such as foot and mouth disease, avian influenza and bluetongue are a continuing threat to our livestock and the outbreak of these diseases always have an enormous impact on agriculture as well as on the economy of the given country.

Most countries within the European Union have so called high security (containment) laboratories. These are highly specialized facilities where work with known and unknown infectious agents is conducted without risk of their escape to the environment, and without health risk to the staff who worked with them. Most of these facilities are commissioned and financed by the local government while some of them are part of e.g. vaccine production units.

It is an irony that facilities that help us to control outbreaks of disease have the potential to cause disease, for example if organisms are accidentally released into the environment. Fortunately, the secure containment laboratories in which these dangerous pathogens are handled have had an excellent (albeit not perfect) biosecurity/biosafety record. This excellent recent safety record, and our current safety procedures, are in large part from investigating and understanding the causes of past accidents and acting promptly to eliminate any identified vulnerability. Appropriate financial and personal resources to allow preventive maintenance and proper training are a key element to safely run such facilities.

2.Biosecurity and biosafety

In facilities which have a certain age, biosafety and biosecurity aspects deserve special attention in particular to maintain a safe operation.The IVI is a facility, which was commissioned in 1992. The approach taken to guarantee the safe operation of the IVI containment laboratory will be presented.

3. Conclusions

An accidental release from a high security containment facility is of obvious concern to the public and raises issues of public trust in both science and government. This is important not only to understand what went wrong and how to prevent it happening again, but also to allow consideration of any more widely applicable issues that this incident raises.

In recent years, the emergence and re-emergence of new diseases which threaten public as well as animal health has been recognized. Therefore we should be alerted to newly identified hazards that may emerge in the future. The facilities which will be handling these agents need to guarantee a safe operation, now and in the future.

ORAL: Baculovirus-based vaccination induces immune responses against foot-and mouth-disease vius in mice and conveys protection from lethal rabbit haemorrhagic disease virus infection in rabbits.

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Key words: BacMam technology, transduction, foot-and-mouth disease virus, rabbit haemorrhagic disease virus

Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV), a member of the family Baculoviridae is used for production of recombinant proteins in insect cells since the early 1980s. In the mid-1990s it was demonstrated that AcMNPV recombinants carrying mammalian cell-active expression cassettes, so-called BacMam viruses, were suitable for gene delivery into vertebrate cells of mammalian, avian and piscine origin. Thus, it appears that uptake of AcMNPV by vertebrate cells is a common event. To evaluate the applicability of the BacMam technology for novel vaccination strategies in mammals, poultry and fish, we generated recombinants expressing major antigens of foot-and-mouth-disease virus (FMDV), African swine fever virus, viral hemorrhagic septicemia virus, avian infectious bronchitis virus, and rabbit hemorrhagic disease virus (RHDV). To test for induction of a specific immune response against FMDV, BacMam viruses expressing P1-2A with or without the 3C protease (3Cpro) were injected intramuscularly into mice. All animals developed a specific antibody response which was higher in rBacP1-2A/3C immunized mice than in mice which received rBacP1-2A which supports the view that 3Cpro-mediated cleavage of the capsid precursor protein is important for an efficient immune response against FMDV. Analysis of the cellular immunity revealed induction of both CD4+ and CD8+ T- cell responses. To investigate whether also larger animals respond to BacMam virus immunization and to examine whether BacMam virus-transduced cells might serve for the same purpose, we immunized rabbits with purified virus expressing VP60 of RHDV or with rabbit kidney cells expressing VP60 after transduction with rBacVP60. Rabbits were challenged 88 days after immunization with 10exp4.5 LD50 of virulent RHDV. Both vaccinated groups were fully protected against the disease whereas unvaccinated control animals developed RHD and died within 48 hours.
These results show that BacMam technology is a promising approach for the development of a novel type of vaccines and demonstrate for the first time that this method is applicable for economically relevant productive livestock. Further vaccination/challenge experiments in pigs, rainbow trouts and chicken are in the planning stage.

ORAL: FMD 2007: prioritizing post-movement ban surveillance in Scotland

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Key words: FMD, livestock movement, risk mapping, veterinary surveillance

1.Introduction and Objectives

The FMD outbreak in Surrey, UK, was declared on 3 Aug 2007. The release of virus in Surrey probably occurred in mid-July. No spread from two index cases was detected within 10 km surveillance zone. The UK was (prematurely) declared FMD free on 7 Sept. Further cases were identified in England later in September. No infected premises were found in Scotland. The outbreak occurred at the time of annual peak in live sheep trade in Scotland, but domestic movements did not return to pre-outbreak regime until 9 Oct and exports to continental Europe - until the UK regained FMD free status on 31 Dec 2007. In mid-Aug 2007, Scottish Government (SG) asked EPIC to assess risks of re-opening Scottish livestock markets. Part of this work contributed to prioritization of FMD surveillance in Scotland after the 4 Aug movement ban and is presented here.

2.Material and Methods

Using the GB Livestock movement records, we traced cattle, sheep, pigs and goats that during 15 Jul-4 Aug i) originated from FMD risk zone (defined to be 20km around index cases) or ii) might have been exposed to the virus in a contact chain and then moved into Scotland, and iii) secondary domestic moves. Prompt veterinary inspections were conducted on all the identified holdings. Using a FMD local spread model, relative risks of spread of virus from the potential seeds in Scotland were quantified and mapped.

3.Results

Contact tracing identified 12 Scottish holdings at-risk due to sheep or cattle on-moves. Field inspections on these 12 identified further 3 undisclosed sheep moves. None of 15 was FMD positive. Risk maps indicated priority areas for surveillance and control should an at-risk holding turn out to be infected.

4.Conclusions

Post movement ban contact tracing using livestock movement databases served as an efficient tool to prioritise veterinary surveillance on the ground. However, we observed that delayed movement reporting, IT complexity and unknown frequency of undisclosed moves limit its utility. Forecasting of local spread of a virus if introduced with livestock movements into a disease free zone is conditional on availability of a-priori thoroughly parameterized risk models and up-to-date knowledge of local livestock demography.

5.Acknowledgements

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ORAL: Reverse genetics of foot-and-mouth disease virus

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Key words: FMDV, Reverse Genetics

Reverse genetics system is an excellent platform for offering an opportunity for analysis and modification of viral genomes at the molecular level and has greatly aided research on virus replication, pathogenesis, and vaccine development. To date ,all of the reverse genetics of FMDV were base on transcribe-in-vitro system, which is complicated and expensive. To overcome the shortcomings, An efficient, convenient and economical method of virus rescue is undoubtedly required for elevating the efficiency of rescuing virus. For this purpose, we developed a method to rescue infectious FMDV by direct transfection of cells with full-length genome cDNA clones. Subsequently, a full-length cDNA clone was generated by assembling 5 cDNA fragments, representing the entire genome, between the T7 RNA polymerase promoter and hepatitis delta ribozyme of a low-copy number transcription plasmid pcDNA3.1/Zeo (+) . Transfection of this plasmid into BSR-T7/5, a BHK-21 cell clone, which express bacteriophage T7 polymerase resulted in the recovery of infectious FMDV, The recovered virus was observed to contain the genetic markers that were artificially introduced during cloning. Characterization of the recombinant FMDV showed that its growth characteristics in tissue culture were similar to those of the parental virus. These results demonstrate that infectious fmdv can be generated entirely from cloned DNA in vivo using reverse genetics techniques and a BHK-derived cell line BSR-T7/5 that constitutively expresses T7 RNA polymerase supported efficient , convenient, and reproducible recovery of FMDV. The system not only provides the basis of the gene function research of FMDV, but is also useful for for various studies involving FMDV molecular biology, pathogenesis and vaccine development.

ORAL: Interaction of Foot-and-Mouth Disease Virus (FMDV) with dendritic cells: role of IL-10.

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Key words: FMDV, dendritic cells, IL-10

1.Introduction and Objectives

Foot-and-mouth Disease Virus (FMDV) is the causative agent of a highly contagious vesicular disease of cloven-hoofed animals. We have previously described that FMDV serotype C infects lymphocytes in vivo, causing a profound lymphoid depletion. This is accompanied by a functional inactivation of antiviral T cells. Such immunosuppression occurs rapidly after infection but eventually the immune response is recovered and able to control the viral infection. The mechanism(s) that initially induces immunosuppression and leads to the loss of T-cell activation is unknown. To understand the mechanisms driving this immunosuppression, we have studied the interaction between T cells and DCs.

2.Material and Methods

Swine DCs were produced from PBMCs with rpGM-CSF and IL-4. The antigen stimulation capacity of DCs was measured by mix lymphocyte reaction. The amount of IL-10 was determined by ELISA.

3.Results

We have found that FMDV infects DCs in vitro and interferes with their maturation. More interestingly, DCs isolated ex vivo from FMDV infected pigs shown a decreased in co-stimulatory molecules. In antigen presentation assays, DCs isolated from FMDV infected swine were not able to activate T cells. The study of cytokines produced in DCs:T cells co-cultures showed a high amount of IL-10 produced. Interestingly, serum from FMDV infected swine at the peak of viremia showed an increased in IL-10. Blocking of IL-10 by anti-IL10 antiboides in DCs: T cells cocultures recovered the antigen capacity stimulation of DCs.

4.Discussion and Conclusions

The in vivo role of IL-10 is generally immunosuppressive although this cytokine plays also an important stimulatory role in the production of antibodies by B-lymphocytes during the development of an immune response against antigens from pathogens. Our data suggest that IL-10 is being produced in FMDV infected swine and may inhibit thymus dependent (TD) antibody responses, reflected in the observed T cell immunosuppression. By contrast, anti-FMDV thymus independent (TI) antibody response may not be affected. We are currently carrying out several experiments to rule out these possibilities.

ORAL: CSFV-replication successfully inhibited in pigs by imidazo[4,5-c]pyridines.

Vrancken, Robert[1]; Haegeman, Andy[1]; Paeshuyse, Jan[2]; Puerstinger, Gerhard [3]; Rozenski, Jozef[2]; Wright, Matthew[4]; Tignon, Marylène[1]; Le Potier, Marie-Frédérique[5]; Neyts, Johan[2]; Koenen, Frank[1]

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Key words: antiviral, pestivirus, classical swine fever virus, in vivo inhibition, virus replication

1.Objectives

Evaluate the antiviral activity of the imidazo[4,5-c]pyridine BPIP (1) in pigs, experimentally infected with classical swine fever virus (CSFV).

2.Materials and Methods

Large White pigs (n=8) received BPIP (75mg/kg/day) in food pellets for 15 consecutive days. One day after first administration, pigs were infected intramuscularly with 105.1 TCID50 of CSFV 'Wingene'. Animals were clinically observed for 33 days and blood sampled 2 to 3 times a week. Tonsils were examined at necropsy. The presence of infectious virus, neutralizing antibodies and leucocytes were examined using standard methods. Viral RNA in blood and tonsils was examined using real-time RT-PCR.

3.Results

Treatment with BPIP resulted in a marked reduction of the period of viraemia and leucopenia. Virus titres at peak viraemia (7dpi) were ~1000 fold reduced ($p=0.00005$) and real-time RT-PCR analysis revealed a significantly lower viral genome load in blood over the whole duration of the trial ($p\leq0.001$). One BPIP-treated/infected animal remained negative on VI and real-time RT-PCR.

4.Discussion and conclusion

BPIP, an in vitro inhibitor of CSFV-replication (2, 3), significantly reduced the virus titre at peak viraemia at 7dpi and the viral genome load in blood of CSFV-infected pigs. This reduction allowed the treated animals to recover from leucopenia, supporting the hypothesis that the progressive decrease of the viral genome load is the result of the synergistic action of the cell-mediated immunity, sustained by the inhibiting effect of BPIP, followed by the viral clearance of the blood by a humoral antibody response. Although blood is cleared, viral RNA, but not infectious virus, was detected in tonsils. This can be attributed to neutralizing antibodies and represents no danger for virus persistence (4). This study provides a proof of concept for the in vivo activity of imidazo[4,5-c]pyridines and suggests that more active analogues of BPIP could further reduce (or prevent) CSFV-replication.

5.Acknowledgements

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ORAL: The residual FMD risk after declaring a region free from disease with an application to vaccinated pigs

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Key words: Bayesian inference; between-herd prevalence; foot-and-mouth disease; test sensitivity; vaccination; within-herd prevalence

After an FMD epidemic a region can only regain its freedom-of-disease status after a 3-month waiting period if no vaccination is applied and a 6-month waiting period if (emergency) vaccination is applied. EU legislation furthermore prescribes a final screening 30 days after the last outbreak or vaccination consisting of clinical inspection and serological testing. When positive results are obtained, the epidemic apparently has not ended yet. When only negative results are obtained, the area is assumed to be free from FMD, although some infected animals might have been missed due to sampling and use of imperfect tests (test sensitivities < 1).

The goal of this study was to estimate the residual FMD risk by calculating the possible within-herd prevalence (PA) and between-herd prevalence (PH) (a) directly after final screening, (b) after a 3-month waiting period, and (c) after a 6-month waiting period, if the area was declared free incorrectly. This is illustrated by calculations for an infected area where all pig herds were vaccinated.

Bayesian inference was used to estimate uncertainty distributions for PA and PH assuming that – despite at least one infected animal being present – all test results were negative. The prior distribution of PA was derived from final size calculations assuming a basic reproduction ratio R0 of 2.42. Distributions of the likelihood that only negative test results were 1 infected animals present in the herd were combined with this≥obtained given prior to infer the posterior PA. Test sensitivity, sample size, and number of repetitions were the main determinants of these likelihood distributions and differed between detection methods. Calculation steps for PH were similar, but an uninformed prior was used. Calculations were carried out in Excel and @Risk (10,000 iterations).

Uncertainty distributions for PA and PH only differed for extreme values when comparing model results after final screening and a 3- or 6-month waiting period, resulting in slightly lower mean values after waiting.

Although this method does not answer the question whether the affected area was declared free from FMD rightly, it gives an estimate of the PA and PH if it was not, providing insight into the residual FMD risk. A 3- or 6-month waiting period did not importantly reduce this risk.

ORAL: Phylogenetic Surveillance of FMD Type O Epidemics in Turkey between 1996–2007

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FMD Institute[1]; Danish Institute for Food and Veterinary Research[2]

Key words: foot-and-mouth disease, molecular epidemiology

1. Introduction and Objectives

Foot-and-mouth disease (FMD) is endemic in ruminants in Turkey causing economic losses and currently representing a threat for disease-free border countries. Approximately 2/3 of all FMD outbreaks in Turkey are due to FMD type O according to the recordings of the diagnostic activities. This suggests that FMD type O might circulate persistently in Turkey (1,3). FMD type O ME-SA topotype has become persistent in many parts of Asia since 1960's. The present study focuses on evolutionary and phylogenic relationships between Turkish and global ME-SA taxa in an attempt to resolve the epidemiological dynamics of type O in Turkey during 1996-2007.

2. Material and Methods

Collection or production of taxa: 242 taxa of 1D part of FMDV genome were either downloaded from NCBI Foot-and-mouth disease virus - type O nucleotide database (taxon ID: 12118) or produced from Turkish field isolates by the methods and primers described previously (1,3).

Phylogenetic and evolutionary analysis: ClustalX and MEGA4 software were used for alignments and phylogenetic analysis (4).

3. Results

ME-SA topotype was divided into four main groups and nine subgroups. Based on the time-series of collected taxa, NPA-01 and NPA-05 of PanAsia C-IV are the only active member in ME-SA topotype. Groups showing pandemic nature were also identified as NPA-05 (3 continent), PA1 (3 continent), Iran2001 C-1 (2 continent). On the other hand alfa and beta subgroups of PanAsia C-IV were confined into Western Asia. Between and within group distances were also determined.

4. Discussion and Conclusions

Strains from FMD type O epidemics in the West and South of Asia could be roughly divided into pandemic and endemic phases where strains became disseminated in large geographical areas or remained more local. Evolutionary analysis indicated that regionally restricted strains were derived from pandemic ones and persisted for longer time compared to those of a pandemic nature. The origins and phylogenic relationships of Beta and Alfa groups defined previously (1) and NPA-05 defined in this study will be discussed.

5.Acknowledgements

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ORAL: Avian influenza surveillance in the EU - Results of epidemiological analysis of surveillance data from 2006 and 2007 and implications for targeting of future surveillance

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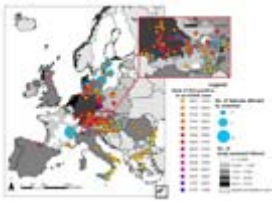
Veterinary laboratories Agency[1]; JNCC[2]; FAO[3]; IZS[4]; European Commission[5]

Key words: EU AI Surveillance, Epidemiology

In 2006 outbreaks of highly pathogenic H5N1 avian influenza virus occurred for the first time in the EU. Incidents in wild birds were detected in 14 Member States (Fig.1). In response to these outbreaks extended data collection was implemented by the European Commission and a database was established to capture epidemiological information on collected birds such as location, species of bird and status at the time of sampling. The objectives of the analysis of the available data were to explore the epidemiology of Avian Influenza with regard to species of wild birds involved, timing and location of infections as well as the applicability of different surveillance types for the detection of infections. Here we present the 2006/2007 results and the epidemiology of the H5N1 HPAI outbreaks in those years and discuss the implications for surveillance and early detection.
NB: Data from 2007 is presently analysed so could not be included here but will be the focus of the presentation at the conference.
Laboratory tests were carried out according to the diagnostic manual (2006/437/EC) . Epidemiological data and test results were collected and submitted to the Community reference laboratory (CRL) at VLA-Weybridge, UK for analysis. Analysis of the results was carried out using Microsoft Access, Microsoft Excel, ArcMap, and R.
In 2006 a total of 144, 805 records of birds of at least 330 species were sent to the CRL.
Results of the analysis indicated that for the detection of H5N1 HPAI passive surveillance of dead or diseased birds appears the most effective approach, whilst active surveillance offered better detection of low pathogenic avian influenza. No carrier species for H5N1 HPAI could be identified and almost all birds infected with H5N1 HPAI were either dead or showed clinical signs. A very large number of Mallards were tested within this surveillance and while a high proportion of LPAI infections was found in this species, H5N1 HPAI was rarely identified in these birds. Species that appear to be very susceptible to H5N1 HPAI are swans, diving ducks, mergansers and grebes, supporting experimental evidence.
Surveillance results suggest that H5N1 HPAI did not establish itself successfully in the EU wild bird population. The impact on these results for the targeting of surveillance systems to improve early detection of H5N1 HPAI infections as well as the comparability of results and future developments of the EU online database are discussed.



ORAL: Improved but still limited HPAI testing capacities in developing countries

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FAO[1]

Key words: HPAI testing laboratories developing countries

Thanks to international support, capacities for HPAI testing have been substantially improved the last three years in many countries. Basic and high technology equipments have been provided to many countries, as well as training to laboratory staff through various projects. However large limitations are still questioning the quality of laboratory results and sensitivity of HPAI surveillance. These limitations are first due to insufficient supply of reagents, especially those used in molecular analysis. Lower cost and quality reagents are sometimes used in laboratories. Besides, improved training of laboratory staff with a critical sense of result interpretation is still generally lacking since only short-term training was mainly provided. Facilities still need improvements in many countries. Biosafety levels are deficient in most countries and maintenance newly build BSL3 facilities is questionable. Quality assurance procedures and documentation is still missing or weak and is difficult to apply in the context of developing countries, in particular equipment maintenance and calibration. The number of samples submitted to laboratories is generally low for surveillance teams are still missing means to undertake thorough field investigations and samples collected dependent on running international projects. Finally, shipment is still cumbersome in many countries.

Various solutions can limit the impact of such deficiencies in laboratory capacities. First the rapid tests can be used under specific criteria, in countries with limited laboratory capacities. The use of rapid antigen detection tests in various countries will be described. Secondly, provision of good quality reagents to countries and of standard protocols (ex. EU protocols) can allow better result quality. Regular proficiency tests should be organised by international reference or regional laboratories. Assistance for international shipment should be provided to national laboratories to encourage diagnosis confirmation and isolate sharing. Long term training of African scientists, in particular, is necessary. Laboratory networks and twinning between laboratories are emerging or being reinforced. Examples of West/Centre Africa and SADC region laboratories networks and the twinning between AAHL (Australia) and 8 Indonesian laboratories will be given.

ORAL: Developing a marker vaccine for rinderpest virus by epitope deletion

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Veterinary Laboratories Agency [1]; Iah Pirbright Laboratory[2]

Key words: rinderpest virus, marker vaccine, phage display, epitope mapping

The current OIE 'gold standard' in serological tests for rinderpest virus (RPV) is a competitive (c) ELISA that depends on competition between RPV H protein specific monoclonal antibody (C1) and antibodies within test sera for virus antigen. However, this test is unable to differentiate between vaccinated animals and those naturally infected within the field. The aim of this project is to produce a positively and negatively marked vaccine for rinderpest virus (RPV) by deletion/alteration of the virus-specific C1 binding site and replacing it with a suitable positive marker sequence.

We have identified the C1 binding site using a phage display library, a technique that has been successfully used in epitope mapping, antibody engineering and vaccine development1. Alterations to the C1 binding sites mapped in the H gene were carried out using both site directed mutagenesis and overlap PCR.

Two regions of the H protein were identified as the putative epitope binding site for C1, indicating that it is a conformational epitope2. Alterations to these regions were assessed by co-expression with RPV fusion (F) protein in an in vitro fusion assay. The first site mapped to amino acids 309-320 (region 1) and these were replaced with corresponding sequences from a related morbillivirus (PPRV). Mutations were confirmed by sequencing and the resulting H gene was functional in the fusion assay. The second site mapped to amino acids 532 – 536 (region 2). Unlike region 1, this mutant was unable to generate syncytia in the fusion assay suggesting that it may be an important functional site and cannot be altered. As expected, altering regions 1 and 2 together was also non-functional. The functional region 1 mutant failed to react with C1 using confocal microscopy and so could act as a potential negative marker.

We have identified the binding sites of C1 which is a conformational epitope with two distinct binding sites. One of the proposed binding sites can be altered without losing protein functionality but prevents detection of H using C1 in immunofluoresence studies. This mutant is now being inserted into the full-length RPV cDNA clone and the rescued virus will be characterised both in vitro and in vivo to establish its usefulness as a marker vaccine.

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ORAL: Development of a novel proteomics-based approach for analysis of stability of FMDV antigens

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Key words: SELDI, proteolysis, vaccine quality control

The "Global Roadmap for Improving the Tools to Control FMD in Endemic Settings" has identified the poor antigenic stability of FMD vaccines (Doel and Pullen, 1990; Razdan et al., 1996) as an important knowledge gap. The molecular basis for this unstability is unclear. Possible causes are dissociation of intact viral particles into distinct smaller particles, aggregation or proteolytic degradation of FMDV antigens.

We analysed FMDV antigen integrity using Surface-Enhanced Laser Desorption Ionization – Time Of Flight – Mass Spectrometry (SELDI-TOF-MS) measurements (Zhu et al., 2005). By this method antigen samples are bound to protein arrays, covered with an energy absorbing molecule and ionized by a laser. The molecular mass of all molecules is then determined using TOF-MS, which is far more accurate than SDS-PAGE. In addition to arrays that bind all proteins, we used protein arrays to which llama single-domain antibody fragments specific for FMDV (Harmsen et al., 2007) were covalently coupled for specific detection of FMDV antigens.

We initially identified all spectral peaks representing the four FMDV structural proteins (VP1-4). VP4 is detected in its myristoylated form as a heterogeneous mixture of 8 molecules differing by about 16 da. VP1 is also identified as a mixture of 3 molecules differing by about 180 da. The molecular basis of this VP1 and VP4 heterogeneity is unclear and to our knowledge not reported previously. VP2 and 3 were identified as single molecules or disulfide-linked VP1-VP2 dimers or VP3 homomultimers, as reported earlier.

We next analysed proteolysis of FMDV antigens using SELDI-TOF-MS. Upon incubation of FMDV antigen for 14 days at 35oC a small amount (<5%) of a VP1 degradation product was detected. Such proteolysis was not observed after 1 year storage at 4oC. This suggests that limited proteolytic degradation can occur when vaccines are not properly stored at 4oC, but does not occur in properly stored vaccines.

Thus, SELDI-TOF-MS proved useful to analyse FMDV antigen stability and identify novel viral protein heterogeneities.

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ORAL: Rapid in depth diagnostics of viral full length genomes using the Genome Sequencer technology

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Key words: sequencing, in depth diagnostics, genome sequencer

The Genome Sequencer technology of Roche/454 Life Sciences facilitates large DNA sequencing projects to be completed in very little time. In a single overnight run of the Genome Sequencer FLX instrument it is possible to generate 100 Mb of raw sequence, sufficient to assemble a complete bacterial or several viral genomes with high quality. Moreover, the Genome Sequencer technology allows sequencing without prior sequence knowledge or cloning regardless of the DNA source (genomic, PCR, cDNA). Thus, the Genome Sequencer technology makes it possible to rapidly analyse any nucleic acid sequence in detail. These advantages enabled the rapidly and detailed characterization of completely unknown samples with regard to their viral RNA/DNA content. Therefore, it is possible to identify pathogens potentially included in a DNA sample by in depth sequence analyses. In addition, we established protocols for the fast in parallel investigation of the complete genome sequences of several viral isolates with very high reliability. Results from rapid full length sequencing of HPAIV H5N1 strains will be presented as an example for the power of this new technology.

ORAL: Experimental infection of mute swans with highly pathogenic avian influenza virus H5N1

Kalthoff, Donata[1]; Breithaupt, Angele[1]; Teifke, Jens P[1]; Globig, Anja[1]; Harder, Timm[1]; Mettenleiter, Thomas C[1]; Beer, Martin[1]

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Key words: HPAI H5N1; swan

Mute swans (Cygnus olor) were often affected with a high case fatality rate during outbreaks of HPAI H5N1 in wild birds. We therefore infected two groups of mute swans with a low and a high dose of highly pathogenic avian influenza virus A/Cygnus cygnus/ Germany/R65/2006 (HPAIV H5N1). After an incubation period of four days clinical signs could be first observed including severe neurological disorders like ataxia, torticollis and opisthotonus, or acute death. Shedding of high viral loads was detected from all swans in oropharyngeal and cloacal swabs for up to six days per animal, and eleven out of twelve naïve mute swans died between five and fourteen days after inoculation. Interestingly, two animals with pre-existing antibodies against avian influenza virus survived, although viral shedding was detected in both animals. In contrast, naïve individuals despite developing high titers of neutralising antibodies after infection succumbed to the disease. Three swans showed widespread endothelial dispersion of viral antigen and succumbed rapidly; these animals did not mount AIV-specific antibodies following virus inoculation. In all swans presenting clinical signs and AIV-specific antibodies, immunostaining was exclusively positive in surface and parenchymal epithelia and neuronal tissue. We concluded that swans (1) are highly susceptible to infection with HPAIV H5N1, (2) excrete significant viral loads for several days and can therefore play an important epidemiological role in the spreading of HPAIV H5N1, and finally (3) can be clinically protected by pre-exposure immunity.

ORAL: NPRO DELETION MUTANTS AS EFFICIENT AND SAFE BOVINE VIRAL DIARRHEA (BVDV) VACCINE CANDIDATES

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Key words: BVDV, vaccine safety, Npro

Bovine viral diarrhea (BVD), a worldwide distributed Pestivirus infection of cattle, causes major economic losses due to reduced fertility, abortions, and the generation of persistently infected calves, which may develop fatal “Mucosal Disease”. According to their cell culture properties, BVDV isolates are divided into two biotypes, referred to as cytopathogenic (cp) or non-cytopathogenic (ncp). Vaccination is an important tool for BVDV-control, claiming not only protection against disease, but also prevention from fetal infection. However, the safety of commercially available modified live vaccines relating to fetal infection is under discussion. The viral N-terminal autoproteinase Npro counteracts IFN-α/-β induction and mediates evasion of the interferon response in infected cells. We constructed Npro deletion mutants based on full-length cDNA clones of an ncp and the homologous cp BVDV type 1 strain. Vaccine safety, immunogenicity, and efficacy were evaluated in vaccination-challenge experiments in calves. Furthermore, both mutants were delivered to pregnant heifers in the first trimester of the gravidity. Four months later, the animals were sacrificed and the fetuses were analyzed for persistent infection. Both recombinants were shown to be highly attenuated and efficacious vaccine candidates. The Npro mutants mediated complete protection from a virulent BVDV-I challenge infection in calves. After application to pregnant heifers, no infectious virus could be recovered from the fetuses, which was in clear contrast to the infection with the parental wild-type ncp-virus. In conclusion, the Npro deletion mutants are modified live vaccines with the potential to induce sterile immunity without the risk of establishing persistent infections of the fetuses.

ORAL: Animal Health Surveillance in the Netherlands

Kock, Petra [1]; van Wuijckhuise, Linda [1]; Mars, Jet [1]; van Schaik, Gerdien[1]

PO box 9[1]

Key words: surveillance, exotic diseases, emerging diseases

In 2002 private and public stakeholders (the Ministry of Agriculture, Nature and Food Quality and the agricultural Boards) aked GD to set up a national animal surveillance system. This was implemented in 2003. Objectives of the surveillance system are:

1. Early detection of OIE list diseases
2. Early detection of new or emerging diseases
3. Analysis of trends and developments in several aspects of animal health.

A number of complementary surveillance components are put in place. They fit in a bottom up structure, involving specialists in various fields of expertise (veterinary medicine, pathology and epidemiology) charged with the aggregation and interpretation of information. Great emphasis is put on gathering the information in the most effective way for each objective. Most effective for early detection of exotic and new disorders are two ways of re-active surveillance, in which participation is voluntary but attractive and rewarding. In case of unusual symptoms in life stock, private practitioners are motivated to call a nationally operating group of health specialists: “GD-Veekijker”. In return, the caller gets free specialist advice on these clinical problems. In case unusual symptoms are accompanied with mortality carcasses can be submitted for post mortem investigation to GD. Direct value for farmer and practitioner is the diagnosis on the individual problem. All results are also analysed for exotic and new disorders. Regular feedback is also provided: important surveillance information for practioners is communicated through publications in magazines, presentations and newsletters. In addition to evaluation of individual cases, each week the reported information and laboratory results are discussed by the specialists involved for their relevance as indicators for exotic or emerging diseases. When necessary, further investigation is instigated by visiting the farm, or by pilot studies. For exotic or emerging diseases close coöperation is established with CVI for laboratory investigation and expertise and with the Food Safety Authority for regulatory aspects. Results are reported quarterly to the steering committee, composed by representatives of the stakeholders including the FSA. When the nature of the finding requires reports are made instantly. Where necessary, GD advises stakeholders on possible actions. The performance of this quick and effective surveillance system will be illustrated by findings on OIE diseases and new disorders.

ORAL: Development of the Classical swine fever (CSF) virus database in Hannover: from compilation of virus isolates to automated genotyping

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Key words: CSFV/ database/ genetic typing

CSF is a highly contagious viral disease of pigs. According to the OIE Classification of Diseases it is classified as a notifiable disease, with the potential for causing severe socio-economic problems and severely affecting the international trade of pigs and pig products. To control the epidemics, fast and reliable tracing of the virus source and its spread proved to be of uttermost importance. This became possible since it was found that particular isolates predominate within certain geographical areas. The inter-relationships among isolates can be demonstrated by genetic typing, which in the meantime has become the method of choice. For this, compilation of epidemiologic data concerning isolates and their relation to individual outbreaks had to be done. This lead to the establishment of the WWW database of CSF virus isolates at the Community Reference Laboratory for CSF. There were three main objectives. Firstly, to keep reference stocks of virus isolates from all epidemics in or near the European Union, and – if available – from all over the world. Secondly, to make the virus isolates available for further characterization. Thirdly, to have a collection of sequences available for genetic typing [1].
Genetic typing of CSFV isolates was being performed in many laboratories, but under different conditions, thus limiting information for inter-laboratory comparison. Genetic typing had to be harmonized. In a cooperative study genotyping using different genomic fragments were compared, and a protocol for calculation of the phylogenetic trees, using public domain computer programs, was developed. In addition, a standardized nomenclature of groups and subgroups was suggested [2].
The database was extensively used, and records increased rapidly. However, this made it difficult for the user to select a standard dataset for genotyping new isolates. As a consequence, the database was supplemented with a module for automated genetic typing, including a graphical display of the phylogenetic tree [3].
The basis for the success of such a database is its acceptance by the scientists involved and their willingness to follow strict protocols. In addition, the database must be easy to maintain, at a reasonable cost.

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ORAL: Fulfilling the potential of molecular assays to detect and characterise FMDV: updated findings from the 2007 outbreaks in the United Kingdom

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Key words: FMDV, real-time RT-PCR, molecular epidemiology

1. Introduction and Objectives

The 2007 FMD epidemic in the United Kingdom was characterised by two distinct outbreak clusters. In total, 8 infected premises were identified (comprising 11 holdings): 2 in the west of the county of Surrey in August and 6 in the north of Surrey in September. This report briefly summarises the results from the laboratory investigation of these cases; using real-time RT-PCR (rRT-PCR) assays to detect FMDV in clinical samples, and full-genome studies to determine the most likely source of the outbreak and to assist real-time epidemiological investigations in the field.

2. Material and Methods

Field samples (epithelial tissues, sera, EDTA blood, oesophageal-pharyngeal fluid, faecal suspension and swabs) were collected by veterinary staff and transported to the IAH. An automated one-step rRT-PCR was used to detect FMDV genome in these samples. Additional antigen detection assays (VI and agELISA) were also used to test some of the material submitted. For high-resolution molecular tracing, full genome sequences were generated for 14 positive samples using overlapping RT-PCR.

3. Results, discussion and Conclusions

FMDV detection: Rapid laboratory-based real-time RT-PCR was used as a front-line diagnostic tool for the detection of FMDV. In total, 3205 samples were tested by rRT-PCR: of which 98 were positive for FMDV. In addition to confirming clinical disease on all the infected farms, the rRT-PCR was also able to provide evidence of pre-clinical infection in a herd of 58 cattle in the first phase of the outbreak. In the second phase of outbreaks, the rRT-PCR supported a targeted surveillance programme comprising 14 high-risk herds in farms surrounding positive cases.
Molecular epidemiology: Within 24 hours, sequence data obtained from the first farm revealed a VP1 gene-identity of 99.8% to FMDV O1 BFS 1860: a widely used reference and vaccine strain. Analysis of full-genome sequences obtained from the infected farms revealed 6-22 nucleotide changes with the closest potential laboratory source. Statistical parsimony (TCS) analysis was used to reconstruct the most likely chain of transmission events that occurred during these outbreaks, clearly showing that the 2 clusters of outbreaks were connected and did not arise through independent sources. Furthermore, these data were used to predict undisclosed FMD infection that was subsequently detected during sero-survey of farms within the control zone.

ORAL: AN ADVANCED FIELD DEPLOYABLE "PEN SIDE" SAMPLE PREPARATION AND PCR DIAGNOSTIC SYSTEM

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Smiths Detection[1]

Key words: RAPID, PEN SIDE, DIAGNOSTICS, PORTABLE, PCR

1.Introduction and Objectives

Efforts to control the spread of naturally occurring highly infectious pathogens, for example Highly Pathogenic Avian Influenza, have been complicated by the need to transport samples to the lab for ultimate identification. This abstract describes a briefcase sized portable sample preparation and PCR system, designed for use in the field, including the ability to sanitise the unit with disinfectant. The system automatically purifies nucleic acids from a wide range of sample types and carries out PCR analysis, reporting either a positive or negative results. The system provides a fully automated portable on-site identification capability in a wide range of weather conditions by a person with no knowledge of PCR.

2.Material and Methods

Operation of the device is extremely simple. A veterinarian suspecting the presence of disease takes a sample from the animal. The veterinarian places that sample in to a single use sample preparation device. The assay profile it automatically selected and automated sample preparation begins. PCR is initiated automatically. This entire process is performed with no user intervention.

3.Results

The sample preparation device was designed to take a lab based process into the field and automate it. Initial results indicate that the device performs as well as the original bench process. There are currently 2 assays being developed and validated on this platform, one to detect all seven serotypes of Foot and Mouth Viruses in a single tube, and one to differentiate high and low pathogenic H5N1 Avian Influenza Virus. These assays are in development/validation using real samples in collaborating reference labs. The platform uses a novel PCR chemistry called LATE-PCR which is able to identify many pathogens or strains of a pathogen in a single tube. The system is currently being prepared for field based trials.

4.Discussion and Conclusions

"Pen side" detection systems offer the promise of rapid detection of disease allowing a more resilient response and more effective outbreak management. A challenge in deploying PCR based analysis systems in the field is the requirement for a robust and reliable sample preparation system. The Smiths Detection's Portable Veterinary Diagnostic System provides the benefit of sensitivity with ease of use.

For more information please visit: www.smithsdetection.com/vet

ORAL: Monitoring of the EBL-free status in the Netherlands

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GD[1]; WUR[2]

Key words: freedom, Enzootic Bovine Leucosis, disease surveillance modelling

In international trade, measures taken to protect animal health should be based on scientific principles and sufficient evidence. Two approaches have been commonly used to provide the required evidence for freedom of disease: a structured, representative survey of the relevant population and a qualitative assessment of multiple sources of evidence.

In the Netherlands, both approaches are applied to demonstrate freedom from Enzootic Bovine Leucosis (EBL). Each year a survey on a random sample of 3.312 Dutch cattle herds was carried out to determine whether the Dutch cattle population is free of EBL. In addition, cattle farmers are required to send in aborted foetuses to be tested for EBL, amongst other diseases.

The goal of this study was to calculate the sensitivity of this annual testing scheme to detect a design prevalence of at least 0.2% of the cattle herds, and 30% of within herd prevalence by means of probabilistic modelling and discuss the effect of the testing of cows that aborted on the sensitivity of the whole system across years.

Recently, advanced disease surveillance modelling presented methods for combining multiple sources of surveillance data to be used to develop a quantitative probability estimate to support claims of freedom from disease or infection (Cameron and Baldock, 1998a,b; Martin et al., 2007). The methodology was used to investigate three different scenarios for EBL surveillance in the Netherlands; randomly testing the bulk milk from 1,739 dairy herds and blood samples from 1,210 beef herds (the Dutch scheme), testing one-third of the dairy herds (7,515) in bulk milk and testing a random sample of beef cattle at slaughter (~26,000) (the Danish scheme), and testing of aborted foetuses only.

The probability to detect an EBL infection in the Netherlands with the Dutch scheme was 96.5% and thus not in agreement with the OIE requirements (>99%). The Danish scheme would increase the sensitivity to detect EBL to >99% also at a lower within-herd prevalence of 10%. Decreasing the number of samples from slaughter cattle in the Danish scheme up to 10,000 would be possible at a within-herd design-prevalence of 30%. At a 10% within-herd design-prevalence at least 17,000 slaughter cattle should be tested. Testing of 12,083 aborted foetuses was a sensitive scheme (99.9%), but the samples may not be representative for the Dutch cattle population.

ORAL: Development and evaluation of a real-time quantitative PCR assay for Culicoides imicola, one of the main vectors of Bluetongue (BT) and African Horse Sickness (AHS) in Africa and Europe

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CIRAD[1]; EID[2]; ULP[3]

Key words: Real-time PCR; Culicoides imicola ss; Bluetongue; African horse sickness

The current microscopy method for identifying the Culicoides imicola Kieffer, 1913 species can be time and labour intensive. There is a need for the development of a rapid and quantitative tool to quantify the biting midges Culicoides imicola ss in light-trap catches. A reproducible and sensitive real-time polymerase chain reaction method that targets the internal transcribed spacer (ITS-1) of ribosomal DNA of C. imicola ss species was developed. This real-time PCR assay was first performed on 10-fold serial dilutions of purified plasmid DNA containing specific C. imicola ss ITS-1. It was then possible to construct standard curves with a high correlation coefficient (r2 = 0.99) in the range of 10-2 to 10-8 ng of purified DNA. The performances of this PCR were evaluated in comparison with morphological determination on Culicoides trapped along the Mediterranean coastal mainland France. ROC statistical analysis was carried out using morphology as gold standard and the area under the ROC curve had a satisfactory value of 0.9752. The results indicated that this real-time PCR assay holds promise for monitoring C. imicola ss population in both surveillance and research programmes because of its good specificity (92%) and sensitivity (95%).

ORAL: INSPIRE: an opportunity to standardise, harmonise and integrate zoonotic data and services

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Key words: INSPIRE, harmonisation, zoonotic data

1.Introduction and Objectives

INSPIRE is an European Directive establishing the legal framework for setting up and operating an Infrastructure for Spatial Information (SDI) in Europe. The first target of INSPIRE is in relation to environmental policies, but it is clear however that once this infrastructure is in place, it will also involve other sectors such as agriculture, human and public health, veterinary data, etc.

Once fully implemented, it will enable data from one Member State to be seamlessly combined with data from all other States. This is particularly important for epidemiological activities relating to the management of epidemic events and surveillance programmes.

2.Material and Methods

A Spatial Data Interest Community (SDIC) for zoonotic data and services will be established to participate in awareness raising and in identifying training needs necessary for both the transposition of the INSPIRE Implementing Rules and the integration of the zoonotic data and services within the I NSPIRE initiative, by addressing the stakeholders to work in compliance with the SDI European standards.

3.Results

The transposition of the INSPIRE rules within the veterinarian-epidemiological context should follow a step-wise approach, starting with unlocking the potential of existing data and national SDI and then gradually harmonising data and information services towards a fully European interoperability system.

4.Discussion and Conclusions

The data are often of unsatisfactory or undefined quality, based on proprietary information systems and not accessible to the public or other users at local, regional, national and international level. Therefore, projects that combine data coming from various sources to provide policy-relevant information and tools are often time consuming and costy. Policies need to be put in place to reduce the duplication in collection, harmonisation efforts and to facilitate and promote wide dissemination of the data. The transposition of the INSPIRE initiative within the veterinarian-epidemiological context is therefore relevant but also a major challenge given the general situation outlined above and the many stakeholder interests to be addressed.

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ORAL: Understanding and predicting between-farm spread of highly transmissible animal diseases: the role of mathematical modelling

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Key words: intervention strategies, transmission model, between-farm transmission, emergency vaccination, preventive culling

1.Introduction and Objectives

In the recent past, large epidemics of Classical Swine Fever (NL 1997/1998), Foot-and-Mouth Disease (UK 2001), and Avian Influenza (e.g. NL 2003) have taught us how difficult it can be to control the between-farm spread of such diseases, especially in densely populated livestock areas. A common feature in these epidemics has been the phenomenon of "neighborhood infections", i.e. the untraced disease transmission between farms despite the presence of movement restrictions and enhanced bio-security measures. I this talk I discuss how mathematical modelling based on the outbreak data and on other data allows a quantitative evaluation of the expected effect of different combinations of control measures on transmission.

2.Material and Methods

Mathematical modeling is used to extrapolate observed epidemiological patterns under past intervention strategies to other epidemic scenarios and possible future intervention strategies.

3.Results

I will explain how the evaluations of intervention strategies can be based on transmission models with farms as individual units, and how the results can be presented concisely in the form of geographical "risk maps". For evaluating control strategies employing emergency vaccination it can be useful to build up the transmission model starting from an explicit animal level, i.e. using animals as individual units and farms as aggregate units. Finally, I discuss how a lack of data currently complicates a quantitative evaluation of the effect of individual bio-security measures.

4.Discussion and Conclusions

I arrive at the following general conclusions about the role of mathematical modelling in analyzing the control of highly transmissible animal diseases:

- Models are necessary instruments to make extrapolations to new outbreak scenarios and new control measures.
- The appropriate level of detail of models is determined by both the type of extrapolation desired and the available data.
- Models can be helpful in identifying priorities for further experimental study or epidemiological data gathering.

5.Acknowledgements

This talk is based on collaborative work with Jantien Backer, Gert Jan Boender, Annemarie Bouma, Michiel van Boven, Armin Elbers, Mart de Jong, Gonnie Nodelijk and Herman van Roermund.

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ORAL: MICROARRAY ANALYSIS OF CSFV VIRULENCE

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Key words: CSFV, virulence, microarray

1.Introduction and Objectives

Classical Swine Fever Virus (CSFV) is a member of Flaviviridae family which causes severe leucopenia and led pigs to death depending on the virulence of the strain. Pathological differences observed with a highly virulent strain (HVS) compared to a moderately virulent strain (MVS) are still not understanding.

We conducted a microarray study intending to identify host gene modulations related to CSFV strains virulence.

2.Material and Methods

Eight SPF pigs, were infected with 106 TCID50 CSFV Eystrup HVS or Paderborn MVS. PMBCs were purified at 0, 24, 48, 72h post-inoculation and frozen in Trizol before RNA extraction. All control pigs RNA were pooled as the uninfected pig reference. RNA were converted to aminoallyl-RNA (aRNA) and either Cy3 or Cy5 labelled using Amino Allyl Message Amp aRNA kit (Ambion) and CyDye (Cy3/Cy5) Reactive Dye Pack (Amersham). Then, each sample from infected pigs was hybridized with a reference sample to a microarray slide from US Pig Genome Coordination Program and scanned using GenePix Pro 5.0 software (Axon Instruments). Real-time PCR of selected genes will be used for the microarray results validation.

3.Results

At the same infection time-point, gene expression comparison in PBMCs isolated from HVS infected pigs to PBMCs isolated from MVS infected pigs revealed many up-regulations of genes implicated in apoptosis, anti-viral and immune responses, cell adhesion and mobility, transcription and translation regulation, signal transduction and metabolism. With the MVS, these expression modulations are time-delay, but without reach the same level for many of them. Interestingly, a lot of IFN stimulated genes (ISGs) are targeted by CSFV. Microarray results are under PCR confirmations.

4.Discussion and Conclusions

CSFV-induced leucopenia, related to apoptosis, are shown to be associated to a strong IFNa production that is time delay and less-level in MVS infected pig serum than in HVS infected pig serum [1]. Microarrays results reinforce the hypothesis that CSFV turns away the IFN anti-viral responses signalling to induce apoptosis and that phenomenon is associated to strains virulence.

5.Acknowledgements

We thank the OUEST-Genopole transcriptomic platform for GenePix output normalization. This work was supported by grants from the Conseil Régional de Bretagne (211-B2-9/2005/ARED /PPCVIRUL) and the NoE EPIZONE (FP6).

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ORAL: Labelled Positive Control (LPC) - improved Safety in real-time PCR assays

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Key words: real-time PCR, positive control

In order to control the function of primers and probes in a real-time RT-PCR assay, positive controls (PC) or standard samples are necessary. However, such positive controls or standards are a serious risk for cross contaminations during the PCR setup, especially if standards with a high viral load are utilized. In the field of conventional, gel-based detection of amplicons artificial PCs with different fragment lengths are often used, but application of such strategy also for real-time PCR assays is not reasonable.

As a consequence, we developed a complete new strategy for clear identification of the PC in real-time PCR assays. The so-called Labeled Positive Control (LPC) was successfully integrated in several real-time RT-PCR assays, e.g. for diagnostics of FMD, Rabies or BVD. Furthermore, the performance of these LPCs as standards for an absolute quantification of viral genome loads was investigated. It could be demonstrated, that there were no differences in the performance of LPCs and in vitro-transcribed standard RNAs. Finally, different strategies for the production of LPC are presented.

In conclusion, the here presented LPC system is a versatile new tool in the field of real-time PCR technology which is e.g. very helpful to avoid contaminations caused by positive controls or PCR standards.

ORAL: HETEROSUBTYPIC PROTECTION AGAINST SWINE INFLUENZA VIRUS. ROLE OF ANTI-NEURAMINIDASE RESPONSE.

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Key words: Influenza, neuraminidase, protection, vaccine

HETEROSUBTYPIC PROTECTION AGAINST SWINE INFLUENZA VIRUS. ROLE OF ANTI-NEURAMINIDASE RESPONSE.

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Three antigenically distinct subtypes of influenza A virus circulate concurrently in the European swine population: H3N2, H1N1 and H1N2. The “human like” H3N2 virus was transmitted from humans to pigs during the 1968 Hong Kong pandemic and the “avian like” H1N1 virus from birds to pigs in 1979. Subsequently, a genetic reassortment between these two subtypes occurred, resulting in an H3N2 virus carrying all the proteins of the avian H1N1 virus except for haemagglutinin (HA) and neuraminidase (NA). The third virus subtype, H1N2, contains genes from three different donor viruses: the HA from a human H1N1, the NA from a swine H3N2 virus and the internal genes from an “avian-like” swine H1N1virus. Therefore, all proteins except for HA and NA are very similar between the three subtypes.

A serosurveillance study in Spain in 2006 demonstrated seropositivity against H1N1, H3N2 and H1N2 viruses in pigs on 70.05 %, 57.48 % and 55.08 % of the farms examined, respectively. Double and triple-seropositive animals are frequently found indicating a lack of cross-protection between viruses of the three circulating subtypes. Heterosubtypic immunity, the immunity induced by infection with a virus of one subtype against a subsequent infection with a virus of another subtype, has been observed in mice and in humans. However, the immune mechanisms responsible for this broad protection are not completely defined and have to be addressed by in vivo challenge studies. We have therefore studied clinical and virological protection against H1N2 challenge in mice with post-infection immunity against swine H1N1 or H3N2 viruses. Infection with a H3N2 virus was shown to protect mice partially to a higher extent than with a H1N1 virus against infection with a virus of H1N2 subtype. Since there is no cross-reactivity between the HA of the three subtypes and the internal proteins are very similar among them, it is deduced that immune response induced by the NA has a major contribution in the heterosubtypic protection.

6.References

ORAL: EPIZONE Databases

Kühne, Sabine [1]; van den Heuvel, Hans[2]; Lens, Bart [3]; Meindl-Böhmer, Alexandra [1]; Blome, Sandra [1]; von Grumbkow, Claudia [1]; Greiser-Wilke, Irene [1]; Zimmermann, Bernd [1]; Moennig, Volker [1]

Institute of Virology, School of Veterinary Medicine[1]; Animal Sciences Group of Wageningen UR[2]; Digital Value (DiVa)[3]

Key words: database, EPIZONE, reference material, experts

Epizone databases are created within the EPIZONE project with the goal to share information between different institutes. The databases contain information from partner organizations on different themes for example on reference material or on experts. The EPIZONE databases are accessible from the central project site (http://www.epizone-eu.net/cps --> Databases).

Some partners provide own websites with already existing external databases, which were linked to the databases website. Other partners provide data, which were collected in central databases.

The advantage of external databases is that laboratories can use it for the management of their own inventory system. They should have an interest to keep the database updated and to maintain it. A major disadvantage is the time-consuming search through several databases.

Therefore, a meta search engine will be developed within the EPIZONE central project site to enable a search with a single query in all external and central databases within the EPIZONE network.

The EPIZONE databases are a good basis to share resources with the positive side effect of the reduction of animal experiments for the production of reference material and the promotion of communication between different institutes.

Databases on reference material

As part of EPIZONE work package 2.2 all participating institutes were asked to contribute information about available reference material for major epizootic diseases especially African Swine Fever, Classical Swine Fever, Foot and Mouth Disease, Bluetongue, and Avian Influenza within their institutes. With the help of this information databases have been created to provide inventories on virus strains, polyclonal sera and monoclonal antibodies.

Expert database

The purpose of this database is to easily find colleagues within EPIZONE with special experiences in certain areas or diseases. It contains information about the country, institute, the core expertise (e.g. bacteriology, biochemistry, clinical aspects), and disease expertise. All participants of the EPIZONE project were asked to fill in their information.

In the future we will help to develop databases for different EPIZONE work packages in cooperation with their leaders and partners. Therefore, we need support and contribution of all participants.

Acknowledgements: We wish to thank all colleagues and institutions who supported the project by providing data on reference material.

ORAL: ASSOCIATION BETWEEN NUMBER OF WILD BIRDS SAMPLED FOR IDENTIFICATION OF H5N1 AVIAN INFLUENZA VIRUS AND PROBABILITY OF OUTBREAK IN THE EUROPEAN UNION

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CISA-INIA[1]; University of California[2]

Key words: Highly-pathogenic-avian-influenza, co-kriging, surveillance, birds, Europe

1.Introduction and Objectives

Subtype H5N1 HPAIV has been isolated in Europe since October 2005,affecting mainly wild birds in 14 EU member states during 2006(1).In the absence of specific regulations on the number of samples to be collected from wild birds,we test that the probability that an H5N1 HPAIV outbreak reported in the EU was located in a given country is similar to the probability that a wild bird sample collected in the EU comes from that country.

2.Material and Methods

Probability co-kriging was used to estimate the probability of occurrence of H5N1 HPAI outbreaks conditional to the distance between outbreaks and to the spatial distribution of the population at risk,based on the method described in Perez et al, 20062.The absolute difference(Dc) between the probability that a wild bird sampled in the EU came from country c(PSc)and the probability that an outbreak in the EU was located in country c(PPc)was computed to identify outliers,applying Grubb's test to estimate if the values of Dc per country that varied from Dc in the EU were statistically significant($P<0.01$). The strength of the association between PSc and PPc was quantified by computing the Spearman's coefficient(Rs).

3.Results

EU countries have generally reached a balance between the probability of disease outbreaks and number of wild bird samples collected for surveillance purposes($Rs =0.715$, $P<0.01$).However, PSc was significantly higher in The Netherlands and Spain than PPc compared to the observed in the remaining EU countries.

4.Discussion and Conclusions

Findings presented here suggest that perception of risk was generally consistent among EU-member countries and that social or political factors may have influenced the collection of a large number of samples in The Netherlands and in Spain.The approach presented here could be useful on the comparison of the relation between probability or risk for disease and intensity of surveillance activities.

5.Acknowledgements

Thanks go the Ministry of Agriculture,Animal Health Division,and to the CISA-UCM joint coordinated project RTA2006-0167-C02-01 for funding.

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ORAL: GENERATION AND CHARACTERIZATION OF CHIMERIC CALICIVIRUS-LIKE PARTICLES DISPLAYING IMMUNOGENIC EPITOPES

Almanza, Horacio[1]; Crisci, Elisa[2]; Cordoba, Lorena[2]; Fraile, Lorenzo[2]; Caston, Jose R[3]; Mena, Ignacio[1]; Montoya, Maria[2]; Barcena, Juan[1]

CISA-INIA[1]; CReSA[2]; CNB-CSIC[3]

Key words: VLPs, Calicivirus, RHDV, epitopes

1.Introduction and Objectives

The capsid protein (VP60) of rabbit hemorrhagic disease virus (RHDV) has been expressed in baculovirus and shown to form virus-like particles (VLPs) (1). We have identified three locations within the VP60 protein where foreign epitopes can be inserted. We aim to explore the feasibility of using RHDV-derived VLPs as antigen-presenting vectors to induce an efficient immune response.

2.Material and methods

Production of RHDV VLPs was performed as previously described (1). BM-DCs generated from cultures of bone marrow cells (2), were incubated with the different VLPs for 4 hours. After extensive washings, BM-DC were incubated with the specific CD8+ hybridoma as previously described (3). Antigenic recognition was determined by IL-2 secretion.

3.Results

We generated a set of recombinant baculoviruses expressing VP60 constructs harbouring different immunogenic epitopes: i) the extracellular domain of M2 protein of influenza virus; ii) a cytotoxic T-cell epitope derived from influenza virus nucleoprotein (NP366–374) iii) a well defined cytotoxic T-cell epitope derived from ovalbumin protein (OVA). All the chimeric VP60 proteins prepared assembled into VLPs. Chimeric VLPs expressing OVA epitope were analyzed to determine whether exogenous antigen was processed and presented by BM-DC in vitro. In fact, OVA antigenic peptide was presented in a dose dependent manner. BM-DC were more efficient presenting the OVA peptide from VLPs displaying the epitope at the N-terminus of VP60 protein.

4.Discussion and Conclusions

Our results indicate RHDV VLPs constitute versatile scaffolds for antigen display. The VP60 protein has been shown to be very efficient in inducing immune response (4). The immunogenic properties of VP60 protein together with the ability of RHDV VLPs to accommodate epitopes in different locations may lead to novel platforms for vaccine development and delivery. Work is in progress to assess the in vivo immunogenic potential of these chimeric VLPs.

5.Acknowledgements

This work was supported by grant AGL-2006-13809 (Spain), and by EPIZONE.

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ORAL: A Quantitative Assessment of the Risk from Imported Live Poultry Infected with Highly Pathogenic Avian Influenza virus to Region of Valencia

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Key words: Highly pathogenic Avian Influenza; risk assessment; trade; poultry

Valencian´s Region is one the most important spanish poultry area [3]. Because of that it is necessary to know the risk of entry of a disease as devastating as HPAI, in order to prevent it. This paper aims to show the modelling of a quantitative assessment of the risk from imported live poultry infected with HPAI virus to Region of Valencia and first results that it has been obtained with this model.

The methodology developed it is summarized in the following points:
• Identification of live poultry supplying countries [1],where HPAI outbreaks had occurred in the last twelve years [4].
• Review the number of: outbreaks, infected animals [4], poultry exported to Region of Valencia [1] and poultry population [2] that they have had in the last time.
• Estimation of the probability that an animal selected for export in country X at any random instant in any random year, is infected and enters in the Region of Valencia. This probability is calculated as follows: R1=Pi•Yf•t·(1-Pd), where: Pi is the probability of an incursion of HPAI virus and subsequent outbreak, in country X, per year; Yf is the proportion of the poultry population affected, per year; t is the duration of infection in years; Pd is the probability of the infected animal will be detected before being exported [6].
• Estimating the Risk of HPAI virus entering to Region of Valencia at any random instant in any random year, from importation of poultry. This risk is calculated as follows: i=n R=∑1-(1-R1i)^Ei.[5]
i=1 Where: R1i is the calculated variable for a country i, and Ei is the number of poultry exported for a country i, per year. The model was developed using the software @RISK 4.5.

The probability of HPAI virus entering to Region of Valencia at any random year, from importation of poultry, has a mean value of 6,91•10-2, a modal value of 2,55•10-2 and associated 95% certainty interval that ranges from 6,72•10-3 and 0,23.

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ORAL: EQUINE INFLUENZA IN AUSTRALIA – THE NEED FOR SPEED:

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Virology Laboratory, EMAI[1]

Key words: High-throughput, real time PCR, robotics

Until 2007 Australia had been free of Equine Influenza (EI). In August 2007, EI was confirmed in horses in a large equestrian centre. A multi-focal outbreak was identified on properties scattered over a 700 km range in the states of New South Wales and Queensland. More than 50,000 horses were affected on 8,500 farms. However, through a combination of rapid testing of diagnostic and surveillance samples combined with strict control measures and strategic vaccination, the outbreak was brought under control within 3 months. Intensive surveillance over the next 3 months has shown that EI has been successfully eradicated.

This presentation will highlight the key features of the testing program and the lessons learned. These include aspects that started with sample collection and identification in the field through to completion of testing and disposal of samples. In the laboratory, rapid high throughput testing was achieved by streamlining sample handling combined with the strategic application of automated test procedures. A real time reverse transcriptase PCR (qPCR) assay was utilised to viral RNA in nasal swabs while serum samples were tested in a blocking ELISA (bELISA).

An existing high throughput PCR capability was further refined during this rapidly evolving outbreak in response to variable but increasing workloads driven by a rapidly changing field situation. The test procedures were streamlined to minimise repetitive tasks and maximise reproducibility from day to day. The system employed was based on a 96 well plate format utilising magnetic bead based total nucleic acid extraction chemistry (MagMAx Viral, Ambion) in conjunction with a magnetic particle processing system (Kingfisher 96, Thermo). Assays were run on either an ABI 7500 Fast or an ABI 7900HT Fast thermocycler. This combination of RNA extraction and qPCR equipment allowed a throughput of more than 2,000 samples per day and a “turn-around” time of about 3 hours for a batch of 300 samples. Urgent samples could be completed in less than 2 hours. At the peak of the testing program 2300 samples were tested by real time PCR in one day and more than 30,000 individual samples in a 4 week period. This high throughput capability has been shown to give highly reproducible results and has been one of the keys to the success of the control and eradication program. This is the first occasion in which a qPCR has been applied to a large scale EI outbreak.

ORAL: Detection of FMDV persistent cattle by salivary IgA test

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IAH[1]

Key words: FMDV carrier animals, IgA test

Introduction and objectives: The new European council Directive 2003/85/EC on FMD has made provision for vaccination and the use of post-vaccination serosurveillance to detect sub-clinical infection. Long-term sub-clinical infection with FMDV has been demonstrated in vaccinated and subsequently challenged ruminants. Since carriers may be considered a risk for transmitting infection, they must be identified by post-vaccination surveillance to substantiate freedom from infection to regain the FMD-free status for the purpose of international trade. Tests for antibodies to FMDV non structural proteins (NSP) have been developed to specifically detect infection in vaccinated herds. The performance of NSP tests is dependent upon the use of inactivated and purified FMD vaccines. Our recent studies indicate that though the induction of NSP antibodies by infection of vaccinated animals correlates with the extent and duration of virus replication, some of the vaccinated carriers may not be detected. Furthermore, confirmatory tests are needed to rule out false positive NSP antibody reactions.
Materials and Methods: A O serotype-specific ELISA had been developed (1) and recently after further refinement it has been extended to detect foot-and-mouth disease virus (serotype O, A, SAT and ASIA) specific IgA antibody in the saliva of persistently infected cattle. Saliva samples were collected and analysed from naïve cattle (n=860), FMDV challenged cattle (n=40), FMDV vaccinated cattle (n=170) and FMDV vaccinated-and-challenged cattle (n=170). Saliva samples were also collected at least from 600 field cattle after FMD outbreak of SAT and A serotype. The FMDV infection status of each of the cattle was determined by virus isolation and RT-PCR tests on oespphago-pharyngeal fluids and the ability of the IgA test to detect viral infection and persistence was compared.
Results: 81% and 86% of PCR positive carrier animals were detected in field and experimental cattle respectively with 98% of specificity. IgA test has also been extended to other domestic animals and to different FMDV serotypes. Final results will be presented in the meeting.
Discussion and conclusion: IgA test is a potentially very promising candidate to identify persistently infected animals in a vaccinated population and could be used as a direct screening test or a supporting test to NSP.
References: 1. Parida et al. 2006. Vaccine, 24, 1107.

ORAL: Wildlife surveillance for lyssaviruses in North-West Europe

van der Poel, Wim H. M. [1]; Müller, Thomas[2]; McElhinney, Lorraine[3]; Freuling, Conrad[2]; Marston, Denise[3]; Kooi, Bart[4]; Fooks, Anthony[3]

Central Veterinary Institute, Wageningen University Research[1]; Friedrich Loeffler Institute[2]; Veterinary Laboratories Agency[3]; Central Veterinary Institute[4]

Key words: wildlife; lyssavirus; RABV; EBLV; CSFV

Many important animal and zoonotic diseases have wildlife reservoirs. Lyssaviruses are probably the most well-known example. In Europe the main wildlife reservoirs for lyssaviruses are foxes (classical rabies virus, RABV) and bats (European Bat Lyssaviruses, EBLVs). Moreover there is an increasing number of Raccoon dogs, which are also a RABV reservoir. In terrestrial rabies wildlife frequently causes spillover infections to farm animals, pets and humans resulting in economic constrains and increased public health risk. Therefore, European countries with endemic rabies have implemented wildlife surveillance programs mainly for RABV. WHO's recommendations on rabies surveillance in foxes have been adopted by EU. Within the EU network of excellence MEDVETNET protocols for reactive and pro-active surveillance for bat rabies have been developed and were adopted by EUROBATS. Since 2005, data on rabies surveillance in foxes, other animals and bats (numbers of animals tested) have been submitted to the WHO Collaborating Centre for Rabies Surveillance and Research at the FLI in an attempt to compare rabies surveillance at a European level. Preliminary data suggest that the majority of European countries do not meet WHO and EU recommendations.

The current surveillance programmes of EBLVs in bats have revealed a prevalence of around 20% in Serotine bats in Northwest Europe and lower but significant prevalences in other bat species like Daubentons and Myotis. Isolated EBLV sequences are compared and stored in a common database. The database now has 109 entries and includes archived EBLV type 1 and 2 cases and sequence data submitted by reference laboratories in the UK (VLA n=14), Netherlands (CVI n=42), Germany (FLI n=27), France (AFSSA n=12), Spain (ISCIII n=6) and Poland (PZH n=5).

Within EPIZONE, a research proposal entitled WILDSURV was launched to (i) harmonise current approaches in rabies surveillance throughout Europe, taking into account the different prevalences between regions and the oral vaccination campaigns, and (ii) evaluate recommendations of both WHO and EU for rabies surveillance concerning practicability, taking into account the experiences of countries with rabies control programmes. As this may also help to optimize surveillance approaches for other wildlife related diseases, classical swine fever is included.

ORAL: DEVELOPMENT OF A NOVEL SEROLOGICAL ASSAY FOR THE DETECTION OF RABIES VIRUS NEUTRALISING ANTIBODIES USING LENTIVIRAL PSEUDOTYPES

Wright, E. [1]; Temperton, N.J. [1]; Marston, Denise A.[2]; McElhinney, Lorraine M[2]; Johnson, Nicholas[2]; Müller, Thomas[3]; Weiss, R.A. [1]; Fooks, A.R. [2]

UCL[1]; VLA[2]; Friedrich-Loeffler-Institute[3]

Key words: Rabies, lyssavirus, lentivirus, pseudotype, neutralisation, serology, assay.

Introduction and objectives

Pseudotypes are viruses that carry the genome and core of one virus and the envelope of another. Studies at UCL have previously shown that retroviral pseudotypes expressing the SARS-coronavirus spike S-protein and H5N1 influenza virus haemagglutinin can be used in sensitive and specific assays for the detection of VNA to these pathogens. We are now developing viral vectors that can be used in neutralisation assays to determine antibody titres for other biohazard level 3 or 4 pathogens. This has two main benefits over existing methods: Assays using retroviral pseudotypes can be undertaken in biohazard level 2 laboratories and the use of either green fluorescent protein, luciferase or β-galactosidase as a reporter mechanism allows the assay to be used at a minimal cost in laboratories throughout the world.

Materials and Methods

The RABV isolate used in this study was CVS-11. The CVS-11 G-gene sequence was amplified using the RT-PCR with primers designed based on the published sequence. EBLV-1 (isolate RV9) and EBLV-2 (isolate RV1787) G-gene sequences, were sub-cloned from existing transfer plasmids using specific PCR primer sets. Once amplified, they were ligated into pI.18, a pUC-based plasmid incorporating the hCMV IE promoter, and sequenced to ensure correct alignment. The Vesicular Stomatitis Virus G-protein (Indiana serotype) expression vector pMD.G was used to create a control pseudotype virus.

Results

We report the analysis of neutralising antibodies to CVS-11, EBLV-1 and EBLV-2 in sera from RABV-vaccinated humans, canines and felines using lentiviral pseudotypes. As the only lyssavirus protein present in the pseudotypes is the G-protein we can accurately determine the precise role that antibodies targeting this protein play in cross-neutralisation.

Discussion and Conclusions

This powerful, novel assay for the detection of RABV VNA using CVS-11 pseudotypes can be adapted to meet the needs of many laboratories worldwide without requiring high containment. Using pseudotypes with the luciferase reporter we established the assay's sensitivity and specificity with serum samples previously characterised using the World Health Organisation (WHO) and Office International des Epizooties (OIE) 'gold standard' fluorescent antibody virus neutralisation test.

Acknowledgements

This study was partially funded by the UK Medical Research Council and the UK Department for Environment, Food and Rural Affairs (grant SEV3800).

ORAL: MOLECULAR DIVERSITY OF RABIES VIRUS STRAINS IN SOUTHERN EUROPE

McElhinney, Lorraine[1]; Marston, Denise[1]; Freuling, Conrad[2]; Harkess, G.[1]; Stankov, Srdan [3]; Johnson, Nick[1]; Velic, Ramiz [4]; Santrac, Violeta [5]; Müller, Thomas [2]; Fooks, Tony[1]

Veterinary Laboratories Agency[1]; Friedrich-Loeffler-Institute[2]; Pasteur Institute Novi Sad[3]; University of Sarajevo[4]; Veterinary Institute of the Republic of Srpska[5]

Key words: rabies, epidemiology, Serbia, Bosnia

1.Introduction & Objectives

The molecular diversity of classical rabies viruses (RABV) has been studied at the global level and reference has been made to the existence of a number of European strains in a range of mammalian species (Ref 1, 2). To further investigate RABV in South Eastern Europe, a molecular epidemiological study was performed on a unique panel of viruses from the Former Republic of Yugoslavia (FRY).

2.Material & Methods

Original brain specimens from RABV cases (n=210), isolated in FRY between 1972 and 2006 from a variety of host species were analysed. Viral RNA was extracted and tested by RT-PCR. The amplified PCR products were subjected to purification and genetic sequencing (N gene, 400bp). In addition, sequence data for other RABV (n=78) either in Genbank or held at the VLA was included in the subsequent phylogenetic analysis.

3.Results

All isolates from this region grouped within the European / Middle East Lineage, the majority of which shared sequence homology to the previously published East European (EE) strains. A number RABV isolates from Bosnia and Herzegovina (BiH) and Montenegro, collected between 1986 and 2006, grouped with the West European (WE) strains, believed to be responsible for the rabies epizootic that spread throughout Europe in the latter half of the 20th Century. In contrast, no Serbian RABV isolates from our panel belonged to this sub-lineage. However, a distinct group of Serbian fox RABV provides further evidence for the southwards wildlife-mediated movement of rabies from Hungary, Romania and Serbia into Bulgaria.

4.Discussion & Conclusions

This study represents the first comprehensive molecular epidemiological review of RABV in the central Balkans region. No Serbian isolates were represented in the previously identified WE, CE or NEE sub- lineages. The presence of BiH and Montenegrin isolates within the WE sub-lineage group may suggest that the fox epizootic that spread to Western Europe, simultaneously moved southwards from Croatia through BiH into Montenegro without contributing to the overall fox rabies epidemiology in Serbia.

5.Acknowledgements

This work was supported by a grant from Defra UK (ROAME SE0420)

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EPIZONE

Poster Presentations

POSTER: Evaluation of a commercial rLAMP-assay for the detection of avian influenza virus RNA

Postel, Alexander[1]; Frischmann, Sieghard[2]; Harder, Timm[1]

Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health[1]; Mast Diagnostica Laboratoriums-Präparate GmbH[2]

Key words: rLAMP, rRT-PCR, AIV

1.Introduction and Objectives

The urgent need for new rapid tests for on-site use in avian influenza virus (AIV) diagnostics prompted us to evaluate commercial isothermic amplification assays (rLAMP) designed to detect RNA of H5- and H7-AIV subtypes.

2.Material and Methods

rLAMP was performed in the Real-time Turbidimeter LA-200© using primer kits „Avian Flu H5 / H7“ (Eiken Chemical, Japan) and compared to validated rRT-PCRs assays.

3.Results

Serial dilutions of AIV strains (subtypes H1, H5, H6, H7 and H10) were used in rLAMP (amplification time 35 min). 21/24 H5 isolates were detected by H5-rLAMP (24/24 rRT-PCR). Pro¬longed amplification (60 min) resulted in detection of the three missing strains but also in false positive results for two none-H5 isolates (H2, H6). 17/25 AIV H7 strains were detected by H7-rLAMP (25/25 rRT-PCR). Prolonged amplification resulted in detection of six further H7 strains and no false-positives for non-H7 strains (H10).

rLAMP detection limits were found at median rRT-PCR Ct-values of 28.6 (H5) and 26.7 (H7). In direct comparisons using serial log10 dilutions rRT-PCR was up to 3 steps more sensitive.

Diagnostic suitability was tested using avain swab samples. H5-rLAMP detected four out of 22 samples positive by rRT-PCR for H5 and one H3-positive but H5-negative sample. Four samples positive by rRT-PCR for H7 were confirmed by H7-rLAMP. Swab samples negative for H5 and H7 by rRT-PCR were also negative in rLAMP.

4.Discussion and Conclusions

The rLAMP primer kits “Avian Flu H5/H7” are currently not fully suitable for diagnostic approaches. In addition to efforts of increasing sensitivity and specificity the development of a subtype-independent rLAMP targeting more conserved genes would be useful. rLAMP is a promising technology for molecular AIV diagnostics.

5.Acknowledgements

MAST DIAGNOSTICA (Reinfeld, Germany)

POSTER: Infrared Thermography – a reliable tool to select suspicious animals during animal disease outbreaks?

Gerß - Dülmer, Hanna[1]; Kramer, Matthias[2]; Beer, Martin[3]; Blome, Sandra[1]; Moennig, Volker[1]

Institute for Virology, University of Veterinary Medicine Hannover[1]; Friedrich-Loeffler-Institute, Institute of Epidemiology[2]; Friedrich-Loeffler-Institute, Institute of Diagnostic Virology[3]

Key words: Infrared Thermography; pigs; fever; CSF; risk based sampling scheme

Introduction:

An increased body temperature poses an early and reliable indicator of various infectious diseases. Therefore, this clinical sign is used for a risk based sampling scheme in cases of animal disease outbreaks. In practice, the procedure of manual rectal temperature measurement is often rather difficult. Problems are mainly caused by temperature increasing stress reactions. Less stressful methods could help to overcome these problems. In the present study, preliminary evaluation of IR thermography was performed in clinically healthy and CSF virus (CSFV) infected pigs under experimental conditions.

Material and Methods:

For this project, the infrared camera ThermaCAM™ P620 (FLIR Systems GmbH, Frankfurt/Main, Germany) and an accompanying ThermaCAM software were used.

To meet hygiene requirements, the camera was protected by a thin (25µm) low density polyethylene plastic bag. In a first step, emission and transmission factors as well as reflected radiation were determined. Afterwards, an animal experiment was designed to investigate the correlation between rectal temperature and skin temperature measured through infrared images. The experimental setting included infection of different age classes of pigs with various CSFV isolates. Images were thus produced from non-febrile and febrile pigs. Additionally, group images were produced in order to see if feverish animals could be found in a group of pigs.

Results:

Preliminary results showed that technical factors like transmission, emission, reflected infrared radiation and taking angle as well as environmental and individual factors greatly influenced the obtained values. Nevertheless, a correlation was found between rectal temperature and different skin areas.

Conclusion:

Against the background of practical experiences during the last CSF outbreaks, amendment of the risk based sampling schemes is desirable. IR thermography could be a promising tool for a non-invasive identification of febrile and suspicious animals.

Further investigations under experimental conditions and in the field are urgently needed.

Acknowledgements:

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Furthermore we want to thank the staff members of the Friedrich Loeffler Institutes in Wusterhausen and Greifswald – Insel Riems for their support. We also thank PD Dr. med. vet. große Beilage for supporting our first field applications.

POSTER: SEROEPIDEMIOLOGY Of THE ARBOVIRUS OF CRIMEAN- CONGO HEMORRHAGIC FEVER IN RURAL COMMUNITY OF BASRA

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Key words: Seroepidemiology, CCHF

1.Introduction and objectives
In 1979 cases of Crimean-Congo Hemorrhagic Fever (CCHF)were recognized in Iraq ,following years several cases of CCHF were diagnosed in Basra, southern Iraq. Aseroepidemiological survey was carried out in rural community of Basra to estimate the size of the enzootic focus in which the CCHF virus is circulating . A total of 682 serum samples were collected from apparently healthy individuals, their ages range from 5 to76 years , 20% of the collected sera were obtained from occupational risk groups (veterinarians , abattoirs workers , farmers).Sera collected from 74 sheep and 48 cattle , 42 tick pools were also gathered parallel to the human and animals sera in the same areas.
2.Material and methods
Enzyme-linked immunosorbent assay (Elisa), was used to detect the prevalence of circulating IgG antibodies in the collected sera.
3.Results
In general IgG antibodies against CCHFV were found in 4.3% of the resident in rural areas of Basra, Seropositive sera were detected in 9.7% of northern residents , while 20% of the sheep sera and 37% of cattle sera were seropositive which indicate that the virus is circulating in the area within the endemic level . The tick pools were identified , the predominant tick species was diagnosed as Hyaloma marginatum.
4.Discussion and Conclusions The existence of enzootic focus for the CCHFV is maintained by ecological , socioeconomic variables , the possibility of emerging infections should always be considered.
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POSTER: AN INCREASED PREVALENCE OF H3N2 SWINE INFLUENZA IN POLAND

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Key words: SWINE INFLUENZA, H3N2

1.Introduction and Objectives
Virological and molecular studies on occurrence of swine influenza (SI) among Polish pigs, undertaken between 2000-2006, have demonstrated that H1N1 subtype is dominant, other 2 subtypes have been only reported in serologic examination (2). In the recent study, the significant increase of antibodies against H3N2 subtype was evidenced. Additionally in 1 farm subclinical, influenza-like respiratory disorders were observed.
2.Material and Methods
A total of 2408 pig sera from 93 herds were collected. None of the herd were vaccinated against SI. Sera were examined in HI test. Additionally 313 biological samples, taken from animals demonstrating respiratory disorders, raised in 30 farms, were used for isolation of SIV, viral RNA and for molecular subtyping.
3.Results
In serological examination 14.4% of serum samples were classified as positive for H1N1, 2.4% for H1N2 and 7.8 for H3N2. SIV was isolated in 20 cases. RNA of SIV was detected in 22 out of 30 tested farms. In molecular subtyping all isolates till 2006, originated from 21 out of 30 tested farms, were determined as H1N1 subtype. The strains isolated during the last outbreak of SI (2007) from pigs demonstrating subclinical signs were determined as H3N2.
4.Discussion and Conclusions
The comparison of the epidemiological situation concerning SI, evidenced in the years 2004 and 2007, shows that prevalence of antibodies against H3N2 increased about 4 times (from 2.2 to 7.8%), while the percentage of positive samples against the dominant subtype H1N1 increased less then 2 times (from 8.2% to 14.4%)(2). The results of this study indicate that the subtype H1N1 is still dominant. An increase of seroprevalence against H3N2 subtype noticed in the examination conducted in 2007 was correlated in time with the first information from the field concerning the “atypical” influenza outbreak with mild, subclinical course of the disease. The “new” subtype - H3N2 in Polish farm was for the first time detected. This study suggests that H1N1 is more related to acute course of influenza and H3N2 more to subclinical one. In the Netherlands (1) swine influenza associated with mild symptoms occur in almost all finishing farms while our case is the first one caused by H3N2 subtype evidenced in East Europe.
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POSTER: Real-time PCR based on LUX (Light Upon Extension) primer – LUX-PCR for rapid, sensitive and convenient detection of PCV2

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Key words: Porcine Circovirus type 2, LUX, real-time PCR

1.Introduction and Objectives

PCV2 (Porcine Circovirus type 2) is a causative agent of PMWS and generally PCVDs in pigs (Allan & Ellis, 2000). Rapid laboratory detection of virus, together with clinical symptoms observation and pathological findings, act a key role in diagnostics of PCVDs. To simplify and accelerate laboratory detection of PCV2, LUX-PCR as a novel molecular-genetic assay based on the real-time PCR format was developed and tested on tissue samples from pigs in Slovakia.

2.Material and Methods

Lymph nodes of 39 piglets suffering from PMWS-like clinical symptoms were analysed. Samples were tested by single PCR employing primers CF8/CR8 (Larochelle et al., 1999) and by real-time LUX-PCR developed in our laboratory. To determine genetic diversity of PCV2 isolates circulating in Slovakia, the sequencing of PCR products originating from ORF2 was carried out and DNASTAR software package was used for phylogenetic analysis.

3.Results

Of 39 samples tested, the specific amplicon was observed in 29 samples with classical single PCR. When compared LUX-PCR with classical single PCR, of 11 samples (7 positive and 4 negative by single PCR) 9 samples were positive by LUX-PCR. The specificity of LUX-PCR products was confirmed by a melting curve analysis. The dynamic range of quantitative analysis carried out with recombinant plasmid by LUX-PCR covered a 8-order interval ranging from 20 to 108 genome equivalents per assay. Phylogenetic analysis of PCV2 isolates confirmed the existence of two separate virus clusters in pig samples from Slovakia.

4.Conclusions

Our study demonstrates that real-time LUX-PCR assay prove to be more sensitive, effective and rapid method when compared with a classical single PCR. Without need of post-amplification process, LUX-PCR is a method of choice for the detection of PCV2 in clinical samples.

5.Acknowledgements

This study was supported by FP project No. NMSACC-PCVD 518432 and APVV No. 20-019-605.

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POSTER: DIVA properties of the chimeric pestivirus CP7_E2gif

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Key words: DIVA diagnostics, pestivirus, chimeric virus

1.Introduction and Objectives

An advantage of the use of chimeric pestiviruses as modified live vaccines against classical swine fever (CSF) resides in their capacity to be manipulated to achieve the characteristics desired for safe and efficacious DIVA vaccines. We have recently described the virulence, immunogenicity and vaccine properties of a chimeric pestivirus CP7_E2gif (Rasmussen et al., 2007). This is an analogous candidate to CP7_E2alf (Reimann et al., 2004), and CP7_E2gif is unique in that no CSFV sequences are present in the chimeric pestivirus genome. We examined the sensitivity and specificity of commercial ELISA assays to identify an ELISA that fulfils the requirements for a DIVA strategy together with CP7_E2gif.

2.Material and Methods

Two commercial ELISAs - one targeting E2 antibodies (Ceditest CSFV) and one targeting Erns antibodies (Chekit CSF marker) - were tested for their ability to detect antibodies against the chimeric virus as well as against CSFV.

3.Results

Ten days after vaccination neutralising antibodies against the homologous virus were observed followed by a 10-fold increase after challenge infection with CSFV (Rasmussen et al., 2007). Testing for E2-specific antibodies using a CSFV specific E2-ELISA revealed no antibodies in vaccinated pigs indicating no cross-reaction to the antibodies induced by the chimeric virus. E2-specific antibody titres could be detected 7 days post challenge infection. In the control group, antibodies were not detected until 14 days post challenge. Serum samples were similarly tested for the presence of Erns antibodies. The vaccinated pigs showed detectable Erns antibody levels before challenge indicating detection of antibodies induced by the chimeric virus.

4.Discussion and Conclusions

DIVA vaccine candidates must be associated with a companion diagnostic test that can differentiate vaccinated from infected animals. This study shows that CSFV-specific ELISAs targeting E2 can be used as companion tests together with CP7_E2gif.

5.Acknowledgements

This study was supported by NoE EPIZONE

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POSTER: Development of new strategies to improve DNA vaccines encoding FMDV minigenes

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Key words: DNA vaccination, immunomodulation, minigenes, FMDV

Introduction

The feasibility of DNA manipulation opens a broad range of possibilities for improvement and/or modulation of the immune responses induced by DNA vaccines. One of the most interesting approaches is to target antigens to the extra-cellular milieu and/or driving them to antigen presenting cells (APCs). We are testing several targeting signals by fusing them to different combinations of B and T-cell epitopes from Foot-and-Mouth Disease Virus (FMDV).

Materials & methods

Groups of 4 pigs received 3 shots of 400 ug of the corresponding plasmid, and 15 days later they were challenged with infectious FMDV. Animals were monitored for development of clinical signs of disease for 14 days. Samples were serially taken at different time points along the experiment in order to analyze: i) the immune response induced by DNA inoculation, both humoral (neutralizing antibodies) and cellular (by ELIspot and lymphoproliferation) responses; and ii) the protective capacity of the experimental vaccines by analysing the viral load after challenge, the kinetic of the antibody response induced, and the lymphopenia caused by the virus, measured by flow cytometry upon PBL staining with diverse surface markers.

Results and discussion

Fusing minigenes to strong signal peptides improved the humoral response induced in mice, while targeting them to APCs enhanced the cellular responses induced. Previous results from the lab also demonstrated: i) that antigenicity and immunogenicity do not always correspond and ii) that cellular responses and FMDV protection conferred by DNA vaccines is very different between mice and pigs. Thus, we are currently doing the experiment in pigs, natural host for FMDV virus. The results obtained will be shown and discussed.

Acknowledgements

We thank M.G. Esguevillas and N. de la Losa for technical assistance, and Ramón y Cajal, AGL2004-07857-C03-01, TRT2006-00035-C02-00 and CONSOLIDER-INGENIO 2010 projects from the Spanish Government as funding sources.

POSTER: Heterologous prime-boost vaccination with DNA and recombinant vaccinia virus Ankara strain expressing outer capsid VP2 and VP5 proteins of bluetongue virus: evaluation of immune responses in mice.

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CISA-INIA[1]

Key words: bluetongue, vaccine, DNA, MVA

1.Introduction and Objectives

Bluetongue is a non-contagious, insect-transmitted disease of certain breeds of sheep and some species of wild ruminants that is caused by bluetongue virus. The virus is transmitted by the bite of Culicoides midges. Bluetongue vaccines play an important role in the control of the disease; however, there are concerns over the use of current polyvalent attenuated vaccines. Recent recombinant DNA technology has provided novel approaches to develop safer vaccines. In addition, the protective efficacy of poxvirus-vectored and DNA vaccines is well known for other diseases and ¨prime-boost¨ has been demonstrated as a good vaccination strategy for the development of protective immunity. The proposed objective is developping reagents and strategies of vaccination against bluetongue virus (BTV) using the MVA vaccinia virus strain that is considered safe and cDNAs.

2.Material and Methods

ORFs from segments 2 and 6 of BTV-4/Menorca, which codified for the outer capsid proteins VP2 and VP5 respectively, were amplified by RT-PCR and cloned into the expression vector pcDNA 3, under the control of the human cytomegalovirus promoter (CMV), and into the vaccinia transfer plasmid pSC11, that allows expression screening using the LacZ marker gene. Recombinant vaccinia virus-Ankara strain (rMVA) were generated by homologous recombination at the thymidine kinase locus and plaque purified.
The analysis of the immune response after vaccination with DNA prime and rMVA boost expressing VP2 or VP5 BTV-4 proteins was evaluated in C57BL/6 mice.

3.Results

High expression levels of VP2 and VP5 proteins were observed by immunoprecipitation and detection of mRNA by RT-PCR. C57BL/6 mice immunized with pcDNA3-VP2/VP5 and rMVA-VP2/VP5 produced high titers of neutralizing antibodies against BTV-4 (log VNT=2), and the presence of these antibodies was maintained at least up to 100 days after immunization. The induction of T cell response after vaccination is still being analysed.

4.Discussion and Conclusions

These data indicate that vaccination with rMVA and DNA may be useful for protective immunization against BTV, and it may avoid the problems inherent to live-attenuated vaccines.

POSTER: Development of a LAMP assay for the detection of African Swine Fever Virus

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Institute for Animal Health[1]

Key words: LAMP, ASF, Isothermal, detection, diagnosis

Introduction

Previous studies have shown loop mediated isothermal amplification (LAMP) to be a simple and effective method of detecting viral genome often with equal analytical sensitivity to real-time PCR1. The simple nature of the test may allow it to be deployed in the field utilising cheap reagents and equipment, making this technology accessible to users in developing countries where African swine fever (ASF) is prevalent. This poster describes the development of a LAMP assay for the detection of ASF virus (ASFV).

Materials and Methods

Publicly available sequence data were used to generate consensus sequences for target regions of the ASFV genome used for primer design (PrimerExplorer2). Primer sets were down-selected on the basis of least mismatch with published ASFV sequences. Seven sets of primers were optimised by altering MgSO4 concentration, betaine concentration, temperature and primer ratio. Once a true amplification had been identified by restriction digest of the product, loop primers were designed and the reaction was transferred to real-time format to allow direct comparison with real-time PCR. The sensitivity of this assay has been evaluated using a wide range of ASFV strains (from the OIE Reference Laboratory).

Results

Of the seven primer sets originally designed, three amplify ASFV DNA. One set was chosen on the basis of its reliability and the flexibility of its reaction conditions. The specificity of the product generated was confirmed by digestion with AvaI. With the inclusion of loop primers, this assay was shown to be faster than real-time PCR, and the resulting assay was able to amplify a wide selection of viruses from all ASFV genotypes.

Discussion and Conclusions

These initial results indicate that the LAMP assay has the potential to be used as a diagnostic tool in the regions of the world affected by ASF. It is has advantages over real-time PCR, since it is more rapid and can be performed using inexpensive equipment. This assay will be tested further on a variety of ASFV genotypes taken from the archives held at the OIE Reference Laboratory (Pirbright) and directly compared with diagnostic real-time PCR.

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POSTER: Interleukin 8 and CK-6 chemokines specifically attract rainbow trout (Oncorhynchus mykiss) RTS11 monocyte-macrophage cells and have variable effects on their immune functions

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Key words: rainbow trout; macrophages; monocytes; chemokines

1.Introduction

In the current work, we have used the rainbow trout monocyte-macrophage established cell line RTS11 to assay the chemoattractant capacity of two chemokines, representative of the CXC (interleukin 8) and CC (CK-6) families. The chemoattractant capacity of IL-8 for neutrophils has been previously demonstrated, however there are only a few studies that point out to a monocyte-attracting role. We have also studied the effect of these two chemokines on the phagocytic capacity, the respiratory burst and the expression of other immune genes.

2.Material and methods

The coding sequences of both rainbow trout IL-8 and CK-6 were cloned into the pRSET A plasmid which produced fusion proteins in which an N-terminal 6x His-tag allowed the purification of the recombinant chemokines. The chemotactic capacity of RTS11 cells towards rainbow trout CK-6 and IL-8 was assayed in chemotaxis chambers (5 mm pore-sized) and flow cytometry. The effect of both chemokines on the phagocytic capacity, the respiratory burst activity, and the levels of expression of different immune genes were measured using standard procedures.

3.Results

Interestingly, two subpopulations of non-adherent cells are distinguishable by flow cytometry that could be identified as immature monocyte- and mature macrophage-like populations, respectively. Whereas IL-8 specifically attracts the monocyte-like subpopulation, CK6 specifically attracts the macrophage-like cell subpopulation.

We found that IL-8 inhibited the phagocytosis capacity of RTS11 cells belonging to the macrophage-like profile. No effect was observed, however, on the respiratory burst. Concerning the effect that IL-8 and CK6 have on the expression of other immune genes, we found that IL-8 and CK-6 had different effects on the levels of expression of different immune molecules.

4.Discussion and Conclusions

In the current work, we describe the effects of two chemokines belonging to either the CXC or the CC family on rainbow trout monocytes-macrophages. These results constitute one of the very few studies in which the effect of IL-8, a CXC chemokine, on monocytes is described. Moreover, it demonstrates that different monocyte-macrophage subpopulations have different reactivity to different chemokines.

5.Acknowledgements

This work was supported by grant AGL2004-07404-C02-02 from the Ministerio de Educación y Ciencia and by the EPIZONE European Network of Excellence.

POSTER: Need for speed: The BioPortal, a web-based near real-time surveillance and analysis tool

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Key words: Spatial-temporal visualizer, HPAI H5N1, Danish wild birds, 2006 epidemic

In 2003, a US Federal interagency working group hosted by the NSF formulated the specifications for a global infectious disease information-sharing infrastructure. The group then commissioned development of a prototype system to demonstrate key elements of the infrastructure. West Nile virus infection and botulism were chosen as the diseases, New York State and California as partners and University of California at Davis and the University of Arizona as information technology facilitators. The system, as developed, includes a high-grade spatial-temporal visualizer (STV) and facilitated acquisition of remote data.

Subsequently, an operational version of the BioPortal system was established at UC Davis for the global surveillance of foot-and-mouth disease. Partnerships were established to acquire access to archival and current disease activity worldwide. Information on the genetic variation was acquired from public and proprietary sources and merged with the epidemiologic and GIS data for visualization using an advanced spatial-temporal-genomic visualizer (STGV). Likewise, data generated by anomaly detection and prospective modeling systems can be integrated and visualized with the epidemiologic and GIS data. The system is available at http://fmd.ucdavis.edu/bioportal.

A generic version of the system with simplified (Excel) data entry is available on request for use by interested parties for the display of their data, including use in biological or other emergency situations.

The system was recently applied to avian influenza data from the EU. Time-space clusters (hotspots) of HPAI H5N1 cases were identified on the wild bird surveillance data collected in Denmark in 2006 using a scan-based algorithm and visualized using the STGV, including the published 8 sequenced wild bird isolates.

Further analysis is required to establish whether “fly out” or “die-out” was the reason for the waning of the epidemic, which took place in May 2006 and which kept the epidemic well behind a frontier crossing through Denmark and through central Europe, leaving the north-westerly parts virtually unaffected. With the present monthly electronic reporting of wild bird surveillance data to the EU the BioPortal could provide up-to-date visualization of the epidemiological situation on-line through the internet.

Demonstrations of the BioPortal and its features using FMD or AI data may be arranged on the spot provided access to the internet is available.

POSTER: Development of Molecular Diagnostic Methods for Detection of Foot-and-Mouth Disease Virus

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Key words: Foot-and-mouth disease ; Multi-RT-PCR ; Real-time RT-PCR ;

In this study, two different RT-PCR were established with different purpose and suited to different samples of FMDV. Among these, Multiple RT-PCR had been developed successfully and made to form a kit, which had been certificated for production by Ministry of Agriculture of P. R. China. And now, the kit was recommended for use in whole country.

(1) For establishment of Multi-RT-PCR, Three sets of primers to detect foot-and-mouth disease virus (FMDV) using multiplex RT-PCR were designed based on several reference nucleotide sequences, and their reaction conditions were determined. By testing ten-fold serial dilutions of FMDV, the sensitivity of multiplex RT-PCR is 100 times higher than conventional RT-PCR. Meanwhile, its specificity was confirmed compared with other related vesicular disease viruses. Furthermore, 30 field samples from different animals were tested, and the results supported the method's potential applications in routine veterinary quarantine and epidemic surveillance of FMDV.

(2) A real-time TaqMan RT-PCR (rRT-PCR) was developed for detection of FMDV RNA。A pair of primer and one TaqMan probe were designed using Primer Expression 2.0 version software after multi-sequence alignment of FMDV. The primers and probe were corresponding to a highly conserved region in 3D gene of O/Akesu/58/2002 (GenBank No. AF511039). Ninety-one base pairs of FMDV gene sequence were amplified with designed primers. We further optimized parameters of rRT-PCR with one-step RT-PCR, which included target sequences, primers, probe and reactive conditions. Specificity, sensitivity and repeatability of one-step RT-PCR was evaluated using control samples and serial dilution of virus from cell culture. A standard curve of rRT-PCR were established with titrated virus RNA from cell culture, and all concentration in the standard curve had a linear correlation. Over 200 samples from tongues, vesicular, marrows and O/P fluids were detected with one-step rRT-PCR. Results showed that the lowest limit of detection was 0.1TCID50 FMDV, more sensitive than RT-PCR, and the results were specific and repeatable.

POSTER: SWINE VESICULAR DISEASE IN LOMBARDY REGION: DIFFUSION IN DENSELY POPULATED PIG AREA

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Key words: SVD, diffusion, pig

Swine vesicular disease (SVD) is a contagious disease of pigs caused by an Enterovirus of the Picornaviridae family. In Europe, SVD outbreaks have been steadily reported in Italy in the last decade, where surveillance and eradication activities are in place.

In the period 2006 - 2007 a recrudescence of SVD was recorded and the disease spread widely in the Italian Northern Regions. Lombardy, a region with high pig stoking density, was most affected.

Even though SVD is considered to be moderately contagious, the 2006 – 2007 epidemic in Lombardy, compare to the previous ones (1989-1989, 2000, 2002), was characterized by a rapid disease spread. In fact, 53 outbreaks were detected and some 150.000 pigs were stamped out.

The SVD outbreaks reported in Lombardy in the period 2006 – 2007 may be grouped in two epidemic periods. During the first one (November 2006 – February 2007) SVD spread among the farms according to the typical routes of transmission of the disease. On the contrary, in the second epidemic period (May 2007 – October 2007), the diseases showed an endemic trend in a small high-density area of the region. The main risk factor for outbreaks in this area, was proximity to a previous outbreak.

To verify the pattern of SVD spread in high-density-areas and to highlight the relevant risk factors, the epidemiological investigations and bio-security questionnaires carried out in the outbreaks were thoroughly evaluated.

Acknowledgments to: "EPIZONE project (European Union, EPIZONE NoE FOOD-CT-2006-016236)".

POSTER: Distribution of Culicoides species in Poland.

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Key words: Culicoides, bluetongue, midges, light-traps, vectors.

Introduction and Objectives

BT is an infectious, non contagious disease transmitted by biting midges belonging to Culicoides. The occurrence of bluetongue in new geographic area where is no evidence of C. imicola, suggest that other Culicoides spp. play role as a virus vector. The aim of the study is to estimate a total number, taxonomy, activity period and geographical distribution of Culicoides species in Poland.

Material and Methods

Light traps (Ondestepoort type) were located in 22 farms all over Poland territory, nearby susceptible animal housings. Collection was made from the end of September to the end of November. Light traps were operated from one hour before sunset to one hour after sunrise, once a week. Trapped insects were collected in labeled jars with 70% ethanol, and were send to laboratory. Culicoides species were separated from other insects, and then classified by microscopic examination.

Results

A total of 96 collections were made. Range between trap locations in number of catches varying from 2 to 8. A total number of 76 161 insects was collected, 30 058 of them belonged to Culicoides complex, and included 6 species. Four of them play a role in BTV circulation; C. obsoletus, C. dewulfi, C. pulicaris and C. punctatus. 70% among all trapped Culicoides species comprised C. obsoletus, found in 83% of all collected samples. 29.6% among all trapped Culicoides species comprised C. punctatus, found in 79% of all collected samples. Only one insect belonging to C. dewulfi was found.

Discussion and Conclusions

The number of Culicoides trapped in Poland was related to the geographical location of the trap. High density was shown in Western part of Poland. Six Culicoides species were found during catching season, most of them belonged to C. obsoletus and C. punctatus. Presence of non blood fed female has demonstated that life cycle of Culicoides is continued even during late autumn, therefore high temperature season can cause an overwintering of bluetongue virus. Entomological research should be continued to obtain more precise data.

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POSTER: A rapid direct whole genome sequencing approach for H5N1Z avian influenza viruses.

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Veterinary and Agrochemical Research Institute[1]

Key words: avian influenza, direct sequencing, genome

1.Introduction and Objectives

Influenza whole genome sequencing using universal primers targeting the noncoding regions for segment RT-PCR amplification followed by primer walking can be cumbersome and requires multiple repeated tests to achieve a proper coverage of each nucleotide. This study aimed to produce a set of H5N1 genotype Z conserved oligonucleotides that amplify overlapping fragments of the influenza genome, reaching a good nucleotide coverage and a near 95% genome coverage in a single experimental procedure

2.Material and Methods

An alignment of 556 representative H5N1 genotype Z sequences was made for each genomic segment, conserved regions were identified and primers designed targeted to these regions generating a total of 78 PCR amplicons ranging in length between 350 and 650 bp. The sequence of these amplicons was determined by direct sequencing.

3.Results

This strategy was tested on 3 whole H5N1 genomes and resulted in a genome coverage of 90-95 % and a nucleotide coverage of almost 3x in a single experimental procedure taking about 3 working days excluding capillary electrophoresis (which depends on the equipment used).

4.Discussion and Conclusions

The presented sequecing approach allows a rapid and standardized sequencing of H5N1 Z whole genomes within the time frame of several working days. Even when only low throughput sequencing facilities are present (e.g. single capilary sequencer), a whole H5N1 genome could be sequenced within a single week.

5.Acknowledgements

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Epizone

6.References

POSTER: MOLECULAR DIAGNOSIS AND EPIDEMIOLOGY OF AFRICAN SWINE FEVER (ASF) OUTBREAKS IN UGANDA

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Centro de Investigación en Sanidad Animal (CISA-INIA).[1]; Ministry of Agriculture, Animal Industry and Fisheries[2]; Centro de Investigación en Sanidad Animal (CISA-INIA)[3]; International Livestock Research Institute (ILRI)[4]

Key words: ASF, epidemiology, p72, p54, CVR

1. Introduction and Objectives

ASF is an important disease of domestic pigs caused by a DNA virus. On October 2007, suspected outbreaks of ASF were notified in three Central Uganda districts. A collection of samples were performed for ASF molecular diagnosis and characterization. The objective of this study was to determine the presence of ASF in Uganda and to uncover epidemiological links between outbreak viruses from Uganda and neighbouring countries.

2. Material and Methods

Sampling collection; 79 different samples were collected in Uganda and submitted to the CISA-INIA.
ASF molecular diagnosis; PCR assay using ASF diagnosis primers PPA1/PPA2 (Agüero et al., 2003) following by virus isolation in primary leukocyte cultures (Malquimst W.A. et al., 1960) was performed.
ASF molecular characterization; i) PCR amplification: partial VP72 was amplified using primers p72U/D (Bastos et al., 2003). Primer pairs ORF9L-F/9L-R were used to amplify the CVR (Nix et al., 2006). Complete VP54 protein was amplified using primers PPA722/PPA89. ii) Nucleotide sequencing and analysis: amplicons were purified by Quiaex gel extraction (QUIAGEN) and sequenced in an automated sequencer. Sequence analyses were performed with CLUSTAL W and MEGA 4.0 package.

3.Results

ASF diagnosis. Detection of ASFV DNA was done by PCR on 79 samples with a 40.5% of positive results. Eleven ASFV Uganda isolates were isolated after three passages in leukocytes prior to molecular characterization.
ASF molecular characterization; P72 and p54 genotyping clustered Uganda 2007 isolates with viruses from Kenya and Uganda collected in 2007 and 2003. CVR characterization placed Uganda 2007 isolates into CVR sub-group XXIV closely related to those obtained in the last outbreak occurred in Kenya in 2007.

4.Discussion and Conclusions

The Uganda 2007 viruses characterized in this study were classified into domestic-pig cycle associated which includes viruses from Uganda and Kenya. This fact may suggests that the disease may be circulating in Kenya-Uganda border districts either through pork products or through live pigs.

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POSTER: SWINE VESICULAR DISEASE IN LOMBARDIA: CASE CONTROL STUDY TO DETERMINE THE ROLE OF RISK FACTORS IN DISEASE DIFFUSION

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Key words: SVD, risk factors, bio-security, case control study

Swine Vesicular Disease (SVD) is a vesicular condition of pigs caused by an Enterovirus of the Picornaviridae family. SVD is reported in Italy and for this reason surveillance and eradication activities are in place.

In the period 2006 - 2007 a recrudescence of swine vesicular disease (SVD) was recorded in Italy and outbreaks were identified in the Italian Northern Regions, as well. Lombardy resulted the most affected region, 53 outbreaks were identified and some 150.000 pigs were stamped out. According to the epidemiological investigations carried out in the outbreaks, bio-security measures were shown to be weak, so that some farms were sometimes exposed to different routes of infection.

To determine the role of the different risk factors involved in the diffusion of the disease, a matched case control study was conducted in the outbreaks and in a selected sample of non – infected farms from the affected area. Matching was performed according to production cycle and herd size. A questionnaire on bio-security was carried out in the selected farms to verify the features considered relevant for the diffusion of SVD.
A multivariate conditional logistic regression model was fitted to the data taking into account the matching of controls to cases. The model building strategy include a preliminary univariate analysis with a significance level of 0.20 and then a stepwise procedure to assess the importance of all the variables. Statistical analysis was performed with R language.

Acknowledgments to: "EPIZONE project (European Union, EPIZONE NoE FOOD-CT-2006-016236)".

POSTER: A novel real-time multiplex RT-PCR for differentiation among virulent and vaccine avian paramyxovirus type 1 (APMV-1) strains

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Key words: real-time multiplex RT-PCR, avian paramyxovirus type 1 (APMV-1)

1.Introduction and objectives

Newcastle disease virus, formally designated as avian paramyxovirus type 1 virus (APMV-1) is a serious pathogen of poultry [1]. The objective of the present study was to develop a rapid, sensitive and specific detection method for the differentiation among virulent and vaccine APMV-1 strains.

2.Materials and methods

The matrix protein gene specific primers and MGB probe were constructed for the detection of wide spectrum of APMV-1 strains. In addition, a second one-step multiplex real-time RT-PCR assay consisting of specific primers and two MGB probes for fusion protein gene were designed for the differentiation among virulent and vaccine APMV-1 strains. To determine the time and distribution of vaccine strain LaSota after vaccination via drinking water in commercial broiler chickens different organs and feces were analyzed.

3.Results

The optimal primers, probe and Mg2+ concentrations for both real-time RT-PCR were determined. The methods were optimized on virulent (pG-442, pG-349, pG-758, C-301/90 and Texas/GB) and vaccine strains (LaSota, Hitchner B1, Ulster). The developed assays show good specificity and sensitivity. Using matrix protein gene real-time approach APMV-1 virus was detected 12 days post vaccination in spleen, trachea and lung. Other organs and feces samples were negative. The results were confirmed with previously published RT-PCR method [2]. In all positive organ samples the vaccine strain was determined with multiplex real-time RT-PCR targeting fusion protein gene.

4.Discussion and conclusions

Our results reveal that the constructed real-time RT-PCR detection methods efficiently amplify specific APMV-1 gene targets and discriminate vaccine from pathogen strains. According to previously published experiments the detection of APMV-1 in broiler chicken organs was relatively late [3]. However, in our experiment the vaccine was not directly applied to individual chicken thus the inoculation dose was not uniform in all tested animals.

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POSTER: Differentiation of two Cyprinid herpesvirus type-3 lineages by multiplex PCR

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Key words: KHV, CyHV3, genotyping, fish, virus

1.Introduction and Objectives

Cyprinid herpesvirus type-3 (CyHV3 or Koi Herpesvirus) has been reported in at least 26 countries worldwide 1 since its first detection in 1996. Three strains of this 295kbp-long DNA virus have been fully sequenced, indicating a very high nucleic acid identity one to another 2. Despite this high similarity, two lineages have been distinguished: one is represented by a strain from Japan (J) while the second includes one strain from the US (U) and one from Israel (I). Based on the aligned sequences of the three strains, we have set up a PCR test to rapidly distinguish lineages J from U/I using two molecular markers.

2.Material and Methods

One CyHV3 isolate from a Koi carp from France (2007) and one isolate from the US (gift of R.P. Hedrick) were produced on the KF1 cell line. In these cases, total DNA was extracted from cells. Another isolate from France was analysed after DNA extracted directly from a dead Koi carp (2007).

Two sets of primers were designed and used simultaneously so that each set amplifies a product of different size depending on the genotype. For the J lineage, products of 168bp and 358bp are expected while products of 130bp and 279 bp are theoretically obtained for both strains U and I. For each PCR reaction, the two expected products are separated on a 2% agarose gel.

3.Results

The method has been successfully applied to two CyHV3 isolates from Koi carps imported in France in 2007. One isolate from France has a genotypic profile similar to a reference strain from the US while the second has a profile expected for J lineage.

4.Discussion and Conclusions

The proposed method is able to differentiate the two CyHV3 lineages J and I/U. Two viral isolates from Koi fishes imported in France in 2007 have been differentiated genetically, suggesting different origins. Altough it should be validated on more CyHV3 isolates, the method has the potential to give clues to the origin of a given isolate in Europe.

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POSTER: THE ANTIVIRAL ACTIVITY OF RECOMBINANT PORCINE INTERFERON-ALPHA AGAINST CLASSICAL SWINE FEVER VIRUS (CSFV)

jing, zhizhong[1]

Z Z Jing[1]

Key words: CSFV, Recombinant interferon, Biological activity, Regular type Fever

1.Introduction and objectives

Interferon （IFN） is first system of body defence against invading pathogens. Studies indicated that IFN can prevention and control several of viral diseases of pig, such as PPRS, CSFV. In this study, we investigated the effect of antiviral on the rabbit by recombinant porcine IFN-α(rpIFN-α).

2.Materials and methods

Thirty rabbits, were randomly divided into six big groups containing three rabbits per subgroup. The rabbits were inoculated with 20 rabbit infection doses of Chinese strain CSFV(C- CSFV) as standard. Groups A, B, C, D and E were infected in ear intravenous. Group A and B were treated at the infection time, Group C and D were treated at three-six hours after infection with 5000U(A1 and C1) or 10000U (A2 and C2) of standard IFN-α (sIFN-α)and 100μg (B1and D1)or 200μg (B2 and D2)of rpIFN-α, respectively. Group E as infection control group and Group F as negative control group. Clinical signs were monitored daily included mental status and body temperature.

3. Results

When treated at the point of infection, 3/3 rabbits of group A1 and 2/3 rabbits of group B1 showed the Regular Type Fever(RTF),the time point of the RTF occurred was later than group E in 0 and 44 hours, the persistence time of the RTF was shorter than group E in 2.6 and 6.6 hours, respectively. On high dose, index showed only 1/3, 16 and 16 hours, and 10.6 and 2.6 hours, respectively. When treated at 36 hours after the infection , 3/3 rabbits in the group C1 and D1 showed the RTF, the time point of the RTF delayed 19 and 24 hours, the persistence time of the RTF reduced 5.6 and 5.6 hours, respectively. In high dose, index showed only 2/3 , 8 and 44 hours, and 2.6 and 6.6 hours, respectively.

4. Discussion and Conclusions

C- CSFV was made by prevalence strain that passed through rabbits successively, which had pathogenic to rabbits. So rabbits inoculated showed the RTF, and the biological activity of the IFN against CSFV can be evaluated by the RTF in rabbits. Comparing with infection control group, treatment groups of sIFN-α and rpIFN-α indicated a better biological activity of antivirus to C-CSFV. However, whether or not have the RTF, are corrected with the biological activity, the time of the treatment and dose of the drug. rpIFN-α has higher antivirus activity than the sIFN-α in corresponding dose whenever of treatment.

POSTER: APPLICATION OF FAST RRT-PCR TECHNIQUES TO THE RAPID DIAGNOSIS OF NOTIFIABLE VIRAL DISEASES

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Key words: RRT-PCR, Viral diseases, Fast RNA amplification, Rapid diagnosis

1.Introduction and Objectives

Bluetongue (BT), African Horse Sickness (AHS), Avian Influenza (AI), and West Nile (WN) are infectious animal diseases caused by different RNA viruses. BT, AI, and WN are significantly emerging in some European regions, while AHS is assumed as a high risk disease to Europe. All of them are notifiable diseases, plus AI and WN have zoonotic potential. Rapid laboratory diagnosis is essential to promptly implement adequated control and eradication strategies. Standardised real-time RT-PCR (RRT-PCR) methods are in use in our lab for BTV (3), AHSV (1), AIV (4), and WNV (2) detection. In this work, established RRT-PCR methods have been modified to a fast format and have been evaluated.

2.Material and methods

Titrated viral suspensions of BTV, AHSV, AIV and WNV, and a panel of cattle, equine, and avian samples were employed in the assays. Nucleic acids were automated extracted from samples using Biosprint 15 (Qiagen). Standard RRT-PCR methods for BTV, AHSV, AIV or WNV detection were performed using QuantiTect Probe RT-PCR kit (Qiagen). Fast RRT-PCR protocols were optimised using QuantiFast Probe RT-PCR kit (Qiagen).

3.Results

Sensitivity assays using serial dilutions of each BTV, AHSV, AIV and WNV were performed by the specific standard and fast RRT-PCR methods. Comparable results were achieved for each virus analysed by both procedures. When clinical samples from cattle, horses, and birds were tested, similar results were also obtained, confirming the specificity of the fast assays.

4.Discussion and Conclusions

The results of an evaluation study of fast RRT-PCR assays for BTV, AHSV, AIV and WNV detection are presented. The similar results obtained when viral suspensions and clinical material were analysed show that fast RRT-PCR systems can be powerful tools for a very rapid, specific and sensitive diagnosis of BT, AHS, AI, and WN. The presented RRT-PCRs only take 80 minutes and can be used in any real-time platform. This, together with the automation of the RNA extraction procedure, makes possible to obtain the definitive results in less than 3 hours, without increasing the cost.

5.Acknowledgements

This work has been funded by INIA projects OT01-002 and RTA2006-00167-C02-01.

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POSTER: Results from the 2007 proficiency testing scheme for RT-PCR methods used to detect foot-and-mouth-disease virus

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Key words: Proficiency Testing, RT-PCR, FMDV

1.Introduction and Objectives

This poster describes the results from the inter-laboratory proficiency testing scheme (PTS) conducted during 2007 to evaluate RT-PCR assays used to detect foot-and-mouth-disease virus (FMDV). This study was organised by the Community Reference Laboratory to assist European Union (EU) and other National FMD Laboratories to deploy accurate and reproducible tests for FMDV. This work also contributes to the goals of EPIZONE WP4.1 and LAB-ON-SITE.

2.Material and Methods

Two panels of coded samples were prepared and sent to participating laboratories for testing by RT-PCR assays. The first ('live') panel was sent to 16 laboratories and consisted of 4 FMD viruses of serotype O, A and Asia 1. In addition to RT-PCR, this panel was also used to assess the performance of virus isolation methods in use by the different laboratories. The second panel sent to 28 laboratories comprised 10 non-infectious FMDV samples prepared by BEI inactivation and included FMD viruses of serotypes O, A, Asia 1, SAT 1 and SAT 2.

3.Results

In total, 30 laboratories agreed to receive at least one of the panels for evaluation of their RT-PCR methods. Twenty-six were located in Europe, 11 of which were also partners in the EPIZONE project. Samples in Panel 1 were detected by all laboratories that reported results for RT-PCR: however dilution studies indicated that these assays differed in analytical sensitivity by as much as 1000-fold. Real-time RT-PCR methods are being increasingly adopted for routine use: in this study, 16 laboratories reported results for Panel 2 using this format. Results for Panel 2 showed that 18/25 of the laboratories successfully detected FMDV in all the positive samples and correctly discriminated the 5 negative samples in the panel. Two laboratories failed to detect SAT viruses, possibly due to nucleotide mismatches with primers and/or probes, while 5 laboratories scored a false positive result for at least one of the negative samples.

4.Discussion and Conclusions

These data contribute to QA programmes and accreditation schemes in place in these laboratories. Future PTS will address important diagnostic activities of National Reference Laboratories and will be designed according to the guidelines set out in the proposed ISO 17043/43 standard.

5.Acknowledgements

Funded by EU CRL, FAO/EUFMD, EPIZONE (WP 4.1) and LAB-ON-SITE

6.References

POSTER: Intra-laboratory evaluation of two commercial DIVA kits for Avian influenza

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Key words: DIVA, Avian influenza, kit validation

1.Introduction and Objectives

The use of vaccination as part of a set of coordinated measures to combat AI is deemed to be successful in achieving the goal of eradication if it allows for the DIVA (Differentiation of Infected from Vaccinated Animals) principle (1). These systems enable the detection of field virus exposure in vaccinated flocks and through this, infected flocks may be properly managed. Several vaccination strategies enabling the DIVA concept have been described. Among these, the application of tests directed to the detection of antibodies induced by antigens only contained in the field virus as markers of field exposure are considered as suitable candidates to implement eradication programmes.

Two commercial ELISA kits are presently available for AI DIVA. The first (ID-Screen ®, ID-VET, France) is a competitive ELISA based on the identification of antibodies to the N1 protein and so can be used to detect antibodies against a circulating field virus of an N1 subtype. A preliminary validation of this kit has been previously presented (2). The second kit (NS1 AIV Ab ELISA, Anigen, Korea) is an indirect ELISA that detects antibodies against the Non-structural 1 protein (NS1). The NS1 protein is only expressed by actively replicating avian influenza virus. It should, therefore, not be present in significant amounts in inactivated vaccines. No data on the sensitivity and specificity of this test is currently available.

The aim of this work was to perform an inter laboratory evaluation of both commercial kits by EPIZONE partner laboratories in the framework of Workpackage 4.3 (DIVA diagnostics).

2.Material and Methods

Both commercial kits were supplied free of charge by the manufactures and sent to each participating laboratory along with a set of reference sera prepared by P13 and a list of recommended sera to be used.

3.Results

Results from this inter-laboratory evaluation will be presented.

4.Acknowledgements

The authors thank ID-VET, France and Anigen, Korea for providing the ELISA kits free of charge.

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POSTER: DNA vaccination against Rift Valley Fever virus: protection studies in transgenic IFNAR-/- mice

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Key words: protective immunity, DNA vaccines,

Rift Valley Fever virus is transmitted by mosquitoes and causes a severe disease in livestock and humans in the form of fatal haemorrhagic fever. RVF is endemic in Sub-Saharan Africa, with a great emerging potential in northern areas. Control strategies alternative to classical vaccines are desirable. Since viral glycoproteins coded in the medium (M) RNA segment have been involved in the induction of immune protection we developed a series of pCMV constructs based on the M segment coding sequence to asses the level of protection conferred after viral challenge in susceptible IFNAR-/- mice. The protective ability of plasmid constructs encoding the viral nucleoprotein was also assayed.

Material and methods.

129Sv IFNAR-/- mice were inoculated intramuscularly with 100 µg of plasmid DNA. Mice received a total of two doses, every two weeks. After the last inoculation, mice were inoculated intraperitoneally with a lethal (2x104 pfu) dose of RVFV-MP12 strain. Mice were monitored for clinical signs during 21 days. Moribund animals were euthanized and samples of different organs were analyzed for virus detection.

Results.

Mice immunized with a construct encoding G2/G1 were fully protected while mice vaccinated with N construct or a combination of G2/G1 and N were partially protected. Mice immunized with a construct encoding Nsm/G2/G1 alone or in combination with N construct succumbed to the lethal challenge as well as mice from control groups (pCMV or naïve group).

Discussion and conclusions.

The results of this study show the ability of DNA vaccines for inducing a protective response against lethal viral challenge. IFNAR-/- mice represents a suitable model for studying the capacity of DNA vaccines to elicit protective immunity and to study the immune mechanisms involved in protection.

POSTER: OCCURENCE OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) AND PORCINE REPRODUCTIVE AND RESPIRATORY VIRUS (PRRSV) IN POLISH WILD BOAR

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Key words: porcine circovirus type 2, PMWS, PRRS, wild boar, phylogenetic analysis

1.Introduction and Objectives

Postweaning multisystemic wasting syndrome (PMWS) and porcine reproductive and respiratory syndrome (PRRS) are important virus diseases of swine. Because wild boars (Sus scrofa) may constitute a reservoir for etiological agents of these diseases, objectives of the study were to investigate presence of antibodies against PRRSV and PCV2 DNA in wild boars and to evaluate phylogenetic relationship between PCV2 strains from Polish wild boars and domestic pigs.

2.Material and Methods

DNA from 121 tonsils and 55 meat juice samples from wild boars was real-time PCR amplified with primers flanking a 494 bp fragment of a PCV2 capsid protein gene (cap) (2). Selected PCR products were sequenced and used for phylogenetic analysis with CLUSTAL W and Neighbour-Joining methods. Samples of meat juice were also tested for the presence of specific IgG antibodies against PRRSV using indirect in house ELISA test (5).

3.Results

All the meat juice samples were negative in PRRSV ELISA. PCV2 DNA was detected in 64 (52.89%) tonsils and in 4 (7.3%) meat juice samples. Phylogenetic analysis revealed that 9 sequences belonged to group 1 PCV2. They were closely related to each other (99.1-100% of nucleotide similarity) and to isolates from Polish pig farms (98.7-100%). The 3 other sequences were clustered together with sequences of PCV2 group 2, in subgroup D, showing only 89.1-89.9% of nucleotide identity to the group 1 sequences from wild boars.

4.Discussion and Conclusions

This study is the first report on presence of PCV2 group 2 in Poland. All previously reported sequences of PCV2 isolated from pigs belonged to group 1 (3). These results indicate that the diversity of PCV2 in Polish wild boars population could be higher than in domestic pigs. High similarity of sequences from wild boars to those from pigs suggests epidemiological relation between these populations.

5.Acknowledgements

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POSTER: NEW ASSAY FOR THE SIMULTANEOUS DETECTION AND DIFFERENTIATION OF WEST NILE AND USUTU VIRUSES BY THREE-COLOUR REAL-TIME RT-PCR

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CISA-INIA[1]

Key words: real time RT-PCR, Usutu virus, West Nile virus, lineages, multiplex

1 Introduction and Objectives

West Nile virus (WNV) and Usutu virus (USUV) are artropod-borne flaviviruses belonging to the Japanese Encephalitis serogroup, having transmission cycles involving birds as amplifying hosts, and zoonotic potential. In recent years increasing WNV activity has been registered in Europe, while, since its first occurrence outside Africa, in Austria in 2001, USUV has spread in central Europe. Most molecular detection methods for WNV (1) are focused on the most widespread lineage (L1), though the lineage 2 (L2) has recently been detected in Europe (2). In a previous study (3) we developed a RRT-PCR detecting L1 and L2 with high sensitivity. In this work we describe a RRT-PCR assay able to detect and differentiate WNV L1, L2 and USUV.

2 Material and methods

Representative strains of WNV: NY99 (L1) and B956 (L2), and USUV (SAAR-1776) were used. Total RNA was automated extracted from samples using Biosprint 15 (Qiagen). Primer sets were designed from conserved sequences of the 3´NTR of the viral genomes. Three TaqMan probes specifically designed to react with each virus-specific amplicon, and labelled with different fluorochromes, allowed three-colour multiplex RRT-PCR analysis.

3 Results

We analyzed the performance of the method for differentiation of lineages 1, 2 and USUV.

The sensitivity of the developed RRT-PCR was determined using three replicates of 10-fold dilutions of titrated viral suspensions of the different WNV and USUV strains, grown in Vero cells. Standard curves were constructed based on the observed Ct values for each viral dilution, showing a linear relationship and wide dynamic range for all the viruses studied.

4 Discussion and Conclusions

This work describes for the first time a real-time RT-PCR method for the simultaneous molecular detection and differentiation of WNV lineages 1, 2 and USUV employing specific TaqMan probes. This method allows typifying the circulating virus in few hours. In summary, the developed multiplex RRT-PCR can be very useful in diagnostic but also in epidemiological surveillance, especially in regions where the above mentioned viruses coexist.

5 Acknowledgements

This work has been funded by INIA project OT01-002.

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POSTER: Genotyping of avian influenza viruses using padlock probes

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Key words: Influenza A, subtyping, padlock probes, microarray

1.Introduction and Objectives

A subtyping assay for both the hemagglutinin (HA) and neuraminidase (NA) surface antigens of the Avian Influenza virus (AIV) has been developed. The method uses padlock probe chemistry combined with a microarray output for the detection. The outstanding feature of this assay is its capability to designate both the HA and NA of an AIV sample from a single reaction.

2.Material and Methods

Padlock probes were designed towards target sequences in conserved or semi-conserved regions within the matrix, hemagglutinin and neuraminidase genes. The probes were added to the samples, ligated then amplified twice by RCA and PCR. Then each reactions were transferred to microarray slides into subarrays and hybridized. The slides were scanned and the images analyzed.

3.Results

A panel of 77 influenza strains was tested representing the entire assortment of the two antigens. 100% (77/77) of the samples tested were identified as AIV and 97% (75/77) were subtyped correctly in accordance with previous examinations performed by classical diagnostic methods.

4.Discussion and Conclusions

This assay is a convenient and practical tool for the study of AIVs, providing important HA and NA data more rapidly than conventional methods.

5.Acknowledgements

This work was supported by grants from EU FP6 program, project SSP3-513 645: New and emerging technologies: improved laboratory and on-site detection of OIE List A viruses in animals and animal products (LAB-ON-SITE) and project FP6-2004-Food-3-A Network of Excellence for Epizootic Disease Diagnosis and Control (EPIZONE).

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POSTER: New and emerging technologies: improved laboratory and on-site detection of OIE List A viruses in animals and animal products

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Key words: TADs, diagnosis, PCR, dip-stick

The goal of this project is to improve the diagnostic tools available for 10 highly infectious transboundary animal diseases (TADs) notifiable to the World Organisation for Animal Health (OIE). The notifiable (former “OIE List A”) diseases targeted are: foot-and-mouth disease, swine vesicular disease, vesicular stomatitis, classical swine fever, African swine fever, bluetongue, African horse sickness, Newcastle disease and highly pathogenic avian influenza. From former “OIE List B” swine influenza is also included. By addressing the recommendations of the Scientific Committee on Animal Health and Animal Welfare, this programme focuses on the development, validation and dissemination of diagnostic tests for the improved diagnosis of the listed TADs. The project has combined the development of improved “front line” diagnostics, such as dipstick tests that can be used in the field, with the production of robust and simple nucleic acid and antigen-antibody detection methodologies. The molecular diagnostic tests generated by the project have exploited the most recent advances in real-time PCR technologies and strategies to detect nucleic acids without thermocycling. Furthermore, the potential of solid phase microarrays (based on padlock probe technologies) and multiplex PCR systems to achieve high throughput diagnosis of the targeted TADs have also been investigated.
LAB-ON-SITE brings together a strong multidisciplinary scientific consortium of nine partner laboratories (representing Reference Laboratories and Collaborating Centres of the OIE, FAO and EU) from seven countries of the EU and includes one SME. The Animal Production and Health Section, FAO/IAEA Joint Division, IAEA, Austria is an external partner. The worldwide dissemination of these results is provided by the multidisciplinary profile and global network of the consortium. Standard operating procedures outlining the new methods are available for use by other livestock diagnostic laboratories.

POSTER: DEVELOPMENT AND VALIDATION OF A REAL TIME RT-PCR ASSAY FOR THE SIMULTANEOUS DETECTION OF AVIAN INFLUENZA VIRUSES BELONGING TO THE H5, H7 AND H9 SUBTYPES

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Key words: avian influenza, H5 subtype, H7 subtype, H9 subtype, real time RT-PCR

Introduction and Objectives

Among avian influenza A viruses, the H5, H7 and H9 subtypes are of major interest to the scientific community because of the serious consequences for the poultry industry and the increasing frequency of direct transmission of these viruses to humans. In certain areas of the world, these subtypes are co-circulating in domestic birds (1), thus confusing the clinical picture and requiring a tailored diagnostic approach. Here, we report of the development and validation of a one step reverse transcription real time PCR (RRT-PCR) assay to detect simultaneously the H5, H7 and H9 subtypes of AIVs from clinical specimens.

Material and Methods

The assay was developed using fluorogenic hydrolysis-probes to detect the H5, H7 and H9 subtypes of AI from clinical samples. Specificity, sensitivity and reproducibility of the method were assessed. The test was also validated on clinical samples of avian origin.

Results

The protocol allows a rapid (less than 2 hrs) detection and identification of each of the three AI subtypes directly on clinical specimens. The H5, H7 and H9 primer and probe sets were able to detect the nucleic acids only of isolates of their respective subtypes. High sensitivity levels were obtained, with limits of detection ranging from 102 to 104 RNA copies. Excellent results were achieved in the intra- and inter-assay variability tests. Results derived from the test validation on clinical samples were in agreement with the gold standard test (virus isolation).

Discussion and Conclusion

The high sensitivity, specificity and reproducibility of this assay together with its short execution time indicate that this method is suitable as a routine laboratory test for rapid detection and differentiation of three prevalent avian influenza virus subtypes in samples of avian origin.
To date, this is the first real time PCR protocol available for the simultaneous detection of AI viruses belonging to H5, H7 and H9 subtypes.

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POSTER: Mabs based platform for neuraminidase Avian Influenza viruses typing.

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Key words: monoclonal antibodies, neuraminidase, Avian Influenza.

Introduction

Avian Influenza (AI) is a disease of poultry diffused worldwide that negatively impacts poultry health and international trade of live animals and their products. Typing of both haemagglutinin (H) and neuraminidase (N) proteins is crucial to study evolution and epidemiology of AI viruses. The aim of this study is typing of AIVs using a Mabs based platform able to identify all the N subtypes.

Materials and methods

AIVs.106 samples of allantoic fluids infected with AIVs of 14 H (from 1 to 14) and 9 N subtypes were used. Several AIVs representative for N1, N2, N3, N4, N7, N9 subtypes were titrated (EID50).

Mabs. Mabs specific for 1-9 N were produced an screened as reported (1). Anti-N MAbs were further analysed in NI assays versus AIVs of the nine N subtypes (2).

ELISA. Sandwich ELISA for N typing were developed using the same or a combination of two different Mabs, specific for the required N subtype, as catcher and tracer. In order to valuate their analytical sensitivity, several ten fold dilution of the titrated strains were analyzed.

Results

Several hybridomas were obtained. Combinations of catching and conjugated MAbs specific for each N were identified on the basis of a wider spectrum of reactivity within homologous N subtypes evidenced by indirect and competitive ELISA. All the MAbs showed subtype-specific NI activity.

Analytical specificity. All 106 AIVs were tested simultaneously, and reacted specifically, exhibiting a strong signal only in the ELISA test for the corresponding N subtype.

Analytical sensitivity. Serial dilutions of titrated AIVs were tested. Considering the EID50, the detection limit for the N-typing ELISAs is calculated to be 105/5,5EID50/0.1ml.

Discussion

The rigorous specificity of the selected MAbs for all N subtypes combined with the capability to recognise all the viruses examined make the developed N-typing ELISAs appropriate for the rapid identification of AIVs isolated in allantoic fluids; however, analytical sensitivities are not proper for a direct diagnosis on biological samples. Great advantages compared with NI assays derive from the simple and rapid ELISA procedure and from the use of MAbs, that ensure assays standardisation and reproducibility.

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POSTER: Sequence analysis of PRRSV from Russia

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Key words: PRRSV, European genotype, molecular epidemiology

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is recognized to consist of 2 genotypes, European and North American. Recent work has shown that East European countries appear to harbor EU genotype viruses of exceptional diversity (2, 3). We have in the present study examined EU-PRRSV sequence diversity in Russia.

2. Material and Methods

Samples were obtained from 37 farms in the Russian Federation, from 1996 through 2006. Additionally, samples from 5 Belarusian farms (from 1999-2001) were obtained. The nested RT-PCR was used to amplify complete ORF7 of PRRSV. Purified, non-cloned PCR amplicons were cycle sequenced.

3. Results

This study provided 57 new sequences of ORF7 of PRRSV strains from 1996 to 2006. All were of European genotype. Most of the new Russian sequences exhibited clustering essentially conforming to the previously suggested subtypes 1, 2 and 3 of EU-PRRSV. The Russian sequences were located in all three subtypes. Interestingly, while reference EU-PRRSV sequences from Western and Central Europe, Asia and North America formed a tight group within subtype 1, the Russian subtype 1 sequences were more diverse. Finally, three Russian sequences from the a single farm formed an independent cluster between subtypes 2 and 3. In the EU-PRRSV ORF7 phylogeny, there was concordance between the size of ORF7, and phylogenetic grouping. Thus, subtype 1 contained viruses with ORF7 size of 387 nt, subtype 2 contained viruses with ORF7 size of 378 nt, and subtype 3 contained viruses with ORF7 size of 375 nt.

4. Discussion

In previous papers examining the molecular phylogeny of EU-PRRSV in Europe, we have continually had to increase our estimate of the diversity of this PRRSV genotype (1, 2, 3). Recently, we suggested that the diversity of EU-PRRSV was sufficient to warrant subdivision in at least 3 subtypes (3). The new EU-PRRSV ORF7 sequences from the Russian Federation grouped in all three subtypes so, the new sequences fully supported the previously proposed subtypes 1, 2 and 3 of EU-PRRSV.

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POSTER: An optimized protocol for the complete genome sequencing of H5N1 highly pathogenic avian influenza (HPAI) virus

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Key words: avian influenza, H5N1, sequence analysis

1. Introduction and Objectives

Since 1997, H5N1 HPAI viruses have reached endemic levels among poultry and have caused severe, even fatal, disease in humans. To better understand the genetic relationship of the H5N1 viruses and to identify the genetic markers associated with increased virulence, transmission and sensitivity to antiviral treatments, sequence analysis represents an extremely powerful tool. The aim of this study is to present optimised protocols for sequencing of H5N1 HPAI viruses.

2. Material and Methods

Viral RNA is extracted from allantoic fluid using the Nucleospin RNA II Kit (Machery-Nagel, Düren, Germany) and is reverse transcribed with SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA - USA). PCR amplification is performed using gene segment specific primers that have the same melting temperature and contain one of two 17 nt "tails" at their 5' end. Primers sequence are available upon request.

The PCR products are purified with the ExoSap-IT kit (USB Corporation; Ohio-USA) and sequenced on both strands using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, Foster City, CA – USA) and two primers complementary to the 17 nt "tails". The sequence reactions are cleaned-up using PERFORMA DTR Ultra 96-Well kit (Edge BioSystems, Gaithersburg, MD - USA) and sequenced in a 16-capillary ABI PRISM 3130xl Genetic Analyzer (Applied Biosystem, Foster City, CA – USA).

3. Results

The sequencing method described here allows for the entire genome of a H5N1 strain to be sequenced in a relatively short time. The extraction of the sample and the amplification of the 8 gene segments can be performed in just one working day while less than three working days are necessary to obtain the final electropherograms.

4. Discussion and Conclusions

The protocols for the entire genome sequencing of H5N1 viruses have been optimised and adapted to a 96 well plate format enabling a TAT (turn around time) of less than 3 days. This method has allowed us to increase the number of individual sequences generated in our laboratory from 3,940 in 2006 to 13,850 in 2007 maximising resources in terms of staff and equipment.

POSTER: Genetic characterization of H1N1 and H1N2 swine influenza viruses isolated in Italy in 1998-2007

Moreno Martin, Ana M.[1]; Barbieri, Ilaria[1]; Chiapponi, Chiara[1]; Foni, Emanuela[1]; Sozzi, Enrica[1]; Canelli, Elena [1]; Luppi, Andrea[1]; Cordioli, Paolo[1]

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Key words: swine influenza, H1N1, H1N2 subtypes, genetic characterization

Introduction

Several changes occurred in the epidemiology of swine influenza viruses (SIV) in Europe in the last years. Classical H1N1 were replaced in 1979 by "avian like" H1N1SIVs. The H1N2 subtype was first described in Great Britain in 1994 and since then spread to continental Europe. In this paper the genetic relationships between 29 Italian H1N1 and, 12 H1N2 SIVs isolated from 1998 to 2007 were investigated.

Material and Methods

Partial HA and NA amplification and sequencing were performed as reported(1). Phylogenetic analysis was conducted by MEGA4(3).

Results

HA sequences of Italian H1N1 SIVs belonged to two groups: 1) strains isolated in the '90s closely related to sw/Fin/2899/82, 2) recent isolates similar to avian-like sw/IV/1455/99 (2). N1 sequences of all recent strains, except three, were closely related to Fin/2899/82 and some earlier Italian strains. The remaining 3 isolates were like IV/1455/99 viruses.

HA sequences of H1N2 SIVs were clearly distinguishable from H1N1 SIVs. Italian strains isolated in 1998-2003 appeared similar to contemporary H1N2 SIVs from Northern Europe and formed a cluster separated from that of the recent Italian strains. Interestingly, one reassortant strain It/5433/01 H1N1 was identified (97% identity to H1N2 Italian SIVs). N2 sequences of the earlier isolates were closely related to current strains isolated in France and Belgium and were in a different cluster from recent Italian strains (human-like sw/Fin/127/99 SIVs). The NA gene of strain It/11271/03 H1N2 exhibited a high similarity to the Italian H3N2 SIVs.

Discussion

These results pointed out homogeneity among the recent H1N1 SIVs as well as distinguishing these from the earlier strains. It should be noted that while HA gene of recent H1N1 SIVs was different from that of the early circulating strains, the NA gene seemed to be more related to them. Also for Italian H1N2 the presence of a homogeneous group, composed of the recent viruses, differing from earlier strains could be described. Finally the occurrence of reassortment events was evidenced by the detection of two reassortant strains. These findings suggest the requirement for improved surveillance activities in the future.

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POSTER: Molecular characterization of highly pathogenic H5N1 avian influenza viruses isolated in Sweden in 2006

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Key words: Avian influenza, H5N1, highly pathogenic, molecular epidemiology

1. Introduction and Objectives: The analysis of the nonstructural (NS) gene of the highly pathogenic (HP) H5N1 avian influenza viruses (AIV) isolated in Sweden early 2006 indicated the co-circulation of two sub-lineages of these viruses at that time(1). In order to complete the information on their genetic features and relation to other HP H5N1 AIVs the further seven genes of eight Swedish isolates were amplified in full length, sequenced, and characterized.

2. Material and Methods: A straightforward protocol for amplifying full length influenza coding sequences was developed. Sequence assembly and alignment were performed with the CLC Combined Workbench 3.0.2. bioinformatics software (CLC bio A/S, Denmark), and phylogenetic tree analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) software v.4.0 (Tamura K, 2007).

3.Results: The presence of two sub-lineages of HP H5N1 AIVs in Sweden in 2006 was further confirmed by the phylogenetic analysis of the nearly the complete genome of the eight isolates. According to the most recent nomenclature(2) six of the analyzed viruses belonged to subclade 2.2.2 and grouped together with German and Danish isolates, while two 2.2.1-like viruses formed a subgroup with isolates of Egyptian, Italian, Slovenian, and Nigerian origin.

4. Discussion and Conclusions: Our data support previous findings on the spread of multiple sub-lineage H5N1 HPAIVs into Europe. The detection of 2.2.1-like viruses in Sweden adds further data regarding their spread in the North in 2006. The close genetic relationship of subclade 2.2.2 Swedish isolates to the contemporary German and Danish isolates supports the proposition of the introduction and spread of a single variant of 2.2.2-like H5N1 avian influenza viruses in the Baltic region.

5. Acknowledgements: This work was partly supported by the Swedish Emergency Management Agency, the EPIZONE, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, the FLUTEST and the Leonardo da Vinci Mobilität Programme.

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POSTER: Evaluation of subclinically infectious status in sheep after FMD outbreak in epidemic regions in China by 3ABC-ELISA complemented with an EITB method

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Key words: FMDV, 3ABC-ELISA, EITB

Introduction

Sheep and goat are always subclinically infected by foot-and-mouth disease virus (FMDV). These are very dangerous if the animal movements are not taken under strict control and restriction. Therefore, this study has investigated subclinical infection in sheep in FMD epidemic regions, and to know how severe of the disease prevalence in sheep and goat in epidemic regions.

Materials and Methods

Total of 2150 sera samples were collected from 22 clinically healthy sheep and goat herds in Asia 1 FMD epidemic region in Ningxia autonomous province in northern-western of China. Sera samples were detected for existence of antibodies against nonstructural protein (NSP) 3ABC. Suspicious and positive samples were further confirmed by an enzyme-immunotransfer blot assay (EITB).

Results

The prevalence of antibody against 3ABC was within 0~50.6% in different sheep herds with an average positive rate of 20.4%. These results indicated that virus activity existed in some sheep herd. The EITB method can be a reliable method for improvement of specificity of 3ABC-ELISA.

POSTER: CHEMOKINE EXPRESSION IN THE MURINE NERVOUS SYSTEM DURING LYSSAVIRUS INFECTION

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Key words: Chemokines, Lyssavirus, Quantitative RT-PCR, Immunohistochemistry

1.Introduction and Objectives

T-cell infiltration into dorsal root ganglion, spinal cord and caudal regions of the brain is a characteristic feature of lyssavirus infection. Chemokines provide a key immunological attractant for lymphocytes although investigation of their role in the pathogenesis of lyssaviruses, has been limited. Two key chemokines, CXCL10 (IP-10) and CCL5 (RANTES) specifically attract T cells and in vitro studies have demonstrated their up-regulation in microglial cells in response to infection with rabies virus (Nakamichi et al. 2005). We and other authors have observed increases in chemokine transcripts in the brains of rabies-infected mice.

2.Material and Methods

To investigate this for non-rabies lyssaviruses we have measured transcript levels of CXCL10 and CCL5 chemokines by real-time quantitiative RT-PCR within the brain during infection with European bat lyssavirus type-2 (EBLV-2). We have corroborated this data through detection of chemokine expression by immunohistochemistry in neural tissue using protein specific antibodies.

3.Results

Following infection with EBLV-2, CXCL10 increased 20-fold whilst CCL5 increased 6-fold prior to the development of clinical signs. These levels increased dramatically to 2500-fold and 2100-fold, over baseline levels at inoculation, respectively. Specific staining detected expression of both chemokines in a range of cell-types within the CNS. Expression was also observed in the endothelial tissue in perivascular cuffs, an inflammatory feature common in EBLV-2 infected mouse brain

4.Discussion and Conclusions

This study simultaneously demonstrates increases in chemokine transcripts and expression of protein within the brain of lyssavirus infected mice. We conclude that the dramatic up-regulation of these chemokines provides the driving force for T-cell infiltration within the lyssavirus infected brain.

5.Acknowledgements

This study was funded by Defra UK through grants SE0524 & SE0528, and supported through Epizone Workpackage 5.3.

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POSTER: Genetic Analysis of the Non-structural (NS) Genes of Avian influenza Viruses Isolated in Mallards in northern Europe in 2005

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Key words: , influenza, avian, wild birds, reservoir, ducks

1.Introduction and Objectives

Our knowledge about the gene pool of influenza A viruses in their natural reservoir in Europe is incomplete, limited information on the prevalence of influenza A viruses in wild birds in Europe has been provided in recent years indicating Mallards (Anas platyrhynchos) as a essential factor of the ecology of influenza A viruses because a particularly wide variety of subtypes isolated from these birds. Therefore in this study we analysed the NS1 gene sequences of 45 influenza A viruses, isolated from Mallard ducks at the major flyway of the Western Eurasian duck population in 2005

2.Material and Methods

Samples were collected from seven hundred and one Mallards (Anas platyrhynchos) as part of surveillance program organized by the Swedish Board of agriculture at Ottenby bird Observatory.

3.Results

Matrix rRT-PCR screening showed that about 24% of examined birds were influenza A positive. From rRT-PCR positive samples a total of 45 influenza A viruses of different subtypes could be isolated. The overall isolation rate was 6 % (45/781). Our results indicate that the most common haemagglutinin subtypes of influenza A viruses in western European mallard ducks are H4, H3 and H6, which accounted for 24%, 21% and 11% of the isolates, respectively

4.Discussion and Conclusions

Analysis of phylogenetic relationships among the NS genes reported in this study clearly show that two distinct gene pools, corresponding to the NS allele A and B, were present at the same time in the same geographic location in the mallard duck population in northern Europe. Out of 45 isolated viruses 39 (87%) belong to Allele A, six (13%) belong to Allele B. Phylogenetic analyses were further revealed three separate clades and multiple sub clades among isolates in allele A and two separate clades in allele B in North European mallard duck isolates.

A comparison of nucleotide and amino acid sequences of viruses reported in this study showed that the degree of variation within the alleles is very low and a substantial number of silent mutations in the nucleotide sequences which results to high degree of homology in protein sequences.Studies of influenza A viruses in their natural reservoir can lead to improvement of veterinary and human medicine. The analysis of nucleotide and amino acid sequence of NS gene has laid foundation for further study of genetic diversity and function of NS 1 protein.

5.Acknowledgements

6.References

POSTER: Development of a magnetic bead microarray for the simultaneous and simple detection of four pestiviruses

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Key words: pestivirus, CSFV, BDV, BVDV1, BVDV2, PCR, magnetic bead, microarray

1.Introduction and Objectives

This study reports a novel method for the rapid detection and identification of the four recognized species in the pestivirus genus of the Flaviviriade family, i.e. classical swine fever virus, border disease virus, bovine viral diarrhoea virus type 1 and type 2. The analysis of pestivirus PCR products was performed on microarrays by means of magnetic bead detection.

2.Material and Methods

The process utilizes an oligonucleotide array, onto which 5´ biotinylated PCR products were hybridized, followed by visualization with streptavidin-coated magnetic particles by the naked eye, microscope or biochip reader.

3.Results

The assay was tested on a collection of pestiviruses that included all four species and allowed a specific and sensitive detection. The results indicate that due to its high sensitivity, specificity and very simple detection procedure, the magnetic bead assay provides a powerful tool for the improved detection and identification of viral pathogens.

4.Discussion and Conclusions

Considering the simplicity of the assay, the protocols for hybridization and magnetic bead detection offer an emerging application for molecular diagnoses in virology that is amenable for use in a modestly equipped laboratory.

5.Acknowledgements

The authors would like to thank European FP6 project, LAB-ON-SITE (SSP3-513 645) for the primary financial support of this project as well as the European FP6 Network of Excellence for Epizootic Disease Diagnosis and Control (EPIZONE) for the contribution of samples used in this study. In particular, Drs. Martin Beer and Bernd Hoffmann, who prepared the ring trial under work package 4.1 "Real-time PCR diagnostics". The authors also would like to acknowledge the European FP6 projects, CSF&WILD-BOAR (SSP1-501599) and EPIZONE (FOOD-CT-2006-016236) for their scientific and financial contribution to this study.

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POSTER: Detection of Borna Disease Virus by in situ proximity ligation assay

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Key words: Borna Disease Virus, proximity ligation assay, diagnostics

1.Introduction and Objectives

Borna disease virus (BDV) is a negative-stranded RNA-virus and the causative agent of neurological disorders in several species, including horses, sheep and cats. BDV persistently infects cells in the central nervous system (CNS). In some animals, like cats, the presence of BDV antigen as demonstrated by immunohistochemistry (IHC) is low. Therefore, there is a need for more sensitive detection methods, such as in situ proximity ligation assay (isPLA).

2.Material and Methods

Brain tissue from experimentally infected animals and from naturally infected horses and cats have been used in the development and evaluation of the BDV-isPLA. Oligonucleotide-conjugated polyclonal rabbit anti-BDVp23 and anti-BDVp40 antibodies were used as proximity probes in the detection of viral antigen, according to the method previously described [1].

3.Results

We have demonstrated BDV antigen in the brain tissue of infected animals using BDV-isPLA. The staining pattern was similar to previous IHC stainings.

4.Discussion and Conclusions

BDV is a neurotropic virus that causes persistent CNS infection in several animal species. The possible presence of BDV infection in humans and its involvement in neuropsychiatric disorders are under debate [2, 3]. In some animals the presence of antigen as demonstrated by IHC is low. Therefore, there is a need for more sensitive detection methods, like isPLA. This study describes BDV detection by isPLA, a method that could prove to be more sensitive than methods currently used. A modified version of this BDV-isPLA could be used to study host-virus protein interactions to further study the pathogenesis of BDV infection.

5.Acknowledgements

The authors would like to thank prof G Gosztonyi, Berlin, Germany for providing brain tissue material, and Mrs Åsa Gessbo for technical assistance. This study was supported by the Swedish Horse Research Foundation.

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POSTER: Comparing pig host responses in vitro and in vivo following infection with pathogenic and non pathogenic African swine fever virus isolates to identify correlates of pathogenicity

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Key words: African swine fever, chemokine, host responses, pathogenicity

Abstract

African swine fever (ASF) virus (ASFV) is capable of inducing a severe haemorrhagic fever in domestic pigs and European wild boar. The virus persists in a natural cycle between the warthog, bush pig and soft tick where it is endemic in sub-Saharan Africa. ASFV had been eradicated from all areas outside this region with the exception of Sardinia until June 2007 when an outbreak was confirmed in Georgia.

Virulent isolates of ASFV cause high mortality with death occurring 5 – 10 days after the onset of clinical signs, less virulent isolates are either moderately virulent with reduced mortality or low virulence causing chronic infections. The main target cells for ASFV replication are monocytes and macrophages which have a key role in activating and orchestrating the innate and acquired immune responses of the host. They orchestrate the acquired response via antigen presenting and lymphocyte activation partly through the ordered expression and secretion of cytokines and chemokines. Many of these soluble mediators have also been implicated in the pathogenesis of major clinical events seen in ASF. Several studies have already examined cytokine expression and production in ASF infections in vitro but limited work has been carried out on chemokines compared to other haemorrhagic diseases.

I aim to compare host responses in vitro and in vivo following infection with pathogenic and non pathogenic isolates of ASFV. Benin, Uganda and Malawi will be used as the pathogenic isolates and OURT 88/3 as the non pathogenic. Host responses to each of these isolates will be examined at the transcription level of selected chemokine and chemokine receptor genes, expression of protein levels and functional assay of chemokine activity. The in vitro experiments will be carried out on blood derived macrophages and total blood leucocytes.

I have selected a number of primers, including for chemokines (CCL2, CCL3, CCL4, CCL5, CCL8, CXCL8, CXCL10, CXCL11) and chemokine receptors (CXCR1, CCR3, CCR1, CXCR3) to enable me to carry out RT – PCR to look at relative gene expression at different time points post infection compared to mock unifected.

POSTER: Comparison of changes in bovine cell transcriptome during infection by rinderpest and measles viruses.

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Key words: microarray, innate immunity, host specificity

Introduction & Objectives: Morbilliviruses all use the same receptor and can infect cells from many different species of mammals, but show strong restriction of pathogenesis to particular host species. It is likely, therefore, that the factors that restrict disease are the nature and type of host response to infection with different viruses. We are using bovine microarrays to examine the changes to the host transcriptome during infection with rinderpest virus (RPV) or the human pathogen measles virus (MV).

Materials and Methods: We have studied the Plowright vaccine strain of RPV and compared it to the highly virulent Saudi/81 strain of the same virus. In addition we have infected cells with wild-type (Dublin isolate) measles virus. All viruses were partially purified by PEG precipitation to remove cytokines or other stimulatory molecules from the virus preparations. We have infected bovine monocyte-derived dendritic cells (moDCs), as they provide a good model for natural infection with these lymphotropic viruses.

Results and conclusions: Cells infected with MV showed rapid induction of interferon and interferon-stimulated genes as well as other innate immune responses. At later time points (8hpi, 16hpi) apoptotic genes were induced and many other effects on transcription (>600 differentially expressed genes at 8&16hpi). In contrast, the virulent RPV induced hardly any alterations in gene expression at any time point. The vaccine strain of RPV showed an intermediate picture, with no changes of gene expression at early time points (2hpi, 4hpi), but some induction of innate immune responses at 16hpi. These results suggest that there is species specificity in the ability of these viruses to avoid or suppress host innate immune responses, and this may govern the ability of the viruses to cause disease.

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POSTER: Complete nucleotide sequence of Italian Chikungunya virus

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Key words: Chikungunya virus, nucleotide sequence

1.Introduction and Objectives

In this work, we describe the complete sequence of Chikungunya virus (CHIKV) detected in Aedes albopictus (1) collected during the Italian outbreak observed in summer 2007 (ITA07-RA1, GenBank EU244823) and the E1 sequences of CHIKV isolates from human sera.

2.Material and Methods

Complete nucleotide sequence was performed employing primers previously described (2). Phylogenetic analysis was performed with MEGA 4 (3) using the neighbor-joining algorithm and the Kimura 2-parameter distance model.

3.Results

The CHIKV genomic RNA was 11788 nt long. The comparison with other CHIKV strains showed that ITA07-RA1 clustered with Indian isolates (IND-06, 99.5% nt identity) and Reunion Islands isolates (RU, 98.8% nt identity), into the ECSA genotype. Analysis of partial E1 (1044 bp, from 10264 to 11307 according to S27 strain) from mosquitoes and human sera shows 100% nt identity. In E1 region, the Italian strain shows two relevant changes (A226V and D284E), also present in >90% of later RU isolates. It was hypothesized that these mutations confer evolutionary success to the virus in its adaptation to the urban vector A. albopictus (2). Moreover, ITA07-RA1 presents the two unique substitutions noted in all of the IND06 isolates, but not in the RU isolates, both in Nsp1 region (T128K and T376M).

4.Discussion and Conclusions

The Italian CHIKV sequenced from mosquitoes and human isolates shows 100% identity. This finding may confirm the role of A. albopictus as vector of the virus in the Italian outbreak (1). Moreover, in the Italian CHIK strain the two relevant substitutions (A226V and D284E) noted in all of the IND06 isolates - but not in the RU isolates - are present. This finding may indicate that the Italian virus comes from India where the CHIKV and the disease are still endemic. This episode indicates that Italy is at risk of introducing arboviruses causing human diseases, such as Dengue, West Nile and Rift valley fever.

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POSTER: Newcastle Disease Virus (NDV): Prevailing Strains and Current Threats.

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VLA Weybridge[1]

Key words: Newcastle disease phylogeny current threats

Abstract: The phylogenetic relationships between recent Newcastle disease virus (NDV) isolates submitted to the International Reference Laboratory (IRL) at VLA Weybridge have been investigated. The isolates analysed in this study have been received from many countries since 2000 and are considered to represent current, prevailing strains of NDV. Partial nucleotide sequences of the fusion (F) protein gene has been determined for all isolates, and analysed in a dataset including reference strains from all currently defined lineages.

Method: Partial nucleotide sequences were determined for a 375bp fragment (47-422) at the 3' end of the fusion protein gene of >100 ND viruses acquired from different host, temporal and geographical origins. Phylogenetic analysis of this data was carried out by Maximum Likelihood.

Results: The phylogeny illustrates overall genetic relationships (Fig. 1) between recent AMPV-1 viruses submitted to the IRL.
All six currently defined lineages are represented.
The most prevalent lineage is lineage 5, with the majority of submitted isolates falling into this one, specifically sub-lineages 5d and 5b.
The next most prevalent lineage is lineage 4; this contains 4b; the variant PPMV-1 strains.

Conclusions: Sub-lineage 5d has caused many outbreaks in the Far East, Middle East & is moving westwards and evolving rapidly.
Sub-lineage 5b is circulating & persisting in Europe and causing sporadic outbreaks of disease.
PPMV-1 (variant NDV) viruses (sub-lineage 4b) are still causing disease outbreaks in pigeons and other species of birds throughout the world.
Lineage 4 isolates from Israel and Cyprus may represent a new sub-lineage (4e).
All isolates in lineage 1 are avirulent except the Australian isolate from 2002, which is consistent with their disease status at that time.
NDV represents a significant threat to poultry throughout the world; only by greater surveillance, education and good biosecurity will the risk of outbreaks of ND be reduced.

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POSTER: Screening for viral dectection of Aedes albopictus larvae hatched from eggs collected during a chikungunya outbreak in Italy

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IZSLER[1]; AUSL Cesena[2]; CAA[3]; Regione Emilia-Romagna[4]

Key words: Aedes albopictus (Asian tiger mosquito), chikungunya (CHIK), vertical transmission (VT).

1.Introduction and Objective

In 2007 the mosquito Ae. albopictus was involved as vector in an outbreak of CHIK in Italy. The disease affected about 250 people, mainly in the village of Castiglione di Cervia (Ravenna).

The risk of overwintering of the CHIKV is represented by infected females transmitting the virus into the eggs (VT). Experiments conducted on VT of CHIKV in Ae. albopictus produced conflicting results: it was excluded by Mourya (2), but Zhang et al. (3) isolated the virus from pools of Tiger mosquitoes born from experimentally infected mothers. Recently, the CHIKV was isolated in 2 out of 500 pools of larvae sampled in La Reunion (1).

The aim of this study was to check the presence of CHIKV in the mosquitoes progeny belongin to the area of the Italian outbreak.

2.Material and Methods

Eggs from ovitraps employed for monitoring of Tiger mosquito were collected. The ovitraps sticks were put in closed glass jars with appropriate solution and let at room temperature for 12-14 h, in order to hatch the eggs. The same sticks after first hatching were conserved for further 3-4 weeks at 4-5°C and re-processed at least other two times, to hatch diapausing eggs.

Larvae were reared at room temperature for 7-10 days, grouped in 15-20 individuals pools and submitted to RT-PCR.

3.Results

402 sticks sampled from August until October 2007 in 158 sites were processed.

A total of 8947 specimens (16% from diapausing eggs) was obtained, 101 were from Castiglione.

The specimens obtained were grouped into 590 pools and subjected to PCR, without any positive result.

4.Discussion and Conclusion

No positive PCR results were obtained, indicating that VT didn't occur in the sampled eggs. Anyway VT is a rare event in CHIK and it might not be easily detectable. For this reason our thought is that re-appearing of the disease from Ae. albopictus larvae is improbable but this event can not be completely excluded.

5.Acknowledgements

Katia Marzani and Jessica Milito (IZSLER-Reggio E).

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POSTER: RealTime (RRT) PCR testing strategies during UK avian influenza (AI) outbreaks in 2007 & 2008

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Veterinary Laboratories Agency (VLA)[1]; VLA[2]

Key words: Avian influenza, RealTime PCR

1.Introduction and Objectives

Three AI poultry outbreaks occurred in the UK in 2007: Two were H5N1 highly pathogenic (HP)AI & one was H7N2 low pathogenic (LP)AI . RRT PCR testing strategies were gauged to these distinct outbreak scenarios. These poultry outbreaks & subsequent H5N1 HPAI swan cases in 2008 demonstrate how RRT PCR provides rapid & accurate information for management of AI.

2.Material and Methods

Clinical samples were collected from poultry for (a) disease diagnosis at the originating infected premises (IP) & (b) screening within the surrounding Protection Zone (PZ). RNA was extracted robotically using a Qiagen BioRobot. AI RRT PCRs for M, H5, H7 & N1 genes are described together with standard methods for AI isolation & serology (Ref).

3.Results

Mgene RRT PCR helped diagnose the index cases. This led to prompt establishment of PZs, Surveillance & Buffer Zones as per EU AI policy. Data will be presented to summarise:

a)Disease diagnosis: Numbers of AI positives at the originating infected premises (IPs).

b)Poultry screening: Extensive testing was conducted in the Protection Zones. These include epidemiologically identified dangerous contact (DC) premises.

c)Key decisions to change from Mgene to respective H5/H7 RRT PCRs will be explained.

d)Differences in strategy between HP & LPAI outbreaks will be emphasised.

e)Environmental specimens from a H5N1 HPAI turkey outbreak also yielded positive results by H5 RRT PCR.There was a clear correlation between H5 positive RRT PCR results in the environment & prevalence of infected turkeys within a given epidemiological unit.

f)Outcomes from accompanying wild bird surveillance by AI RRT PCR will be noted.

g)AI RRT PCR use in UK H5N1 HPAI swan cases in 2008 will be included.

4.Discussion and Conclusions

Virus isolation remains the gold standard for identifying & reporting the index case from any new AI outbreak, but widespread use of AI RRT PCRs in EU labs has demonstrated advantages since 2006. The etiology & epidemiological details of these LP & HPAI UK poultry outbreaks in 2007 necessitated use of appropriate RRT PCR testing strategies.

5.Acknowledgements

The authors gratefully acknowledge the contribution of all scientific & veterinary colleagues in diagnosing & controlling these UK outbreaks.

6.Reference

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POSTER: Rapid Detection and Identification of Avian Influenza Subtypes in a Single Tube Using RT-LATE-PCR

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Key words: avian influenza, penside assay, LATE-PCR, subtyping, fast test

1. Introduction and Objectives

The spread of Avian Influenza (AI) throughout Eurasia and Africa has caused the death of millions of birds. Further, genetic changes could lead to efficient human transmission and a devastating global pandemic. Rapid detection and subtyping of AI's is needed urgently and would be greatly facilitated by a portable diagnostic device.

2. Material and Methods

Using our new technologies (LATE-PCR, PrimeSafe™) we are building a fast RT-PCR assay for field detection and analysis of the low/high pathogenic variants of AI subtypes H5 and H7. The assay includes N1/non N1 subtyping and also detects Newcastle Virus. Internal DNA and external RNA controls are present to prevent false negatives. All possible outcomes are generated in a single tube containing 7 pairs of primers and 10 fluorescent probes (4 fluors). LATE-PCR primers have different concentrations: exponential amplification of double-stranded DNA is carried out until each Limiting Primer is exhausted, followed by ten or more cycles of linear amplification that generate single-stranded amplicons. This assay can be used in laboratory cyclers but is designed to work on a battery operated point-of-care instrument (BioSeeq Vet, under development at Smiths Detection, Inc, Watford, U.K.).

3. Results

For proof-of-principle experiments we utilized viral RNA sequences transcribed in vitro from plasmid constructs. Each of these RNAs was hybridized to one of the LATE-PCR primers (RT primer) and reverse transcribed. The second primer of each set was then added to the cDNA, along with the other PCR reagents. Sets of 1-3 cDNAs, simulating different viruses, were amplified and detected based on their patterns of hybridization to the probes. Probe-to-target hybridization was determined at end-point by reading at 70/50/35oC. Our data confirmed that LATE-PCR end-point analysis is semi-quantitative. We successfully identified H5N1-like, either high or low pathogenic, RNA multiplexes. Tests on low and high pathogenic H7 are completed at the DNA level and underway at the RNA level.

4. Conclusions

In the BioSeeq Vet viral RNA isolation and reagents addition will occur in a hands-off canister atop the thermal cycler. We aim for a total elapsed time (sample-to-read out) of less than one hour.

5. Acknowledgements

Supported by Smiths Detection, Inc, Watford, U.K.

6. References

See http://www.brandeis.edu/projects/wanghlab/

POSTER: Quantification of transmission of FMDV strain Asia-1 Turkey among vaccinated and non-vaccinated lambs

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Key words: FMDV, transmission, sheep, modelling

1.Introduction

Quantified transmission parameters of FMDV are needed for epidemic models used for control and surveillance. For sheep, limited quantified information on transmission of FMDV is available while especially in sheep an FMDV infection can remain unnoticed and thus can spread unknowingly.

2.Material and Methods

We performed transmission experiments in which we quantified transmission of FMDV strain Asia-1 Turkey using a SIR model. We used 12 groups of four lambs, six groups of non-vaccinated lambs and six groups of lambs vaccinated with FMDV Asia-1 Shamir vaccine at two weeks before inoculation with FMDV Asia-1 Turkey 11/2000. From each group, two lambs were infected intranasally and the other two were contact exposed. Oropharyngeal (OPF) swabs were collected daily until 21dpi when the experiment was ended. With the final size, i.e. the total number of contact infections, we quantified the reproduction ratio R. Also, using the virus isolation results of the OPF swabs, for both groups the infectious period T was calculated using survival analysis. For the non-vaccinated group the transmission rate ß was calculated using generalized linear modeling, subsequently, R was calculated using the results of ß and T.

3.Results

In the non-vaccinated group, all inoculated lambs became infectious after inoculation. In two groups two of the contacts, in one group one of the contacts and in three groups none of the contacts became infected. In four of the vaccinated groups two inoculated lambs became infectious after inoculation and in two groups only one. None of the contacts became infected. The reproduction ratio as calculated by the final-size method for the non-vaccinated group was 1.14 but not significantly > 1 and for the vaccinated group the reproduction was 0 and significantly < 1. The estimated transmission rate, infectious periods and reproduction ratios plus 95% confidence intervals are summarized in Table 1.

4.Discussion

In the non-vaccinated group infection with FMDV Asia-1 Turkey 11/2000 resulted in an R that was close to 1 which means that mostly minor outbreaks can be expected. Vaccination with a commonly used Asia-1 Shamir strain reduced R significantly, which confirms the potential of vaccination as important intervention tool during an outbreak. The expected duration of virus shedding of the infectious non-vaccinated and vaccinated lambs was quite long. Whether or not this is strain dependent remains to be investigated.

Groups	Transmission rate (day^{-1})		Infectious period (days)		Reproduction ratio		Reproduction ratio (final size)	
	beta	CI	T	CI	R	CI	R	CI
Non-vaccinated	0.07	0.03-0.17	52	22-125	3.6	0.66-22	1.14	0.3-3.3
Vaccinated -14dpi	n.a.	n.a.	37	13-76	n.a.	n.a.	0	0-0.8

POSTER: Development of a real-time PCR for detection of Cyprinid herpesvirus 3 (CyHV-3) in carp (Cyprinus carpio L.)

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CVI[1]

Key words: Carp, Herpesvirus, KHV, Diagnostics, real time PCR

The common carp (Cyprinus carpio L.) has a long history of domestication. Culture of this species originates form East Asia and the first written description of culture dates back to 473 BC. At the present it still is an important fish for human consumption and is one of the most aqua-cultered species worldwide. In addition to this, koi carp is considered as a highly valuable species kept for ornamental purposes.

In 1998 a herpesvirus (Cyprinid herpesvirus 3; CyHV3) was isolated from diseased koi in Israel and the US. This virus is the causative agent of koi herpesvirus disease (KHVD). In subsequent years mass mortalities due to CyHV3 were reported in carp and koi cultures at global scale: e.g. US, Israel, Japan, Indonesia, Poland, Germany, UK and The Netherlands. The global trade in koi is suspected to have had a major contribution to the current worldwide spread of CyHV3.

The global emerging of the disease urged the need for specific diagnostic tools to detect CyHV3. For most fish viruses isolation of the virus on cell lines is the prescribed method for viral detection. Unfortunately, the sensitivity of the cell lines available for culture of CyHV3 is too low for diagnostic purposes. A number of PCR-based assays have been described to detect CyHV3 DNA and are now widely used for clinical diagnosis of CyHV3. The majority of these assays is based on conventional PCR techniques. The use of real-time PCR detection systems has a number of advantages for the diagnostics compared to conventional PCR assays: (i) high sensitivity, (ii) no post-PCR handling of the products and (iii) reduced risk of contamination.

Two real-time PCR assays were developed targeting the CyHV3 polymerase gene: one based on TaqMan chemistry and on SYBR green chemistry. The assays specifically detects the CyHV3 polymerase gene. No amplification was observed with the closely related herpesviruses CyHV1 and CyHV2. A similar sensitivity was observed in both real-time assays. Compared to a conventional single round PCR assay targeting the viral thymidine kinase gene both assays seem to have a higher sensitivity.
The results from the developed real-time PCR's show the potential application of the assays for rapid and real-time detection of CyHV3 in clinical and sub-clinical infections. Currently the TaqMan assay is used in our laboratory as the routine diagnostic method for detection of CyHV3. The SYBR green assay is used for confirmation of the results of the TaqMan assay.

POSTER: LATE-PCR Detection of Foot and Mouth Disease Virus (FMDV) using the BioSeeq Vet

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Brandeis University[1]; Smiths Detection[2]; Institute for Animal Health[3]

Key words: asymmetric PCR, one-step RT-PCR, RNA virus detection

1. Introduction and Objectives

Foot and Mouth Disease is caused by a positive-strand RNA virus and outbreaks in domestic animals are highly contagious and very costly. Detection of FMDV is complicated by high sequence variation between strains. Here we describe a novel pan-FMDV assay that can detect virtually all strain variants and will be used in a sophisticated field instrument, the BioSeeq Vet.

2. Material and Methods

Limiting and excess primers were designed for the relatively conserved sequences in the FMDV 3D (RNA polymerase) gene according to the criteria of Linear-After-The-Exponential (LATE)-PCR. Primers are pre-incubated with RNA targets, and then amplified using a one-step RT-PCR reaction. LATE-PCR generates an abundance of single-stranded DNA that is freely available to hybridize with a fluorescently labeled probe. The FMDV probe is mismatch tolerant and therefore able to hybridize over a range of temperatures to sequence variants. Separate probes for co-amplified RNA and DNA control sequences guard against false negatives.

3. Results

Preliminary experiments using synthetic DNA templates demonstrated that the fluorescent intensity of the probe at end point was proportional to the initial concentration of the templates over a wide concentration range, 10 to 1 million copies. Subsequent testing showed that viral RNA from each of the 7 FMDV serotypes could be amplified and then detected with the mismatch tolerant probe. All samples with unrelated viruses remained negative for FMDV signals. The non-FMDV samples and other control samples without virus showed amplification of an internal DNA control, confirming amplification. Additional tests of a modified assay with an RNA control are ongoing and will be presented.

4. Discussion and Conclusions

LATE-PCR with a single mismatch-tolerant probe insures detection of virtually all variants of the FMDV virus. The presence of both RNA and DNA control targets insures against false negatives due to inhibitors or technical failures. This quantitative-end-point assay has been designed for a field instrument, the BioSeeq Vet, enabling rapid detection of an FMDV outbreak.

5. Acknowledgements

This research is supported by Smiths Detection, Inc.

6. References

See http://www.brandeis.edu/projects/wanghlab/publications.html

POSTER: Genetic analysis of the haemagglutinin of Avian Influenza (AI) H7N1 viruses circulating in poultry during the 1999-2001 Italian epidemics

Milani, Adelaide[1]; Beato, Maria Serena[1]; Cattoli, Giovanni[1]; Capua, Ilaria[1]

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Key words: Genetic analysis, H7N1, haemagglutinin

Introduction and Objectives

From 1999 to 2001, Italy experienced four epidemic waves in poultry caused by an avian influenza virus of the H7N1 subtype. The first epidemic was caused by a Low Pathogenicity (LPAI) virus, which mutated into the highly pathogenic form (HPAI) nine months later. Stamping out and vaccination were applied to control and to eradicate the infection. However two subsequent waves of LPAI occurred prior to the complete eradication of the H7N1 virus in 2001. In the present study, phylogenetic data on viruses collected throughout the epidemics (1999-2001) are presented.

Materials and methods

Fifty-seven H7N1 viruses were selected considering: date of isolation, species and pathotype. Partial sequences of the HA1 coding region were aligned and genetically analysed.

Results

Phylogenetic analysis confirmed the existence of 2 genetic lineages co circulating prior to the emergence of the HPAI viruses, as reported previously. They were characterised by distinct additional glycosilation sites (AGS) in position 123 and 149. Following the emergence of the HPAI virus, the subsequent LPAI waves were caused predominantly by viruses belonging only to one of the two lineages.

Discussion and Conclusion

Phylogenetic and molecular analysis revealed that only one H7N1 genetic lineage became predominant and persisted until the end of the epidemic. The factors that have driven the predominance of one lineage versus the other remain unclear, and could be related to the immunological pressure, virus- host adaptation dynamics or intrinsic viral characteristics. .

References

J. Banks, et al. Archives of Virology,2001, 146: 963-973.

POSTER: Avian Influenza surveillance in wild birds in Germany

Staubach, Christoph[1]; Globig, Anja[1]; Harder, Timm[1]; Kowalczyk, Stefan[1]; Richter, Sven[1]; Unger, Fred[1]; Wilking, Hendrik[1]; Conraths, Franz J[1]

Friedrich-Loeffler-Institut[1]

Key words: Avian Influenza, surveillance, wild birds, database, Germany

1.Introduction and Objectives

As a consequence of the first outbreaks of highly pathogenic Avian Influenza (AI) H5N1 Asia in wild birds in Germany in February 2006, wild bird surveillance activities have been increased. Within the national AI wild bird surveillance, more than 69,000 samples were investigated in 2006 and more than 25,000 wild birds tested in 2007. For the management of the surveillance data and for epidemiological analysis, a web-based database was developed and established.

2.Material and Methods

The database was implemented as a protected internet website. Veterinary authorities at the local or state level can record or modify data via a web form or import data via a defined text file thus allowing to import data from the data bases of regional laboratories. A user interface enables data export as MS Excel, dBASE, PDF or XML files to facilitate the reporting e.g. to the European Commission AI database. Security mechanisms on dataset level fully reserve the rights of the competent authorities regarding data up- and download. Each sampled wild bird is assigned a single database record that includes ornithological, topographical and diagnostic data. Data visualisation through any internet browser provides table views and summary reports that can be stratified for instance by time period, NUTS levels, bird species, active or passive monitoring, virological and serological results. Automatic calculation of estimated prevalences and their confidence limits for various criteria (e.g. bird species or time series) is possible. A map server displays the surveillance data on topographical maps in different resolutions using vector and raster maps.

3.Results

Epidemiological analysis of the monitoring data revealed that the vast majority of HP H5N1 cases in wild birds were detected in birds found diseased or dead (533/31,808), while only a single infection was detected in a freshly shot bird (1/24,369).

4.Discussion and Conclusions

The surveillance database on Avian Influenza in wild birds facilitates the reporting of the results of extensive monitoring activities in the wild bird population conducted by different parties of all Federal States. However, epidemiological analysis of the results suggests that the random active surveillance in live and freshly shot birds is inefficient regarding H5N1-detection, whereas a targeted monitoring of waterfowl in Ramsar sites is efficient for the detection of low pathogenic AI viruses.

POSTER: ENTIRE GENOMIC CHARACTERISATION OF SIX LOW PATHOGENIC H5 SUBTYPE AVIAN INFLUENZA VIRUSES COLLECTED IN FRANCE BETWEEN 2002 AND 2006

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AFSSA[1]

Key words: Avian Influenza, low pathogenic H5 subtype, genotyping

1.Introduction and Objectives

H5 low pathogenic avian influenza (LPAI) virus has the potential to become highly pathogenic and to pose serious problems in both animal and public health. Surveillance and characterisation of AIV in wild and domestic species is a necessity. Previously, several French isolates from poultry were partially characterised (1). We have analysed the entire genome of 2 additional AIV isolated from decoy ducks in 2006, to acquire molecular information and to identify possible reassortment events in 3 H5N3, 2 H5N2, and 1 H5N1 LPAIV.

2.Material and Methods

The 2 recent AIV were processed as previously mentioned (1). DNA sequences were obtained and compared with LP and HP AIV entire sequences available in databases. Phylogenetic analyses were conducted using the neighbour-joining (NJ) method and the maximum likelihood method (ML). Genotype was defined for each gene according to its position on specific NJ phylogenetic trees within a cluster supported by a bootstrap value over than 80% and confirmed by ML trees.

3.Results

The 6 H5 LPAI virus genes were clustered within Eurasian avian influenza lineage. Genomic sequence of some viruses showed specific characteristics such as, insertion of 1 amino acid (aa) in the PB1 gene of a H5N3 duck isolate, the PB1-F2 protein of a H5N2 chicken isolate showing 11 aa-long instead of 90 aa-long, and for a H5N1 domestic duck isolate, the insertion of 8 aa in the NS1 gene and the I28V mutation in the M2 gene as previously reported in H5N1 HPAI viruses. All of the genes fell into at least 2 phylogenetic groups, except the matrix protein genes that were closely related each other. We identified some Danish, Dutch, Italian, and Asian H5 and H7 AIV as major donor of genes of the French viruses.

4.Discussion and Conclusions

The results gave prominence to genomic particularities and reassortant H5 LPAIV. Since all the studied isolates came from either free-range poultry or decoy ducks in contact with wildlife, these results confirmed works showing that reassortment events in the wild waterfowl reservoir occurred more frequently than previously thought (2).

5.References

1. Cherbonnel et al., 2007. Avian Dis, 51, 408-13

2. Campitelli et al., 2008. J Gen Virol, 89, 48-59

POSTER: ACHIEVEMENT OF AVIAN INFLUENZA VIRUS-LIKE PARTICLES THAT COULD BE USED AS SUBUNIT VACCINE AGAINST LOW PATHOGENIC AVIAN INFLUENZA STRAINS IN DUCKS

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AFSSA[1]

Key words: Avian Influenza, low pathogenic H5N3 subtype, Virus-like particles

1.Introduction and Objectives

In a previous work, we attempted to develop a baculovirus expressed VLP H5N3 influenza vaccine composed of three structural proteins (H5, N3 derived from a recent French LPAI virus strain, and M1 derived from an Italian LPAI virus strain) but we did not display the formation of VLPs (1). In the present study, we have changed the molecular construction to improve the expression of VLPs.

2.Material and Methods

Triple recombinant plasmids were produced as previously mentioned (1), except that the NA gene was placed under the control of the p10 promoter (2). Subcellular distribution of the 3 expressed proteins in Sf9 cells infected with the recombinant bacmids was performed by IFI. Cell supernatant was purified onto a sucrose density gradient and protein expressions were analysed by western blotting or neuraminidase inhibition assay. VLPs were visualised by electron microscopy and the presence of HA and NA proteins on their surface was investigated by immunogold labelling.

3.Results

We generated a triple recombinant baculovirus and we demonstrated that the 3 proteins were successfully expressed in rB-infected cells and were localized to the level of the cell membrane. Analyses of the purified supernatant showed that the 3 proteins were co-released and were present at the same sucrose densities. Electron microscopy examination confirmed the formation of VLPs that were morphologically identical to wild-type virus. Immunogold electron microscopy demonstrated that H5 and N3 proteins were present on VLP surface.

4.Discussion and Conclusions

We described the achievement for the first time of H5 avian influenza VLPs that should allow developing safe and effective subunit vaccines to control the spread of LPAI in ducks. We will assess the protection afforded by these VLPs in ducks following a LPAI virus challenge.

5.Acknowledgements

We thank B. Grasland and A-C. Nignol for Sf9 cell culture.

6.References

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POSTER: Development of novel serological and virological diagnostic tools for West Nile virus infection in horses

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AFSSA[1]; Institut Pasteur[2]; ENVA[3]

Key words: serology; quantitative RT-PCR; horses

West Nile (WN) fever has become a major public health and veterinary concern in Europe and the Mediterranean Basin since 1996 romanian outbreak. Clinical infections in humans and horses can range from uncomplicated WN fever to fatal meningoencephalitis, and call for active or passive surveillance of WN virus (WNV) circulation, whenever possible at every level of the transmission cycle, i.e mosquitoes, birds, horses and humans.

Confirmation of WN infection can be inferred from direct identification of WNV or from detection of anti-WNV antibodies. Recent interlaboratory assays have underlined limitations of current diagnostic tests: a poor specificity of ELISA tests due to cross-reactive antibodies between closely related flaviviruses, and a difficult amplification of some WNV strains by quantitative RT-PCR (qRT-PCR). Indeed WNV exhibits considerable genetic variations, displaying as much as 30% nucleotidic divergence. Genetically divergent strains have been detected in Europe and adapted molecular tools for the sensitive amplification of every strain of WNV is therefore highly desirable. With that aim, we designed and implemented a new protocol of qRT-PCR amplifying conserved regions of WNV genome (3' non coding region). Good sensitivity of WNV RNA detection from horse blood or brain specimens has been achieved and a multiplex protocol for simultaneous amplification of cellular and viral RNA has been developed. Evaluation of qRT-PCR specificity is in progress.

Moreover, purified WNV antigens (envelop (E), domain III of E, non structural 1 proteins) derived from bacterial or insect cell cultures were produced in order to supplement the available range of serological diagnostic tools. An indirect IgG ELISA based on E has been validated on 30 horse sera, by comparison with the gold standard "Plaque Reduction Neutralization Test".

Novel ELISA and qRT-PCR tools have been validated and should allow for standardization and improvement of detection methods for WN infection. Cell-culture derived antigens are now available for the development of additional diagnostic tools, such as competition ELISA for screening of multispecies sera.

POSTER: Risk factors of Salmonella spp. in Spanish pre-slaughtered pigs: from the fattening farm until the slaughter; preliminary results.

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Key words: risk pig salmonella slaughter farm

1.Introduction and objectives

Salmonella has been recognised as one of the most important foodborne pathogens(1)Salmonellosis outbreaks have been associated with the consumption of pork.This has stimulated studies reporting risk factors along the food chain(2).Positive Salmonella finishing pigs in farms increased the risk of asymptomatic intestinal carriage of Salmonella by pigs at slaughter(3).In these cases contamination of carcasses may occur(4).In a study,trailers used from swine transport and the holdings of slaughters were contaminated(75% and 100%)before they get in contact with pigs(5).Our aim is to identify and quantify the risk factors of Salmonella in fattening pigs until the slaughter.

2.Material and Methods

Our study involves 15 Spanish farms.Pigs are in the fatteninghouse from 20 to 110kg.We collect samples from pens,water,feed,environmental,farmer, from the trailer(before loading and after unloading)and from the slaughter.We use standard method ISO 6579 to isolate salmonella.

3.Results

422 samples have been analysed.Salmonella has been isolated from 107 of them.The most contaminated places were:environment(32,7%)farmers(28,6%) andvectors(28,6%)(Fig1).In Fig 2,we show the positive samples from the farm to the lairage room.During the fattening period,faecal samples tested positive were 18%(Fig2)This percentage rises to 39%when the animals are loaded.We find Salmonella in 50%of cleaned and disinfected trailers. In the slaughter, before getting in contact with the unloaded pigs,the percentage of positive samples has been 96,7%.Trailer samples after unloading are positive in 92,3% of cases.

4.Discussion and Conclusions

A Salmonella-contaminated farm environment contributes to the persistence of Salmonella infections on the farms(2,6)Positive faeces increase during transport(5)The presence of Salmonella spp. in trailers is an important risk factor(4).When pigs reach the slaughterhouse,they found it contaminated.The lairage is an important risk factor(7).Our results emphasise the need for control measures in farm, transport and slaughter.

5.Acknowledgements

Thanks

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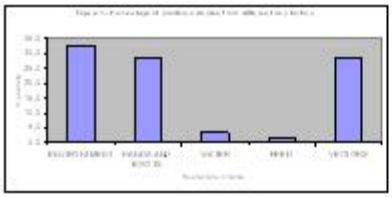

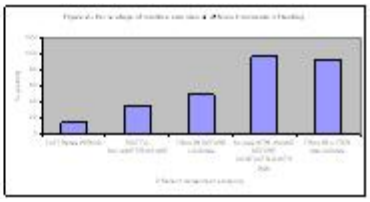

POSTER: Bluetongue virus (BTV)-8: Cloning of full-length genome segments NS1, NS2, NS3, VP4, VP6, and VP7

Petkovic, Sonja[1]; Hoffmann, Bernd [1]; Keil, Günther[1]; Vahlenkamp, Thomas[1]

Friedrich-Loeffler-Institute[1]

Key words: BTV-8 genome

1.Introduction and Objectives

Bluetongue (BTV) is an insect-transmitted viral disease of ruminants. BTV genome is composed of ten segments of ds-RNA which encode seven structural (VP1-VP7) and three non-structural (NS1-NS3) proteins (Mertens, 1989; Roy, 1989, 2005). Twenty-four distinct serotypes have been identified (Erasmus, 1990). The objective of the project is to gain more insights into the pathogenesis and to generate vaccines with cross-protecting properties.

2.Material and Methods

BTV GenBank® alignments were used to design primers compatible to the 5' (GUUAAA) and 3' (ACUUAC) ends of all ten RNA segments. Primer also contained recognition sites for EcoRI, PstI, HindIII, and BamHI.
BTV-8 RNA was prepared and used for RT. Amplification was done with Platinum® Pfx DNA polymerase using 30 cycles with 94°C for 30 sec denaturation, 43 to 55°C for 30 sec annealing and 68°C for up to 3 min amplification. PCR products were ligated into the vector pX8ΔT.

3.Results

We amplified BTV-8 full length genome segments encoding the non-structural proteins NS1, NS2, NS3 and the structural proteins VP4, VP6, and VP7. PCR products of about 1769 bp (NS1), 1124 bp (NS2), 690 bp (NS3), 1981 (VP4), 1046 (VP6), and 1156 bp (VP7) were cloned into pX8ΔT. Enzyme digest confirmed the specificity of the cloned products.

4.Discussion and Conclusions

We cloned all segments encoding the non-structural proteins NS1, NS2, and NS3 which are involved in viral replication, the formation of inclusion bodies, and viral release, respectively. Among structural proteins, we cloned VP4, VP6, and VP7 which display among others functions viral capping and helicase activities, and are part of the viral capsid, respectively. We currently work on the highly variable capsid proteins VP2 and VP5 (Bonneau, 2001). Especially VP2 will be of interest as it harbours most of the neutralization epitopes.

5.Acknowledgements

We thank S. Finke for providing the plasmid pX8ΔT and K. Wink for the technical assistance.

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POSTER: Structure and sequence motifs of siRNA linked with in vitro down-regulation of morbillivirus gene expression

Servan de Almeida, Renata[1]; Keita, Djénéba[1]; Libeau, Geneviève[1]; Albina, Emmanuel[1]

CIRAD[1]

Key words: siRNA, RNAi, morbillivirus, sequence motifs, secondary structure, target sequence selection

The most challenging task in RNA interference is the design of active small interfering RNA (siRNA) sequences. Numerous strategies have been published to select siRNA. They have proved effective in some applications but have failed in many others. Nonetheless, all existing guidelines have been devised to select effective siRNAs targeting human or murine genes. They may not be appropriate to select functional sequences that target genes from other organisms like viruses. In this study, we have analyzed 62 siRNA duplexes of 19 bases targeting three genes of three morbilliviruses. In those duplexes, we have checked which features are associated with siRNA functionality. Our results suggest that the intramolecular secondary structure of the targeted mRNA contributes to siRNA efficiency. We also confirm that the presence of at least the sequence motifs U13, A or U19, as well as the absence of G13, collaborate to increase siRNA knockdown rates. Additionally, we observe that G11 is linked with siRNA efficacy. We believe that an algorithm based on these findings may help in the selection of functional siRNA sequences directed against viral genes.

POSTER: Development of real-time RT-PCR assays for the detection of peste des petits ruminants virus

LIBEAU, Geneviève[1]; KWIATEK, Olivier[1]; KEITA, Djénéba[1]; KAMISSOKO, Badian[2]; ALBINA, Emmanuel[1]

CIRAD-BIOS[1]; LCV[2]

Key words: PPRV; Q RT-PCR;

1.Introduction and Objectives

Peste des petits ruminants virus (PPRV) is the etiological agent of a serious, often fatal, disease of sheep and goats (1) shown to be different from rinderpest virus (RPV) and other members of the genus Morbillivirus (2). RT-PCR has been applied successfully to the diagnosis and the molecular epidemiology of PPRV (3, 4). In this study we report the determination of PPRV RNA abundance performed with a TaqMan probe and a pair of primers designed for the PPRV nucleoprotein (N) gene for rapid, sensitive and specific diagnosis of PPRV.

2.Material and Methods

These assays targeted the N genes of PPRV using the same region than for previously published primer pairs of the current RT-PCR (3) and both types were compared. A primer set for ribosomal sub-unit 18S RNA was employed as an indicator of the RNA integrity. Total RNA was extracted from all samples according to NucleoSpin®RNA Virus kit procedure (Macherey-Nagel). Enhanced sensitivity was found using mastermix One Step-RT" AgPath-ID™ (Applied Biosystems) that contains ROX for signal normalization.

3.Results

Real-time PCR was conducted on all samples to identify those positive for viral RNA. A standard curve was produced to determine the absolute copy number of the virus in relation to the starting amount of total RNA. The relationship between Ct values and RNA concentration was linear within a range of 10–106 RNA copies/reaction. The real-time assay detection limits were significantly lower than those of standard RT-PCR and could detect RNA from strains representing each lineage of PPRV. No false positives were identified when testing other morbilliviruses.

4.Discussion and Conclusions

Real-time RT-PCR must support routine PPR laboratory surveillance as well as research projects. It will be linked to the robotisation of RNA extraction in order to improve diagnostic capabilities.

5.Acknowledgements:

EU Markvac project of the Community's 6th Framework Program.

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POSTER: Confirmatory tests for Foot-and-Mouth Disease DIVA serology based on profiling of antibodies to non structural proteins

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Key words: foot-and-mouth disease, DIVA, confirmatory test, NSP profiling serology

Introduction

The vaccinate-to-live policy for foot-and-mouth disease (FMD) is based on the availability of validated screening assays to differentiate vaccinated from infected animals based on the detection of antibodies to FMDV non structural proteins (NSP). The need of confirmatory tests for NSP-serology has encouraged this study for the determination of serum profiles against multiple NSP.

Materials and Methods

Indirect ELISAs for the measurement of antibodies to the NSP 3A, 3B and 3D of FMDV were developed using monoclonal antibodies to trap the respective recombinant antigens. Diagnostic performances were studied using known sera derived from experimental cattle with a know FMD status. Data were analysed in comparison with results of a validated 3ABC-trapping ELISA (1).

Results and discussion

Early seroconversions against 3A, 3B and 3ABC were similar, while antibodies to 3D occurred two days earlier; however, antibodies to 3B were low or not detectable in few animals. Duration of antibodies was evaluated up to 400 days after infection: results showed that antibodies detected by the 3ABC-ELISA were the most persistent, followed by antibodies detected by 3A-ELISA, while antibodies detected by 3B- and 3D-ELISA decayed more rapidly. Specificity for 3A-ELISA was 98.3%, sensitivity reached 100% in infected cattle and 83% in cattle that were vaccinated prior to infection. These sensitivity estimates are similar to those found for the validated 3ABC-trapping ELISA, consistently with the good correlation observed between the two tests. The specificity for 3B-ELISA reached 99%, whilst sensitivity was significantly lower in both categories of infected (75%) and vaccinated/infected (72%) cattle, possibly due to either “3B-non-responder” cattle or to a faster decay of antibodies to 3B.

Further research is needed to improve sensitivity and specificity of the 3D-trapping ELISA, Conclusions

3A- and 3B-ELISA may be considered as prototype NSP-ELISAs useful for NSP-Ab profiling. They do not reach individually the diagnostic performance of the 3ABC-ELISA; however, combined in a “profiling test” comprising multiple antigens, they may have interesting applications, included a potential use as confirmatory system.

Acknowledgements

EU projects FMD_ImproCon, Epizone, National grant Ministero Salute PRC99004

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POSTER: TRANSCRIPTIONAL RESPONSE IN THE TONSILS AND RETROPHARYNGEAL LYMPH NODES OF PIGS AFTER ORONASAL INFECTION WITH CSFV STRAINS RIEMS AND EYSTRUP

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Central Veterinary Institute of Wageningen UR[1]; Animal Science Group[2]; Animal Sciences Group[3]

Key words: CSFV, virulence, transcriptional response

1.Objectives.

The transcriptional response in tonsils and retropharyngeal lymph nodes of pigs oronasally infected with CSFV vaccine strain Riems and highly virulent strain Eystrup was studied using cDNA microarrays.

2. Material and methods.

Two groups of 6 pigs (6 weeks old) were housed in separate stables and oronasally infected on day 0 with 105 TCID50 of CSFV vaccine strain Riems or with strain Eystrup. In a separate stable 8 additional pigs were mock infected at day 0. At 2, 4, 6 dpi 2 pigs per group, with the same genetic background, were sacrificed to collect tissue samples. All EDTA blood and tissue samples were tested for the presence of CSFV RNA by real-time PCR.

Using a home-made cDNA microarray prepared from porcine spleen and jejunum tissue mRNA expression levels in RNA pools prepared from the tonsils or lymph nodes collected from the two infected pigs in each group that were slaughtered at the same day (n=2) were all compared to the mRNA levels in an uninfected reference pool prepared from homologue tissues collected from all eight control pigs (n=8).

3. Results and Discussion.

Pigs infected with virulent CSFV strain Eystrup rapidly developed fever (≥40 °C) and symptoms of acute CSF from 3 dpi until the end of the experiment (6 dpi). All blood, tonsil and lymph node samples collected from Eystrup infected pigs were tested positive for CSFV RNA by RT-PCR. In contrast, pigs infected with avirulent vaccine strain Riems showed no signs of CSF nor developed fever. All tonsil and lymph nodes collected from Riems infected pigs at 4, 6 and 12 dpi were tested positive for CSFV RNA. All pigs in the control group showed no signs of disease and were negative for CSFV RNA in all tests.

Microarray comparisons detected several clusters of genes for which mRNA expression was specifically regulated by replication of CSFV in the tonsils and lymph nodes. QRT-PCR of five selected genes confirmed the expression data of the microarray experiments. Regulated genes were functionally clustered in dsRNA mediated antiviral response genes, IFN-γ induced genes, genes involved the complement system and differentiation/maturation of B-cells, and genes involved in the metabolism of glutathione. Functional differences in gene expression patterns were observed between Riems and Eystrup. Biological significance of these differences are discussed in relation to CSFV pathogenesis.

POSTER: Onset and kinetics of antibody responses against the neuraminidase N1 and the M2e peptide in chickens after infection with two different LPAIs

Marche, Sylvie[1]; Lambrecht, Benedicte[1]; Descamps, Francis[2]; Saelens, Xavier[2]; Van Borm, Steven[1]; van den Berg, Thierry[1]

veterinary and Agrochemical Research Institute[1]; Ugent[2]

Key words: influenza, low pathogenic, neuraminidase, M2e, DIVA

1. Introduction and Objectives

A good control and prevention strategy based on vaccination also need a good surveillance plan of the vaccinated birds with DIVA tools. Tests based on the detection of the anti-neuraminidase antibodies seem promising and for this reason we decided to develop a test based on the inhibition of the neuraminidase activity. The test was evaluated on birds either infected with a H3N1 A/duck/Belgium/02216/06 or H7N1 A/ck/it/1067/v99 LPAIs and the kinetics of the antibodies production against the N1 neuraminidase protein was established using this test. This kinetic was compared with the production of antibodies against the M2e peptide.

2. Material and methods

The N1 neuraminidase inhibition test was developed in the laboratory (S. Marché, unpublished data) and L. Lambrecht developed the ELISA M2e (Lambrecht et al. 2007).

3.Results

In the H3N1 infected birds, no M2e antibodies and only small amounts of N1 antibodies were detected. In the H7N1 infected chickens, the antibody responses were rather high against the two proteins but the production of antibody against M2e peptide was rather short and disappeared rapidly compared to the kinetic of antibodies against N1 neuraminidase.

4.Discussion and conclusions

Our results indicate difference in expression of the N1 protein and M2e peptide according to the virus. Furthermore, the kinetics of production of the M2e and N1 antibodies is quite different. Therefore, the detection of antibodies against M2e could be a good tool to detect recent infection with avian influenza whereas the neuraminidase inhibition test could be used as a more sustainable DIVA test.

5.Acknowledgements

We would like to thank M. Sayouti and M. Boschmans for their excellent technical assistance. This study was funded by the Federal Agency for the Safety of the Food Chain.

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POSTER: Development of an antigen capture ELISA for detecting Rabbit Hemorrhagic Disease Virus

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Key words: Rabbit Hemorrhagic Disease Virus,ELISA ,McAb

1.Introduction and Objectives

Rabbit haemorrhagic disease (RHD) is an acute fatal disease of rabbits, which cause high economic losses in rabbitries as well as high mortality in wild rabbits. There are great significances to estabilsh a rapid,sensitive and specific method for the diagnose .In this test , An antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA)for detecting rabbit hemorrhagic disease (RHD)virus was developed to detect RHDV.

2.Material and Methods

BALB/c mices were immuneized with RHDV-TP strain. Six strains of hybridoma were obtained,one of which named DE2 with high titre and affinity was used as capture antibodies to establish a ELISA method.

Ag-ELISA for detecting RHDV was developed with McAb DE2 to capture virus ,while the rabbit anti-RHDV serum was used as the second antibody to identify virus. Working conditions of the Ag-ELISA were optimized and its capabilities were evaluated.

3.Results

Hybridoma AD4、AG10 、BC9 、BE8、 BH3 、DE2 were obtained and their antibodies titres in ascites ranged from 1:4000 to 1:3×104 in ELISA tests. McAb DE2 with high titre and affinity could cohere with a linear epidope stable,which showed McAb DE2 could be as a diagnostic reagent.

the optimum working concentration of McAb was 1μg/mL and that of rabbit anti-RHDV serum was 4μg/mL. 67 liver tissue samples from probably infected rabbits in local farms were detected. 62.7% of 67 liver tissue samples had positive results in contrast to 55.2% by HA test. The detection limit of this assay is 26ng/mL to purified RHD virus.

4.Discussion and Conclusions

The Ag-ELISA based on the McAb-DE2 could detect RHDV fome liver samples infected by RHDV correctly. The Ag-ELISA displayed excellent specificity ,sensitivity and repeatability .Also it's easy to operate and could save time and labour. Ag-ELISA was confirmed a excellent method for rapid diagnosis of RHD. In addition, 6 strains hybridoma cell secreting MAbs of RHDV stably obtained in this test would be used in the related study of RHDV ,suchi as screening the epitopes and discriminate detection.

5.Acknowledgements

Thanks to PH.D. Liu Huai-ran for Providing RHDV TP strain.

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POSTER: Investigations on Mx induction in bovine, human, and feline cells after treatment with different type I interferons

Neumann, Stephanie[1]; Wink, Kerstin[1]; Vahlenkamp, Thomas[1]

Friedrich-Loeffler-Institute[1]

Key words: IFN, bovine, human, feline

1.Introduction and Objectives

Type 1 interferons (IFN) are crucial in the innate immune response against viral infections. Both, antiviral and immunomodulatory functions are described. Human and feline type I IFN are commercially available. The objective of the study is to analyse the cross species reactivity of bovine, human and feline type I IFN in cells of bovine, human, simian, canine, and feline origin.

2.Material and Methods

Cells of bovine (Madin-Darby bovine kidney, MDBK), human (human rectum adenocarcinoma, HRT-18), simian (embryonic African green monkey kidney, MA104; African green [vervet] monkey kidney, Vero), canine (Madin-Darby canine kidney, MDCK), and feline (early whole embryo, KE-R; Leopard epithelial kidney, Leo) origin were grown to confluency and treated with dilution ranging from 10000 to 10U of recombinant bovine IFN-α, human IFN-α, and feline IFN-ω. Cells were harvested after 24h and analyzed by Western Blot for the expression of Mx using the anti-Mx antibody M143 (Flohr et al. 1999).

3.Results

Western Blot analysis revealed that bovine IFN-α and feline IFN-ω induced measurable Mx expression only in cells of bovine and feline origin, respectively. Surprisingly human IFN-α showed a broad range of Mx induction as protein expression was measured in cells of human, simian, bovine, canine, and feline origin.

4.Discussion and Conclusions

Type 1 IFN is currently been used in treatment regimen against some viral infections in humans. Due to the receptor specificity IFN usually are species specific (Müller-Doblies et al. 2002). This was confirmed for the recombinant bovine and feline IFN investigated. In contrast, the human IFN-α showed to have a broad range of cross-species reactivity.

5.Acknowledgements

We thank G. Keil (FLI, Germany) for providing the recombinant bovine interferon-α and G. Koch (University Freiburg, Germany) for providing the anti-Mx antibody M143.

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POSTER: An overview of experimental transplacental transmission of foot-and-mouth disease virus

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Key words: Foot-and-mouth disease virus, transmission, sheep

There are field reports of foot-and–mouth disease virus (FMDV) causing severe abortion in sheep. The lack of previous experimental data regarding the possible transmission of FMDV transplacentally, its role in causing abortion and the potential of the foetus and associated fluids to act as a reservoir of infection has hindered understanding of the disease. Our recent study demonstrated that FMDV can cause transplacental transmission in sheep at 45, 75 and 90 days gestation, causing foetal death. But no histopathological lesions were detected in the foetuses or placenta. Infectious virus and viral RNA were isolated from amniotic fluid and foetuses from 2 dpi onwards. Using ISH, viral RNA was visualised in tongue epithelium, myocardium and skeletal muscle. Infectious virus was still present in foetal fluids collected at 18 dpi, indicating that the potential for FMDV-induced abortion to cause further disease transmission, an epidemiological factor previously overlooked. In exploring the persistence of FMDV in foetal lambs, viral RNA was detected in foetal tissues at 42 dpi but no infectious virus was isolated from these tissues. Further studies are required to characterise the pathogenesis in detail.

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POSTER: Optimisation of viral vectors for oral vaccine delivery

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UMR Virologie INRA-Afssa-ENVA[1]; Mayo Clinic College of Medicine, Departments of Medicine and Immunology, Division of Infectious Diseases [2]; UMR Immunology Cancerology[3]; R&D Laboratory of the Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois[4]

Key words: oral, vaccine, vector, adenovirus, dendritic cell

Orally delivered vaccines are highly desirable for countering infectious diseases of domestic animals. Upon oral vaccine delivery, it is probable that the induction of immune responses (IR) is limited both by the extent to which the digestive epithelium is breached and by the efficiency of antigen capture and presentation by dendritic cells (DC). By consequence, the tropism of a vectorised vaccine has important implications for vaccine efficacy. In an effort to optimise the tropism of vaccinal vectors, we have adopted a strategy based on recombinant adenoviruses (Ad) designed so as to facilitate retargeting. In such vectors, one of the viral proteins, the fibre, is metabolically biotinylated subsequent to the insertion of a biotin-acceptor peptide (bap). This modification permits coupling of Ad to almost any ligand of interest, via an avidin bridge, so long as the ligand can be biotinylated. The tropism of such AdF-bap can be modified in an almost limitless fashion, and thus facilitate assessment of the utility of retargeting Ad toward different populations of cells as regards induction of IR.

Initially, the retargeting of AdF-bap towards intestinal epithelial cells (EC) and DC will be evaluated in models elaborated in vitro using either biotinylated antibodies directed against receptors present at the apical pole of intestinal EC or at the surface of DC, or biotinylated versions of the natural ligands of these receptors, when these are available. An AdF-bap vector expressing the marker protein luciferase will be used to study how digestive mucosa is breached in polarised monolayers of intestinal EC. An AdF-bap vector expressing GFP (« green fluorescent protein ») will be used to evaluate the efficacy of gene transfer in DC by flow cytometry.

The interactions between Ad retargeted towards selected receptors of the digestive epithelium and DC of the intestine or the draining lymph nodes will then be explored in vivo, initially after oral delivery in mice, and compared with their capacity to elicit local and systemic IR. To this end, the construction of Ad bearing wildtype or biotinylated fibers and expressing one of two model antigens, the C fragment of the tetanus toxin (TTFC) or chicken ovalbumin (ova), are underway. Ad expressing TTFC, a potent mucosal antigen, will be used to establish a proof of concept, while Ad expressing ova, for which a large number of immunological tools are available, will be used for mechanistic studies.

ORAL: CSF Vaccine & wild boar: a DIVA approach

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[1]Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, [2]Joint R&D Division in Virology, The National Veterinary Institute & The Swedish University of Agricultural Sciences, [3]Friedrich Löffler Federal Research Institute for Animal Health

Classical swine fever (CSF) is a recurring disease of domestic pigs in Europe, mainly due to re-introduction from wild boar reservoir of the disease. Current strategies for control of CSF in wild boar are hunting and vaccination with C-strain. However this vaccine does not allow differentiation from wild type viruses. For a better control of CSF in wild boar, an EU project "CSF Vaccine & wild boar" has been granted, supporting development of a marker vaccine and acompanying diagnostic assays. Based on marker vaccine (CP7_E2alf) nucleotide sequence, gel-based and real-time RT-PCR assays have been developed and evaluated for differentiation of infected from vaccinated animals (DIVA). Both DIVA assays are highly specific and sensitive. As the vaccine virus replicates much less efficiently compared with wild type viruses in vivo, a two-step protocol has been proposed for earlier detection of vaccine virus and differentiate it from wild type viruses. Such an approach is useful in controling CSF in wild boar where a marker vaccine is available.

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