EPIZONE

New generation researchers in pig viral diseases
Building bridges from labs to policy and the farm

Madrid, 12-14 July 2010

Abstracts, Conclusions and Recommendations

EUROPEAN COMMISSION
European Research Area

ConFluTech

The workshop *"New generation researchers in pig viral diseases: building bridges from labs to policy and the farms"* was held in Madrid from 12th to 14th July 2010 hosted by the Faculty of Veterinary Medicine of the Universidad Complutense.

This workshop is a follow up of the successful meeting held on 7th - 9th July 2008 in Shanghai *"Research in Swine Viral Diseases Building Bridges"* (http://www.epizone-eu.net/Paginas/EPIZONE%20Downloads/Brochure%20Building%20Bridges.aspx)

The workshop gathered a large number of young and senior scientists working in swine viral diseases from European countries, P.R. of China and also from Russia.

The purpose of the workshop was to allow young scientists to interact and exchange views among them and with senior scientists on their research activities in a relaxed and fruitful atmosphere. The spirit of the meeting was to raise awareness among the young researchers of the importance of the research carried out in the laboratories for generating knowledge and tools to detect, prevent and control viral diseases which devastate pig production and trade in different parts of the world. In this context the setting of the scene was made by Dr Kazuaki Miyagishima, Deputy Director General and Head of the Scientific and Technical Department of the World Organisation for Animal Health (OIE), Dr Juan Lubroth Chief Veterinary Officer of the Food and Agriculture Organisation (FAO) and Dr Shishan Yuan Director of the Department of Swine infectious Diseases of the Shanghai Veterinary Research Institute. The need for collaboration and coordination of researchers and research funders at international level was also highlighted given the challenges faced by animal production at global level and the limited funds for research. To cover this aspect, Dr Alex Morrow from the Department of Environment, Food and Rural Affairs presented the ERA-NET EMIDA, a scheme currently on-going in the EU to coordinate research funders in animal health and the future network STAR-IDAZ which will pave the way for a coordination at international level.

The workshop was divided into different sessions focused on specific diseases or group of diseases of major concern in particular PRRS, ASF, FMD, CSF, PCV2 swine influenza. Other diseases, some of them zoonotic, were also included in the agenda such as those caused by, hepatitis E, Japanese encephalitis, parvo, pox, corona, paramyxo, citomegalo and picorna viruses.

As for Shanghai, the workshop in Madrid is the result of the cooperation and coordination between the Institutes of the CAAS and relevant Chinese Universities, the Directorate General for Research of the European Commission and EU supported research projects in FP6 and FP7 in particular the EU funded Network of Excellence for Epizootic Disease Diagnosis and Control EPIZONE[1], ConFluTech[2], ASFRISK[3], PRRSCon[4], CSF go_DIVA[5], FMD-DISCONVAC[6], FLUPIG[7], PCVD[8] and EMIDA[9].

I would like to acknowledge the support of young colleagues Petra van der Laag from EPIZONE and Dr Eefke Weesendorp from YOUNG EPIZONE and the young "SUAT" team of the Faculty of Veterinary Medicine of Madrid for the remarkable deployment of efforts during the workshop and the contribution to its excellent atmostphere. I would specifically like to thank Raquel Vargas for her outstanding dedication to ensure the

best logistics and social events in Madrid for all the participants in the most economic way. Equally, I would like to thank our young researchers from Europe, P.R. China and Russia for their participation hoping that they have made connections and that they will maintain them in view of future collaborations.

I would also like to acknowledge the contribution, enthusiasm and young spirit of the senior scientists and their readiness to share their knowledge and experience with the younger generation. Those who have recently retired or are about to retire should not forget that there they are needed. We count on them. They are "real public goods".

Special gratitude to Prof Jabbar Ahmed, Prof Hong Yin, Dr Shishan Yuan and Prof Hans Nauwynck for their help in the preparation of the workshop and to Dr François Madec for the final "touch" of the workshop to guide us on the research needs to face the challenges of pig production and where the younger generation of scientists and research funders should target their efforts.

I want to take this opportunity to thank our colleague from Nashville, Tennessee, Dr Kitty Parker, with whom I shared the university benches in the 70s, who kindly provided us with the picture of the happy pigs for the poster of the workshop.

Finally, I would like to acknowledge the support of Prof Joaquin Goyache, Dean of the Faculty and of Prof José Manuel Sanchez-Vizcaino who demonstrated once more that serious work is more productive in a friendly and trustworthy atmosphere.

I am confident and hopeful that we can give a continuation of this type of events in the coming years.

Isabel Minguez Tudela, DVM, PhD
Principal scientific officer
Research Directorate General
Directorate of Biotechnology, Agriculture, Food
European Commission

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[1] EPIZONE FOOD- "Network of Excellence for Epizootic Disease Diagnosis and Control" http://www.epizone-eu.net/default.aspx
[2] ConFluTech: SSPE-CT-2006- 044462 "Capacity building for the control of avian influenza through technology transfer and training"
[3] ASFRISK: GA 211691 *"Evaluating and controlling the risk of African swine fever in the EU"* http://www.asfrisk.eu/
[4] PoRRS-Con: GA 245141*"New tools and approaches to control Porcine Reproductive and Respiratory Syndrome (PRRS) in the EU and Asia"*
[5] CSFV_goDIVA: GA 227003 *"Improve tools and strategies for the prevention and control of classical swine fever"* http://www.csfvaccine.org/
[6] FMD-DISCONVAC: GA: 226556 *"Development, enhancement and complementation of animal sparing, foot-and-mouth disease vaccine-based control strategies for free and endemic regions"* http://fmddisconvac.net/
[7] FLUPIG: GA 258084 *"Pathogenesis and transmission of influenza viruses in pigs"*
[8] PCVD: Contract FOOD-CT-2004-513928: *"Control of porcine circovirus disease (PCVDs): towards improved food quality and safety"* http://www.pcvd.org/
[9] EMIDA: 219235 *"Coordination of European Research on Emerging and Major Infectious Diseases of Livestock"* http://www.emida-era.net/

Begoña Valdazo-González

Institute for Animal Health (United Kingdom)

Transmission pathways of FMDV using full genome sequences
She showed how the analysis of complete FMDV genome sequences from the 2001 and 2007 outbreaks in UK helped to identify sources and transmission pathways of the infection and predict undisclosed infected premises. Laboratorial and analytical methods to use the full-genome sequencing approach for fine-scale epidemiological investigation of FMDV epidemics are currently being developed in collaboration with different entities from Turkey and Malaysia. Preliminary results encourage the idea that this approach can be used in endemic countries. Further collaboration with Tanzania and Chine are expected to perform the current protocols.

Aldo Dekker

Central Veterinary Institute Lelystad (The Netherlands)

FMD the global dimension
Aldo gave an overview of the history of FMD control in Europe leading to the status freedom without vaccination and the FAO / OIE initiative to set up regional roadmaps to achieve similar results in other regions with the ultimate aim to control FMD world-wide. At a national level the progressive control pathway can be used to monitor progress. The OIE is considering to adopt the several stages of the progressive control pathway in their regulations. This initiative will lead to new (or often old) research questions. Several examples were given and the discussion was started how researchers could contribute to this initiative.

David Levebvre

Veterinary and Agricultural Research Institute (Belgium)

Validation of antibody-based methods to Refine, Reduce and Replace animal testing in foot-and-mouth disease research
David presented data on validation of antibody assays to replace challenge experiments in cattle. These studies are currently funded by the European framework 7 programme with the project FMD DISCONVAC. It was shown for type O that different types of serological tests are at least as precise as the *in-vivo* vaccine potency tests and could thus replace the *in-vivo* test. He showed how the validation principles from the OIE can be used in trying to standardise these tests, further work is necessary to get similar results for the other FMD serotypes. In the discussion it was mentioned that the standardisation was done only for a standard fresh vaccine, it was not clear if the same would also apply if vaccine 146S antigen would disintegrate into 12S antigen. More research is needed on this issue, although the results of the study in Argentina might be used as there was indication that the shelf life of that vaccine was limited. The results showed lower protection 11 months after production of the vaccine.

Discussion

The general discussion focussed on the what kind of research would be necessary in the future. Although the chairman indicated that research on the social economic impact in developing countries is surely needed to convince local farmers to invest in control programmes, most participants were thinking about production of other types of vaccines. Not all participants were knowledgeable on FMD and some suggested solutions, like peptide vaccines, that have been showed not very effective. Vaccines that contain the whole virus capsid seem to be the only effective candidates. So this means inactivated virus particles or empty capsids produced in other systems outside the host, like baculovirus, or by the host, like the adenovirus recombinants. In the discussion which species should be vaccinated, it became clear that this would depend on the local situation when applying prophylactic vaccination. In Europe e.g. only cattle was vaccinated, but in Taiwan pigs had to be vaccinated to prevent spread of the disease. But it was clear that it would be wise to vaccinate all susceptible species when a ring vaccination was applied around an outbreak.

African swine fever (ASF)

African swine fever (ASF) is a lethal, hemorrhagic disease of domestic pigs and has serious socio-economic implications, affecting the livelihoods of pig keepers in affected countries. The disease also has adverse effects on both regional and international trade and food security. The epidemiology of ASF is complex and the ASF virus has been maintained in eastern and southern Africa for an unknown period of time, in an ancient sylvatic cycle involving soft ticks (*Ornithodorus* genus) and asymptomatic infected warthogs and bushpigs and red river hogs, (*Potamochoerus spp.*). Two additional cycles have been described in endemic areas, namely a domestic pig/tick cycle, without warthog involvement, and a domestic pig/pig cycle in which the virus persists in domestic pigs in the absence of other vertebrate or invertebrate hosts. Epidemiologically is well established that the entrance of ASF virus in a free country is related to the entrance of infected or carrier animals as well as of uncooked infected pork through international ports or airports where garbage containing uncooked pork can be found and used for pig feeding. Once ASF is established in domestic swine, infected carrier-animals are the most important source of virus dissemination for susceptible pigs.

Summary of the presentations of:

José Manuel Sánchez-Vizcaíno

Complutense University of Madrid (Spain)

The last adventure, So far

Carmina Gallardo

Complutense University of Madrid (Spain)

A general view on ASFV molecular epidemiology and diagnostic Research

Alexander Malogolvkin

National Research Institute for Veterinary Virology and Microbiology (Russia)

Current ASF epidemiology in Russia

Currently, ASF is endemic in the majority of sub-Saharan Africa. Within Europe was confined to Sardinia until June 2007 when ASF was notified in Georgia. The ASFV responsible of this outbreak was closely related to East-African genotype II viruses instead to genotype I historically circulating in Europe. Since 2007, ASF progressed to neighbouring countries Armenia, Azerbaijan and Russian Federation, reaching the near border with Ukraine. During the period of 2007-2010 on the territory of South-Russian and Northern Caucasus regions a total of 122 cases of the disease has been declared both in domestic and wild boar and this confirms that endemic foci are developing in given region.

Current situation of ASF threatens the EU countries, Eastern Europe, the Black Sea basin countries and – in the worst case scenario – central Asia and even China, which has the largest pig population in the world. Since there is no vaccine currently available rapid laboratory diagnosis is essential in control strategies. But, are we ready to combat with ASF? Would current diagnostic techniques be sensitive enough taking into consideration the new circulating genotypes in Europe?

From 2000 to date, reliable, specific, and fast PCR tools have been developed and validated for virus detection. They have been shown to be highly sensitive for the detection of the current circulating isolates in Europe and Africa. However, serological surveillance studies performed in East Africa among 2004-2009, have shown low seroprevalence with, in contrast, a high incidence of virus in domestic pigs. This could be related to genome variability of antigenic ASF proteins in Eastern African isolates, the more variable and genotypically distant. To what extent current serological diagnostic techniques might be missing some of these new variants?

To answer this question a recent study has been focused on the development of new serological techniques based on different ASFV selected on the basis of genome variability criteria and taking into consideration the current European circulating genotype II. A comparative study through the analyses of a wide variety of samples collected in different epidemiological situations has been done. The results obtained could be the first approach demonstrating the capability of the formal diagnostic techniques to perform a serological diagnosis with high sensitivity, specificity and confidence, adapted to current epidemiological situations.

Porcine reproductive and respiratory syndrome (PRRS)

During two sessions (Session 1: Pathogenesis and Immunity and Session 2: Epidemiology and Control) several European and Asian scientists gave the results of their recent work on different aspects of PRRS. An overview of the contributions, given during the two sessions, and the combined discussions/ conclusions are summarized.

Pathogenesis and Immunity

Wander Van Breedam

Gent University (Belgium)

Virus-macrophage interactions or where the pathogenesis of PRRSV infections starts
The last two decades, tremendous efforts have been made to get better insights in the way PRRSV infects its target cell, a subset of porcine macrophages. The Laboratory of Virology at the Ghent University has done pioneering work on the way PRRSV is entering and infecting the host cell. It all starts with the interaction of PRRSV with heparan sulphate, followed by the binding to the cellular receptor sialoadhesin. The GP5/M complex is mediating these first contacts with the cell. Sialic acids on the viral envelop glycoproteins that link to sialoadhesin play a crucial role in this binding process. Upon binding, sialoadhesin is pulling the virus particle in the cell by clathrin-mediated endocytosis. Once inside the cell, the pH inside the virus-carrying endosome drops and proteases get active, such as cathepsin E and a not yet identified serine protease. CD163 is playing a role during the desintegration phase but up to date the clear mechanism remains unresolved. The GP2a/3/4 seems to play an important role at this stage. Future work will be done on the desintegration of the virus particle.

Lei Zhou

Shandong Agricultural University (P.R. China)

The 30-amino-acid deletion in NSP2 of highly pathogenic PRRSV emerging in China is not related to its virulence
Recently a highly virulent PRRSV emerged in China. This virus is characterized by a 30 amino acid deletion in NSP2. Most Asian scientists were convinced that this deletion was associated with the highly increased pathogenicity and virulence. In order to examine this, several strains were developed by reverse genetics and their pathogenicity/virulence was compared. One highly virulent strain, one low virulent strain, one highly virulent strain with a restored NSP2 (originating from the low virulent strain), one low virulent strain with a deletion in NSP2 and revertant strains. With these strains, animal experiments were performed. From the results, it could be concluded that the deletion in NSP2 was not responsible for the differences in pathogenicity. In the future, further work will be done on identifying the genes determining pathogenicity and virulence of PRRSV isolates. A better knowledge will allow to attenuate PRRSV by reverse genetics and to create new generation PRRSV vaccines.

Enrique Mateu

Universitat Autònoma de Barcelona (Spain)

The fight between PRRSV and the pig's immunity
The immune response against PRRSV is characterized by several abnormalities. First of all, interferon-alpha, an important cytokine in starting up the whole immune response is not well activated. Further, although PRRSV-specific antibodies are induced starting from 7-8 days post inoculation, neutralizing antibodies appear much later during infection (4-5 weeks post inoculation). Also the cell-mediated immunity is activated late during infection. All these hampered branches of the immunity are leading to a long persistence of the virus in the pig. Interleukin-10, an immune suppressive cytokine is believed to be involved in the malfunction of the immunity. Clear differences were found between PRRSV isolates in their capacity to induce IL-10. In vivo work is ongoing to compare isolates that induce high levels of IL-10 with isolates that induce low levels of IL-10. Cross-protection studies revealed that previous exposure to one strain does not give a full protective immunity upon challenge with another strain. Based on our present knowledge, it can be stated that PRRSV has a complex interplay with the host's immunity. On the one hand, it is paralyzing the immunity and on the other hand it is evading from immunity. Further work is necessary to fully understand this complex game. Better insights are essential for the development of fully protective vaccines.

Yaxin Wang

Shanghai Veterinary Research Institute (P.R. China)

T-cell epitopes of the matrix protein of PRRSV
With this work T-cell epitopes were identified on the matrix protein of PRRSV. Defining epitopes (T-cell and B-cell) on all viral proteins and testing their protective power (CTL activity and neutralizing antibodies) is crucial for the development of effective vaccines.

Helen Singleton

Veterinary Laboratories Agency (United Kingdom)

Immunomodulatory effects of EU-PRRSV on porcine monocyte derived dendritic cells and macrophages
Porcine monocytic derived dendritic cells (MoDC) and macrophages can be infected with PRRSV. The effect of a PRRSV infection in MoDC and macrophages on surface antigen (MHCI, MHCII, CD14, CD80/86 and CD163) expression and cytokine production profile was studied. The antigen expression pattern was clearly different between MoDC and macrophages. However, by repeating the experiment, it became clear that it was impossible to reproduce the results. Therefore, standardization imposes itself. It was hypothesized that the supernatant of macrophage-grown virus caused different effects in between the experiments. In the future, virus will be purified to solve the problems. Based on this study, PRRSV scientists should be warned on using raw macrophage grown PRRSV in *in vitro* tests.

Epidemiology and Control

Shishan Yuan

Shanghai Veterinary Research Institute (P.R. China)

PRRSV vaccine development by reverse genetics, where to start and what to expect
An attenuated virus has been made from the highly pathogenic PRRSV strain JX143 by 100 serial passages in MARC cells. Infection of MARC cells with the obtained JMX100 virus resulted in higher titers, and smaller plaque sizes in comparison with the parental virus. The JMX100 virus was characterized for genetic changes. It was shown that a unique 88 amino acid deletion of nsp2 was related to the cell adaptation. An antibody against this 88 aa deletion was developed. This peptide is recognized by antisera from immune pigs and as a consequence this cell-adapted virus can be differentiated serologically from the parental strain. An infectious cDNA clone of JXM100, pAJXM is developed. This cDNA clone is now promising for further development of a DIVA vaccine.

Xiaofang Hao

Lanzhou Veterinary Research Institute (P.R. China)

What lessons can be learnt from the genetic analysis of Chinese strains
Several PRRSV strains isolated in China before 2006 were investigated. The studied isolates all belong to the North American type (NA-PRRSV). They were further divided into two subgroups, based on GP5 phylogenetic analysis. The HP-PRRSV strain belongs to subgroup 1, and became the dominating virus in China since 2006. Computer analysis revealed that this virus has 5 potential glycosylation sites, while other virus isolates of subgroup 1 have 3-4 potential glycosylation sites. Whether the number of glycosylation sites is related to the increased virulence of HP-PRRSV should be further studied.

Tomasz Stadejek

National Veterinary Research Institute (Poland)

The complex epidemiology of PRRSV in Europe
In Europe, both European type (EU-PRRSV) and NA-PRRSV type viruses can be found. Although before 1996 it was suggested that EU-PRRSV genetic diversity was lower than NA-PRRSV, it has been shown more recently that the diversity of EU-PRRSV is extremely high in comparison to the NA-PRRSV. Especially the Lithuanian strains are more intermediate between prototypical EU-type and NA-type. This could result in difficulties with the diagnosis of PRRSV. The effect of strain diversity on the serological diagnosis was tested with serum from pigs infected with different subtypes of European strains. Four different ELISA assays were used. It was shown that all ELISA tests detected antibodies induced by any of the EU-PRRSV types, but they differed in sensitivity. A larger problem could be observed with the sensitivity of current RT-PCR assays. Due to divergent EU-PRRSV strains they might be missed in diagnostic RT-PCR assays. Therefore, we should proceed with the characterisation of virus strains, since we may not have the complete picture of the diverse strains distribution yet. Important is that Europe not only focusses on EU type strains, but also of NA type strains.

Sophie Morgan

Veterinary Laboratories Agency (United Kingdom)

Sequencing of PRRSV isolates, a useful tool in epidemiology
A serological study was performed to investigate the PRRSV situation in the UK in 2004. It was shown that 56% (366) of the herds was seropositive for PRRSV. To control the PRRSV infections in herds, and find sources of infection, veterinarians request sequence data of isolated samples. This is currently performed with ORF5 sequencing. It was questioned whether ORF5 is the best choice, or whether more ORF regions should be included. This would be helpful to provided more reliable epidemiological data on the spread of the virus. Including another ORF would, however, increase the cost for the farmer, which is undesirable. Informing veterinarians about the risk of obtaining only ORF5 data seems valuable. From the data obtained with sequencing 121 isolates, it was concluded that UK PRRSV strains become more diverse, which has implications on vaccination. Furthermore, the discovery of a vaccine-like strain from a clinical submission highlighting dangers of using live-vaccines.

Eefke Weesendorp

Central Veterinary Institute Lelystad (The Netherlands)

Development of GP2-mutated PRRSV by reverse genetics for vaccine purposes
An attenuated PRRSV recombinant of the LV strain, with two amino acid changes in GP2, and the wild-type reverse genetic virus, were studied in vitro. For this, the cytokine production (IL-1, IL-10, TNF-alpha, IFN-beta) and phenotypic characteristics (CD14, CD80/86, SLAI, SLAII) of bone-marrow derived dendritic cells (BM-DC) after infection were used to compare the strains. It was shown that there were no differences in phenotypic characteristics. Differences were observed in cytokine production. Furthermore, BM-DC were cultured in FBS or homologous pig serum. Almost no differences were observed between these serum types. It was suggested that in these in vitro studies, infection of DC should be confirmed, because DC can also take up virus without replication. Furthermore, these types of studies might be improved by selecting specific cell types.

Athipoo Nuntaprasert

Department of Veterinary Medicine Bangkok (Thailand)

Herd situation and experience in Thailand to control PRRS
In Thailand, the control of PRRS differs among herds. It is dependent on different farm management practice. One of the strategies of farmers is to vaccinate all pigs at the same time with live-modified vaccines. In pregnant sows this could result in abortions. Sequence data were obtained from 10 PRRSV isolates originating from 10 different herds. The results showed that both EU and US genotypes are still circulating in pig-producing areas of Thailand with predominantly the US genotype. Also infections with multiple viral agents are observed. This was studied in 10 farms in a pig dense area in 2008-2009 with post-weaning multi-systemic wasting syndrome (PMWS). In these farms, mortality was observed up to 50%. PCR analysis on serum and tissues was performed for Classical swine fever, PRRSV and PCV2. Most of the farms were PCR positive for at least two of these agents.

General discussion and conclusions

• Pathogenesis

It is very important to get a full knowledge on the replication cycle of PRRSV in its target cell. Binding and internalisation in alveolar macrphages are fully elucidated. However, desintegration, the formation of a replication complex, transcription/translation, assembly and egress are still poorly understood. Further, the cross-talk between viral and cellular proteins and the cell signaling pathways that are activated during the different stages of replication should come into the picture. It is essential that the interaction of PRRSV with susceptible dendritic cells should get more attention. Indeed, it is very well possible that there are clear differences in the replication cycles of PRRSV in alveolar macrophages and dendritic cells. Better insights in all these aspects of the viral-cell interactions will allow a better understanding on how the virus manipulates the central players of the immunity and will facilitate the development of adaptable efficient vaccines.

• Immunity

It became clear that PRRSV is not a virus that recently appeared out of nowhere into the pig population. The ingenuity with which the virus hampers the immune response and escapes from different attacks from the immunity demonstrates a long co-evolution between the virus and the immunity. Only a few immune branches remain, such as neutralizing antibodies, although they appear late in infection and a certain type of NK cells are still able to "control" the infection. Recent highly virulent strains demonstrate that even the latter branches are not fully effective anymore. It is very well possible that this virus heads to a full persistent infection in pigs.

It is important to get a detailed map of the epitopes on all viral proteins. Full attention should be paid on the neutralizing epitopes. Further, more research should be done on the identification of non-CTL immune cells that are still able to eliminate PRRSV-infected cells. And finally, it is important to completely understand the way PRRSV escapes from immunity. All these efforts will show the direction to develop new generation vaccines.

• Epidemiology and control

For Europe, the higher virulent East European strains might possess a danger. It is not known how pigs of other European countries will react when they get infected with these viruses. Pigs are often subject to several viruses or bacteria. It is important to understand the effect of PRRSV vaccination on PRRSV-associated diseases.

More surveillance and diagnosis should be performed to monitor the PRRSV situation in a country. For Asia, it is important that more time is spent on the diagnosis especially the ability to diagnose European type strains. For Europe, emphasis should be put on the diagnosis of non-traditional European strains and American type strains.

A problem could be observed with the sensitivity of current RT-PCR assays. Due to divergent EU-PRRSV strains they might be missed in diagnostic RT-PCR assays. Therefore, we should proceed with the characterisation of virus strains, since we may not have the complete picture of the diverse strains distribution yet.

In the last years, emerging and re-emerging zoonotic viral diseases posed a great challenge to animal and public health at a global level with a massive impact on the socio-economy of affected areas. Many of them emerged from Asia and arrived in Europe and many other parts of the world. Among these diseases, the Hepatitis-E-Virus (HEV), the mosquito-borne Japanese encephalitis virus (JEV), and Swine and Avian Influenza were very prominent. This situation afforded a close collaboration between Asian and European scientific groups aiming at development of integrated control strategies for these diseases and other relevant infections. Thus, national, regional and bi-regional collaborative networks were established. The European Union Commission provided considerable funds to support these collaborations among others Epizone, ConFluTech, Flutrain and ASEM-Dialog. One of the most important outcomes of these projects is a network linking Chinese and European scientists, particularly young scientists.

Alessandra Berto

Veterinary Laboratories Agency (United Kingdom)

Hep E virus
Accumulating evidence indicates that HEV transmission may be zoonotic in developed regions with swine and perhaps other animal species serving as reservoirs for the virus. The exact transmission routes are unclear, largely because HEV is extremely difficult to propagate in vitro, but retail pig products have been shown to contain HEV RNA.The Rotary Cell Culture System (RCCS) is a technology for growing cells without gravity. The design of the RCCS allows the cell lines to more realistically resemble in vivo structure of their tissues of origin.
The aim of this study was to grow swine HEV in PLC/PRF/5 in 3D cells culture to test the sensitivity of this system. The results obtained by qPCR demonstrated that the virus was able to replicate in this system. The general examination of the data indicated that the 3D system is an efficient system to evaluate HEV. RCCS could represent a powerful tool in the study of HEV pathogenesis and virus inactivation.
It has been shown that commercial pig livers purchased from local grocery stores as food in Japan and the United States are contaminated by HEV and that some of the HEV-contaminated commercial pig livers still contain infectious virus.
Using the developed 3D cell culture we are now trying to mimic Feagins's experiment, infecting the cells with heat-inactivated virus from HEV positive pig liver sample. At present no results are available as this experiment it is ongoing. If successfull, the 3D cell culture system will be dimostrated to be an useful tool to replace in vivo with in vitro system in HEV studies.

Xu Zongke

Lanzhou Veterinary Research Institute (P.R. China)

Japanese Encephalitis virus Epidemiology situation in China
In the first part of his talk, Xu Zongke gave an introduction to Japanese Encephalitis virus Epidemiology and its current situation in China. He mentioned that with the exception to Tibet, Qinghai and Sinkiang, all other regions of China

are endemic areas, especially the most Southern regions, such as Kwangsi. In close association with the prevalence of Culex tritaeniorhynchus, sporadic or epidemic measles of Japanese encephalitis occurs from July to September every year. From December to April in the following year, the incidence of the disease is decreasing.

In the second part he described the research activities on JEV in China. Thus, pathogen differentiation and characterization at the molecular level are in the focus of the research. Monoclonal antibodies have been raised and used in rapid diagnosis of the virus. Researchers from China cloned and sequenced the full-length genome of porcine Japanese Encephalitis virus strain SXBJ07 and established an ELISA for virus detection. In addition, a multiplex RT-PCR for detection and differentiation of the Classical Swine Fever Virus and Japanese Encephalitis Virus has been developed.

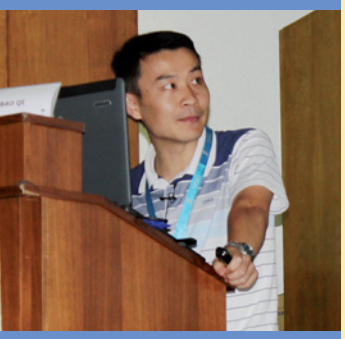
In the last part of his talk, Xu Zongke talked on the methods of control measures and on the national policy about it in China regarding JEV. He mentioned that Japanese Encephalitis belongs to Category B notifiable diseases.

Davide Lelli

Istituto Zooprofilattico Sperimentale Lombardia ed Emilia-Romagna (Italy)

Pandemic influenza (A/H1N1) virus in pigs
The pandemic influenza A/H1N1 virus (H1N1v) is characterized by a unique combination of gene segments from both North American and Eurasian swine lineages. Swine plays an important role in the ecology of influenza A viruses being susceptible to viruses of both avian and mammalian lineages. The study reported by E. Sozzi provided preliminary information about pig susceptibility and potential ability to act as an intermediate host for H1N1v viruses.
Two trials were conducted using SPF swine housed in BSL-3 laboratory facilities and infected intratracheally. Other SPF pigs were introduced for contact infection. The two trials showed that pigs are susceptible to H1N1v and spread virus fast since contact pigs became infected and started to shed virus 4 DPI. Based on these results it could be assumed that H1N1v will spread fast and efficiently if introduced into pig farms increasing risk for establishing of endemic infections. So far, pigs have not been demonstrated to be involved in the epidemiology of the H1N1v.

Furthermore, one H1N1v was isolated from a pig farm in North Italy, the same area where several H5 and H7 avian influenza epidemics were recorded. The simultaneous circulation of swine, avian and human influenza viruses could generate potential changes in the virus characteristics through reassortment with co-circulating viruses and produce a significant impact on human health and global economy.



Wen-Bao Qi

College of Veterinary Medicine Guangzhou (China)

Isolation and Characterization of H4N8 Avian Influenza Virus from Pigs with Pneumonia in southern China

Wen-Bao Qi and colleagues reported on the isolation and characterization of H4N8 Avian Influenza Virus from pigs with pneumonia in southern China They referred on the results of surveillance studies on swine influenza virus in southern China carried out since 2001 till 2009. A H4N8 influenza virus was isolated from pigs with pneumonia on a commercial swine farm in Guangdong province. Based on phylogenetic analyses of the sequences of all eight viral RNA segments, the authors considered that this virus belongs to avian influenza virus of the Eurasian lineage. Accordingly, they considered their data as the first report of interspecies transmission of an avian H4N8 influenza virus to domestic pigs under natural conditions. After a detailed analysis of the literature, the authors mentioned that the appearance of avian influenza viruses among pigs poses concerns for both veterinary and human health.

Huijun Lu

Changchun Veterinary Research Institute (P.R. China)

Isolation and Molecular Identification of a Novel Paramyxovirus Isolated from Pigs in China

In 2000, an outbreak of fever and dyspnea with 50% incidence and 15% fatality occurred in a pig farm in Jilin province, China. The clinical signs of infected piglets included fever, dyspnea, diarrhea, and delirium and sows showed abortion, premature delivery, fetal death, and infertility. The paramyxovirus was detected from the lungs, spleens, and kidneys of the dead pigs by electronic microscopy (EM).

The pathogenic paramyxovirus was isolated and purified by 3 passages of plaque-purification in CEF cells prepared from 9-day-old embryonic SPF eggs, and designated as JL01. The virus was characterized and the results showed that MDT (mean death time) of the isolate was 51.2 h and the EID50 was 10-7.5/0.1 ml. After isolation the virus was returned to experimental pigs at 40 days old, which caused similar clinical signs (fever, dyspnea and delirium) in all 6 inoculated pigs, 3 of which died 10 days p.i. The virus could be detected in blood, liver, spleen, lungs and kidneys of all experimental pigs by EM.

Sequence analysis showed that the F gene of the isolate JL01 has 91% to 99% identities with reference strains of Newcastle disease virus (NDV). The cleavage site of the F protein of JL01 was found to be G-K-Q-G-R-L (112~117aa), the typical amino acid sequence in avirulent NDVs. The length of the complete HN gene of JL01 was 1848 bp, encoding 616 aa HN protein, highly consistent with the HN gene length of avirulent NDV. Phylogenetic analysis classified JL01, along with two other paramyxoviruses isolated from pigs in Shanghai and Fujian provinces in China, into NDV genotype I.

In conclusion, current isolates of paramyxoviruses causing pig disease in China should be considered as Newcastle disease virus-like viruses and further study is needed to focus on cross-species transmission of NDV to pigs or other mammals.

Discussion

A number of specific questions were addressed to each speaker. The most relevant points were related to the detection and differentiation systems, current status and molecular basis of the pathogenesis of these diseases. Another part of the discussion was related to the following issues:

1. A better linking of young Chinese scientists to their European colleagues and vice versa.
2. Priority setting regarding research taking into consideration that infections are a complex of interactions which involve more than one molecule or pathogen. It was recommended to give more attention to epidemiology of these diseases and to establish more efficient surveillance systems.
3. Establishment of functional links to scientific communities, universities, public authorities, companies, farmers and consumer organisations
4. Importance of managing veterinary services on a sustainable financial basis, which reflects partnerships between public authorities, the farming communities and private sector.

PCV2 session

Ken McCullough

Institute of Virology and Immunoprophylaxis (Switzerland)

The immunology of PCV2 infections and disease development
Dr McCullough emphasised the role of PCV2 as an immunomodulating agent and noted specifically the role of the double stranded DNA replicate intermediate in modulating the pig immune system. However Dr McCullough also made it very clear in his presentation that there are still many things we do not know about the interactions of PCV2 with the host and how this leads to development of full blown disease.

Jinxue Long

Shanghai Veterinary Research Institute (P.R. China)

Genetic analysis of PCV2 strains in China
This presentation outlined the variation in PCV2 viruses that have been recovered to date from diseased and non diseased pigs in PR China. He suggested that 4 genotypes of PCV2 have been indentified in his country (PCV2a, b, d and e). Dr Long outlined the possible role of PCV2 as a co-factor with PRRSV in the development of disease syndromes in PR China.

Paolo Martelli

Università degli Studi di Parma (Italy)

Control of PCV2-associated diseases by vaccination
presented some data from field trials on the control of PCV2-associated diseases by vaccination. The results of these trials showed convincingly that both sow and piglet PCV2 vaccines work very well.

Liping Huang

Harbin Veterinary Research Institute (P.R. China)

Development of an ELISA for the detection of anti-PCV2 antibodies
Liping presented on the development by her of a blocking ELISA for detection of antibodies to PCV2. The ELISA was based on a neutralising PCV2 monoclonal antibody and may be adapted for screening for vaccine responses.

Tamas Tuboly

Szent István University (Hungary)

PCV2 in rodents.: Epidemiology of possible vectors for PCVD
Tamas from Budapest presented data on recovery of PCV2 strains from rodents and wild boar and the possible implications for epidemiology and spread of the virus in domestic pigs. PCV2b was the most prevalent virus in wild boar.

Katarzyna Podgarsky

National Veterinary Research Institute (Poland)

Profiles of seroconversion to PCV2 in herds of different clinical status
Poland presented results on PCV2 seroprofiles and concluded that quantitative or semi-quantitative serology should be considered as an aid to diagnosis of porcine circovirus disease in the field. This stimulated some debate on methods of diagnosis in PR China and elsewhere.

Beatrice Grasland

Agence nationale chargée de sécurité sanitaire de l'alimentation, de l'environnement et du travail (France)

PCV2 capsid mutants and experimental infections
France presented some very interesting data on the relationship of the PCV2 capsid protein to virulence in swine. She proposed that the results of her experiments had shown that PCV2b was more virulent that PCV2a and this was due to a mutation in the capsid protein. This was challenged in discussion and a debate took place on this matter.

Hans Nauwynk

Gent University (Belgium)

PCVD: Everything solved or just started
Belgium presented a stimulating summary of his thoughts on what is known and still not known about PCV2 and PCVDs. He concluded that, although PCVD were now being controlled by good vaccines a lot of basic information on the immunology and disease processes associated with this virus is still not known.

Conclusions

Prof Allan closed the PCV2 session by thanking the speakers and reminding the delegates from EU and PR China who attended the session that PCV2 is still an important co-factor in disease syndromes of swine and its importance in interactions with viruses such as PRRSV should not be underestimated, especially in the current outbreaks of HFD in Asia.

Dong Yan Huang

Nanjing Agricultural University (P.R. China)

Use of Swine Pox Virus as gene vector
Dr Dong Yan Huang from the college of Veterinary Medicine, Nanjing Agriculture University, presented the first paper. It dealt with Swine Pox Virus used as a transporter for foreign genes. Theoretically the use of a low virulent and pig-specific virus as transporter could help propagation and proper immune response. Indeed the preliminary results seem to corroborate the point. However several questions remain especially those related to the results that could be expected in pig populations where Swine Pox Virus has still some activity.

Jianfei Chen

Nanjing Agricultural University (P.R. China)

Differential diagnosis of Coronavirus infections pigs
Dr Jianfei Chen, from the National key laboratory of veterinary biotechnology (C.A.A.S), in Harbin spoke about the differentiation between TGE virus (Transmissible Gastro-Enteritis) and PED Virus (Porcine Epidemic Disease) and those of different live vaccines, in use in China. Different molecular methods were tested in an attempt to develop dedicated specific and sensitive assays. It was reminded that an epidemic diarrhea is currently causing severe losses in Asian pig herds.

Ren Xiaofeng

Harbin College Veterinary Medicine (P.R. China)

Characterization of target-cell penetration by coronaviruses
Dr Ren Xiaofeng, from the lab of microbiology, Department of Preventive Veterinary Medicine, Agriculture University in Harbin talked about the first steps of cell infection by coronaviruses. The role of molecules like cholesterol was given special consideration. The role of specific proteins and of temperature was also assessed.

Jinxue Long

Shanghai Veterinary Research Institute (P.R. China)

Detection and isolation of a novel parvovirus
The fourth paper was given by Dr Jinxue Long from the Veterinary Research Institute (CAAS) in Shanghai. It was about recently described porcine bocavirus and novel porcine parvovirus. Both seem to be present in Chinese herds. They were detected from tissues of sick pigs but their pathogenic role in disease is not yet fully demonstrated. The researcher showed that one of his cell lines could be used for the isolation of the new PPV.

Huijum Lu

Changchun Veterinary Research Institute (P.R. China)

A paramyxovirus in pigs
Dr Huijun Lu from Changchun Veterinary Institute (CAAS) presented the fifth paper. It dealt with the isolation and the molecular characterization of a virus found in pigs suffering from severe illness. The isolate was very close to Newcastle disease virus (a paramyxovirus). No causative relationship can, at this stage, be established between infection and illness. Other pathogens could also be involved in addition to this virus. Hence, its presence might be fortuitous. It was said that an attenuated live vaccine was used against Newcastle Disease in poultry in the past and the latter virus could have spread from poultry to pigs.

General comments

The session was well attended and we had lively exchanges with the speakers. Numerous questions were raised and for most they could get an answer or a clarification. Relevant comments were also made. It was especially helpful to have in the audience Prof. M. Pensaert from Gent University (Belgium), the "father" of coronaviruses in pigs. At the end of the session, the chairman outlined the broad perspectives which are now open for viral investigations through molecular technologies. On the other hand he said that step by step from biologists we are becoming chemists, performing refined, subtle chemistry. In our hands we have highly performing devices, modern technologies that allow to go fast. Additionally there is some economic pressure. Even in this rather exciting context, the chair recommended the scientists dealing with genes like those of viruses to not rush, in order in particular to ensure safety at a given step before launching the next. He also advised the researchers to remain in touch with the surrounding world of livestock production.

Chanchun Tu

Changchun Veterinary Research Institute (P.R. China)

The Proteomics of CSF infection
Prof Tu has carried out a comprehensive study on the altered transcriptome and proteome within peripheral blood leukocytes (PBLs), including peripheral blood mononuclear cells (PBMCs), consequent with CSF infection, utilising microarray, 2-D gel electrophoresis and mass spectrometry.
Of all 20,201 pig genes arrayed in the chip, nearly 1,800 showed altered expression after infection. Alterations in genomic expression were confirmed by real time RT-PCR of selected cellular genes and Western blot of a selected protein. In addition, proteins of PBMCs from the CSFV-infected pigs were resolved by 2-DE followed by mass spectrometry to uncover the change at proteomic level. Quantitative intensity and MALDI-TOF-MS/MS analysis 34 unique protein spots showing altered expression. The cellular functions of these proteins included cytoskeletal, energy metabolism, protein translation and processing, antioxidative stress, heat shock and blood clotting. Western-blot analysis confirmed the up-regulation of annexin A1 and down-regulation of cofilin - a family of actin-binding proteins which disassembles actin filaments.

Anastasia Lange

Tierärztliche Hochschule Hannover (Germany)

***In vitro* and *in vivo* determination of cytokine gene expression profiles after infection with classical swine fever virus**
Dr Lange described a comprehensive study of the changes in cytokine expression of a pig kidney cell line, following *in vitro* infection. In one approach, using Serial Analysis of Gene Expression (SAGE), up-regulation of interleukin 8 (IL-8) was detected. In a second approach, cytokine mRNA expression profiles in PBMCs were measured, *ex vivo*, using quantitative real-time PCR. In this experiment, only TNF-α was upregulated, and only with a virulent strain of CSF and younger animals greater increase than older animals.

Yan Zhu

Changchun Veterinary Research Institute (P.R. China)

Antigenic analysis of Chinese CSFVs
Dr Yan presented the results of monoclonal antibody profiling of a number of isolates of CSF from PR China. Twenty-one CSFV field strains isolated between 1996 and 2006 were characterized, along with two Chinese reference strains: Shimen and the vaccine strain, HCLV, using 28 monoclonal antibodies (mAbs) against four pestiviruses. The reactivities were consistent with the diversity of CSF isolates found elsewhere in the world, all showing reactivity with pan-CSF mAbs WH303 and WH302, but with variable reactivities with other CSF mabs. This study provided the first evidence for the existence of antigenic differences among Chinese CSFVs and comparisons with isolates from other parts of the world.

Denise Henrych

Tierärztliche Hochschule Hannover (Germany)

Epitope mapping of the structural glycoprotein Erns of Classical swine fever virus

Dr Henrych presented her work on epitope mapping of the CSF viral protein E^{rns}. Using five chimeric E^{rns} constructs with combinations of the different E^{rns} gene fragments from different pestiviruses, the reactivity of 13 monoclonal antibodies (mabs) were analyzed in the peroxidase linked antibody assay. Analysis of their cross-reactivity revealed that the epitopes studied were all discontinuous, with one CSFV-specific epitope at aa 55-110 and one BVDV-specific epitope at aa111-167. Additionally, two mAbs were identified as candidates for a DIVA ELISA.

Robert Vrancken

Veterinary and Agricultural Research Institute (Belgium)

Antivirals and epizootic infections: CSF as a proof-of-concept

Dr Vrancken presented data on novel compounds, imidazopyridines, which specifically inhibited the viral replication by interacting at the top of the finger-domain of the viral encoded RNA-dependent RNA polymerase (NS5B). *In vivo*, a compound of this class (BPIP) showed that a daily treatment of CSFV-infected pigs resulted in a reduced period of viraemia and in a highly significant reduction of infectious virus (genome) titres ($p \leq 0.001$). There was also a degree of reduction in virus transmission from BPIP-treated/CSFV-infected animals to untreated sentinels. This proof-of-concept indicates that a potential reduction of the viral spread of an epizootic disease could be achieved using an antiviral treatment, without compromising existing control strategies.

Chao Tong

Zhejiang University (P.R. China)

Construction of chimeric CSF virus as candidates for development of CSF vaccines

Dr Tong has been attempting to develop an improved vaccine for use in PR China, which more closely resembles the viruses currently circulating in that country, contending that genetic and antigenic diversity of the immunogenic glycoprotein E2 between vaccine C strain and group 2 field isolates may compromise the vaccine efficacy. A recombinant virus, RecCHZ-CSFV, derived from C-strain, was created, containing the antigenic region of E2 representing prevalent field isolates. The virulence and immunogenic characteristics of this recombinant were evaluated in rabbits and pigs. In rabbits, the engineered virus showed the same effects as the parental C-strain. In pigs, there were no clinical signs or pathological changes or significant leukopenia observed. A low level viraemia was detected in vaccinated pigs 12 days post-inoculation. High antibody and neutralizing antibody titre specific to group 2 field strains developed in pigs inoculated with the recombinant, compared to that of C strain inoculated pigs. Therefore, the recombinant virus RecCHZ-CSFV may serve as the candidate strain for further DIVA vaccine development.

Xia Feng

Lanzhou Veterinary Research Institute (P.R. China)

Genetic marker vaccine against classical swine fever
Dr Feng presented her work on the development of DNA vaccines for CSF. Three candidate recombinant recombinant plasmids were constructed: pcDNA Es1-11, with a single copy of CSFV E2 gene; pBudCE Es1-11/Es2-22, with a dual copy of CSFV E2 gene; pBudCE lacZ/Es2-22, with a copy of CSFV E2 gene and reporter gene lacZ. In a challenge trial in rabbits, following three vaccinations a month apart, the ES1-11 vector expressing a single copy of the E2 gene provided the best protection, with 3 of 4 animals producing detectable antibody and all animals were protected against challenge. The conclusions of the study also highlighted that the choice of plasmid was also an important factor.

Eefke Weesendorp

Central Veterinary Institute Lelystad (The Netherlands)

Airborne spread of CSF
Dr Weesendorp described a study designed to assess whether true airborne transmission of CSFV was possible. In a first trial, virus titres in the air were determined in rooms housing pigs infected with three strains of virus of varying virulence – Zoelen, Paderborn and Brescia. No virus could be detected in air samples taken from the low virulence Zoelen-infected pigs. Virus was detected in the air of rooms housing the pigs infected with the Paderborn and Brescia strains, and the higher the dose or virulence of the virus strain, the sooner virus could be detected in the air samples.
A second trial was conducted in an isolation unit with two rows of three pens, housing four pigs each. The rows were 2,5 meters apart of each other, allowing only contact through the air. Two pigs in a corner pen were inoculated with the Paderborn strain, and transmission through the air was found to be very efficient.

General impressions from the session

The session was a lively one, with much discussion surrounding many of the papers which exhibited a broad spectrum of subject areas. It is clear that much of the focus in developing novel tools for control still surrounds the development of marker vaccines. We were also given a glimpse of the antigenic makeup of Erns, which might yet prove of value within a DIVA differential test. The paper on antivirals was notable for its originality and may provide a glimpse of its role in the control of epizootics in the future. It was interesting to note that there is still much focus induction of antibody to E2, even though the role of T cells in immunity to CSF is known to be significant. The subject of airborne spread of CSF provides the first clear scientific proof of this phenomenon and raises the possibility even of farm to farm transmission of virulent viruses, where infection rates are high and premises are close together. The tranche of papers focused on understanding elements of pathogenesis showed that our understanding of its basis is still in its infancy. The global diversity of CSFV of CSFV is always of concern, both in the context of detection and vaccine protection, so it was interesting to hear of the mAb reactivity patterns of Chinese strains – also with some relief, since they were shown to be of conventional genotypes.

Conclusions and recommendations

1. It is clear that the need for a DIVA vaccine for CSF is shared by both Europe and PR China. We should continue to share our experiences and knowledge, also considering whether a collaborative project could be mutually beneficial to the scientists and their sponsors.
2. The C-strain is one of the best vaccines ever created and, despite the diversity of CSFV, is remarkably effective at inducing rapid protection – so any novel vaccine must be at least as good.
3. Vaccines designed to induce antibody as the sole mechanism of protection are unlikely to provide sufficiently rapid or long-lasting immunity, compared to vaccines which incorporate a mechanism of viral replication or active protein expression within the animal.
4. Research into effects of viral proteins and cytokine responses is of value in understanding the basis of pathogenicity and immunity to CSF, which are themselves critical to engineered attenuation and development of new generation vaccines.
5. The development of antivirals as a potential tool within a control scheme, is increasingly becoming a reality. Those responsible for such planning should begin to consider how they might be used and the consequent constraints to international trade that might emerge. Of course, development of such tools should also consider the safety of the chemicals involved, where it is intended that meat from treated animals are destined to enter the food chain.

Participants

Organising institutions

European Commission, Directorate General Research
ec.europa.eu

Veterinary Faculty of the Universidad Complutense de Madrid
www.sanidadanimal.info

EPIZONE, Network of Excellence for Epizootic Disease Diagnosis and Control
www.epizone-eu.net

Conflutech: Capacity Building for the Control of Avian Influenza Through Technology Transfer and Training
www.conflutech.net

Participating institutions

World Organisation for Animal Health (OIE)
Food and Agriculture Organisation of the United Nations

P.R. China
- Lanzhou Veterinary Research Institute
- Harbin Veterinary Research Institute
- Shanghai Veterinary Research Institute
- Beijing Institute of Animal Sciences
- Beijing College of Veterinary Medicine
- Changchun Veterinary Research Institute
- Nanjing Agricultural University
- Zhejiang University
- Shandong Agricultural University
- Yang Zhou University
- South China Agricultural University

Vietnam
- National Institute of Veterinary Research

Thailand
- Chulalongkorn University

Russia
- National Research Institute of Veterinary Virology and Microbiology, Russia

Belgium
- Veterinary and Agrochemical Research Centre
- University of Gent

France
- Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

Germany
- Free University Berlin
- University of Veterinary Medicine Hannover

Hungary
- Szent Istvan University

Italy
- Università degli Studi di Parma
- Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna

The Netherlands
- Central Veterinary Institute, part of Wageningen UR

Poland
- National Veterinary Research Institute, Pulawy

Portugal
- Faculdade de Medicina Veterinária, Lisboa
-

Spain
- Centro de Investigación en Sanidad Animal
- Centre de Recerca en Sanitat Animal

Sweden
- Swedish University of Agricultural Sciences
- National Veterinary Institute, Uppsala

Switserland
- Institute of Virology and Immunoprophylaxis

United Kingdom
- Department for the Environment Food and Rural Affairs
- Veterinary Laboratories Agency
- Queens University Belfast
- Royal Veterinary College
- Institute for Animal Health, Pirbright

Contact:
Web: www.epizone-eu.net
E-mail: epizone.cvi@wur.nl
Phone: +31 320 23 88 83

Mission of EPIZONE
EPIZONE is an EU funded Network of Excellence for Epizootic Disease Diagnosis and Control to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of Foot-and-mouth disease, Classical swine fever, Avian influenza, and other relevant epizootic diseases like Bluetongue and African swine fever, through increased excellence by collaboration.