EPIZONE

Summary of Presentations

Conclusions and Recommendations

SHVRI CAAS

The workshop "Research in Swine Viral Diseases –Building Bridges" aimed to bring together scientists from China, Southeast Asian countries, Russia and EU in order to debate on major pig viral diseases. It was held in Shanghai from 5th to 9th July 2008 and hosted by the Shanghai Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (CAAS).

The workshop builds on the outcome of the EU-China Meetings on Biotechnology, Food and Agriculture Research held on 30th March – 1st April 2005 and 2nd – 8th July 2007 in Beijing between the Directorate of Biotechnologies, Agriculture and Food of the Research Directorate General of the European Commission and CAAS as well as the workshop of ASEMDialog held also in Beijing on 13th -18th August 2007. These meetings aimed at identifying areas of common interest to increase effective cooperation in the last phase of FP6 and in FP7[1].

The workshop is fully in line with the spirit of the EU-China S&T Cooperation Agreement, the scientific cooperation between ASEAN and the EU and in general with the opening of the European Research Area to international partners.

This workshop constitutes a good example of international coordination and cooperation between Shanghai Veterinary Research Institute (SHVRI) and Langzhou Veterinary Research Institute (LVRI) - both from CAAS - and the EU funded Network of Excellence for Epizootic Disease Diagnosis and Control (EPIZONE)[2]. Additional contributions provided by three other EU funded projects ASEMDialog[3], ConFluTech[4] and PCVD[5] as well as by the Research Directorate General of the European Commission has allowed to further widen the scope to Bhutan, Cambodia, Myanmar, Thailand, Vietnam and Russia.

The topic of the workshop focuses on major infectious swine diseases which cause devastating losses in a key food sector in both parts of the planet. In order to better respond to the global challenges of transboundary animal diseases, international cooperation in research should be strongly encouraged for the delivery of scientific knowledge and improvement of the disease prevention and control tools.

[1] FP6 (2002-2006) and FP7 (2007- 2013): 6th and 7th Framework Programmes of the European Community for research, technological development and demonstration activities (http://cordis.europa.eu/en/home.html)

[2] EPIZONE "Network of Excellence for Epizootic Disease Diagnosis and Control" http://www.epizone-eu.net/default.aspx

[3] ASEMDialog: Contract SSPE-CT-2006-044266 "The EU, China and South East Asia Dialog for the Development of Research Areas in Animal Health of Mutual Interest"

[4] ConFluTech : Contract SSPE-CT-2006-044462 "Capacity building for the control of avian influenza through technology transfer and training"

[5] PCVD: Contract FOOD-CT-2004-513928: "Control of porcine circovirus disease (PCVDs): towards improved food quality and safety"; http://www.pcvd.

Building Bridges Workshop

This workshop is an important milestone in international S&T collaboration in animal health. It has successfully accomplished its goal of "*building bridges*" in the research of swine viral diseases by sharing experiences in the epidemiology, pathogenesis, diagnosis, vaccines and control strategies, by providing information about on- going research activities and identifying needs and common areas of interest for future international collaborations in particular in the 7th FP.

I would like to acknowledge the invaluable work of Dr Shishan Yuan, Dr Hong Yin, Dr Trevor Drew and Dr Johan Bongers in the preparation of this workshop. I would also like to highlight the help provided by Prof. Jabbar Ahmed to allow a wider participation of Chinese scientists. Thank you to all the speakers and participants. Finally I would like to specially thank all the young scientists from SHVRI who so enthusiastically, generously and efficiently helped with the logistics and organisation. In this regard, I encourage you all to start thinking on further "building bridges" ….for example between the younger scientists from Europe and Asia.

Isabel Minguez Tudela, DVM, PhD
Scientific Officer
Research Directorate General
Directorate of Biotechnology, Agriculture, Food
European Commission

Trevor Drew

Isabel Minguez Tudela

Session 1. Porcine reproductive and respiratory syndrome virus: disease situation, epidemiology, diagnosis, prevention and control, ongoing and future research

Tomasz Stadajek:

Molecular epidemiology of EU-genotype PRRSV in Europe: clues to PRRSV emergence and implications for disease control

Dr Stadejek presented some compelling data that challenged the prevailing view that EU-PRRS is less genetically diverse than its American counterpart. He also postulated that Europe was the origin of the virus, with only one of the EU subtypes now prevailing globally, with others still restricted to Eastern Europe. He explained that the likely source of the virus was the result of breeding indigenous breeds of pig, which might have harboured the progenitor virus, with conventional, Western European breeds in the late 1950s. Dr Stadajek highlighted the possible future effects of such diversity on diagnostics and prevention tools and strategies that were configured and targeted only to the currently prevailing genotype.

Hans Nauwynck:

Pathogenesis of porcine reproductive and respiratory syndrome

Dr Nauwynck emphasised that, while there was much concern about the apparently overt virulence of the Chinese variant of PRRS, there were other strains of the virus, of both the EU and American genotype, that could be regarded as equally pathogenic, giving the example of a Belarus isolate. He reviewed the current knowledge on receptors and the role they likely play in binding and internalisation of virions, along with data on the response of various arms of the immunological defence of the pig to infection and the degree of effectiveness they provided. He posed a number of questions regarding the apparent manipulation and avoidance of immunity exhibited by PRRSV, including the varying effects of exposure at different stages of pregnancy. He presented some impressive data on the course of disease and virus distribution and excretion, tying these with the epidemiology of the disease at the herd level. Finally, Dr Nauwynck then reviewed the strategies for control of PRRS in herds, taking account of the results of these experimental findings.

Emmanuel Albina:

Epidemiology and global mechanisms of spread of porcine reproductive and respiratory syndrome virus

Dr Albina presented a review of the current knowledge of the diversity of PRRSV and the epidemiological factors that play a role in spread of PRRSV among breeding systems worldwide. He also highlighted the effects of increased intensification and artificial breeding on spread of PRRSV and other diseases. Finally, he reviewed some of the strategies currently employed to prevent spread and reduce the losses due to PRRS, along with biosecurity measures aimed at keeping regions free.

Sujira Pancharyanon:

Diagnosis and control of PRRS in different husbandry systems

Dr Pancharyanon reviewed the current methods used by laboratories to diagnose PRRS in herds, along with the pros and cons of specific tests and how to interpret results. She also described the strategies used by swine practitioners in controlling and eliminating PRRSV from pig production systems in Thailand. Dr Pancharyanon emphasised that these strategies were rarely easy to apply and were usually a combination of husbandry strategies and good biosecurity, sometimes in combination with vaccination.

Hanchung Yang:

An overview of re-emergence of atypical PRRS in China

Prof Hanchun described the emergence of "pig high fever disease" in China in June 2006 and the spread of the disease in the following months. He highlighted the profound clinical effects and mortalities seen, through direct losses and abortions, also illustrating the gross lesions and their similarity to swine fevers. Unique to PHFD, however, were the lung lesions, which, in some cases, also clearly showed secondary bacterial involvement. He then described the consistent finding of a particular strain of PRRS virus from clinical cases and the discovery of a deletion in the nsp2 region of the genome within this strain. He acknowledged the significant role played by other pathogens, both viral and bacterial, in field cases, however, including PCV2, CSFV, *H. parasuis*, *S. Suis*, *P multocida* and *Salmonella spp*. He did, however, assert that this variant PRRS was the primary pathogen and that vaccination provided some protection from clinical signs and mortalities. He also asserted that live vaccines were better than killed ones.

To Long Thanh:

Diagnosis and research on PRRSV-related disease in Viet Nam from March 2007 to June 2008

Dr To described the appearance of PHFD in the northern provinces of Viet Nam in February 2007 and the rapid detection of PRRSV from clinical cases, by PCR. He outlined the approach made in confirmatory diagnosis and the control measures that were applied to contain the disease. He described the huge pig losses that farmers suffered – particularly devastating since approximately 70% of pig production is at village level. Dr To then described the re-emergence of the disease in 2008 and collaborations with scientists in the US in determining the effects of the virus on pigs in experimental conditions. Unlike the results of experiments with Chinese isolates, an isolate of PRRSV from Viet Nam did not kill pigs, though a homogenate prepared from the tissue of affected pig did. Dr To concluded that the high mortality seen in pigs in Viet Nam is due to a combination of co-infections, in which the variant PRRSV is a major factor.

George Fu Gao:

Molecular analysis of highly-virulent PRRS virus variants from China and Viet Nam in 2006-2007

Prof Gao described the whole-genome analysis of isolates of PRRSV from cases of PHFD in China and Viet Nam and the revelation of a consistent discontinuous deletion of 30 aa in the nsp2 region, comparing it to other strains from North America, such as MN184 and P129, also with deletions in this region, but at different positions and lengths. He also revealed other differences among the structural proteins of the viruses isolated. He outlined the current work, involving reverse genetics approach to analyse the role of the nsp2 deletion and other changes, along with structural genomics to determine the structural basis of the PRRS virion, its assembly and replication.

Shishan Yuan:

Reverse genetics of porcine reproductive and respiratory syndrome virus

Prof Shishan described his impressive work in producing infectious clones of normal EU and American isolates of PRRSV, along with the variant strain JX143. Experiments producing chimeric viruses have revealed the similarity in function of many regulatory elements of the two genotypes, despite their relative lack of similarity at the genome level. He also presented compelling data on animal experiments with variant virus rescued from the infectious clone and the severe clinical effects and high mortalities that were seen.

Enmin Zhou:

A potential novel PRRSV receptor on MA-104 and porcine alveolar macrophage

Dr Enmin described the discovery of a novel receptor which was associated with the induction of anti-idiotype antibody. He demonstrated that, for some pigs, the rapid production of auto- anti-idiotype antibody correlated with rapid viral clearance. In contrast, virus persisted in those pigs which developed anti-idiotype antibody later. Dr Enmin concluded that it remains to be clarified whether this is a cause or effect relationship, but it might be possible to utilise the anti-idiotype network to manipulate the immune response.

Recommendations and priorities:

PRRS & PHFD

Control of PRRS infections on-farm and at regional level is a complex task and compounded by the apparent persistence of the virus in apparently immune animals.

The Workshop recommends that research priorities wrt PRRS & PHFD are:

1. To determine the basis of immunity to PRRSV and how the immune response of the pig can be enhanced to enable a more vigorous, targeted response, that more rapidly suppresses viral replication and achieves long-lasting and broad, sterile immunity.
2. Specific studies aimed to determine the molecular basis of pathogenicity. In this area, it is particularly important to collate and share sequence information, as a common resource for all researchers. Collaboration between scientists in EU and Asia countries in HP-PRRSV and PHFD in areas of pathogenesis should be encouraged, with the aim of identifying molecular markers and the molecular basis of virulence.
3. To develop improved vaccines, taking into account the findings of 1 and 2 above.
4. To elucidate the co-functions of the various confirmed and putative receptors should be elucidated, in relation to virus binding, receptor epitope mapping and mechanism of entry.
5. Host genetics resistance, reduction of susceptibility of populations, and targets for receptor-blocking agents may also yield clues as to the basis of virulence and provide opportunities for control.
6. To clarify the role of co-infecting viruses and bacteria in clinical disease of PHFD.
7. To continue to monitor PRRSV diversity at the global level and the consequent ramifications for detection and control.

PRRS/PHFD summary and conclusions:

It is clear there is still much we do not understand about the pathogenesis of this virus and interactions with the host.

The degree of pathogenesis seen with PRRSV infections is remarkable, ranging from mild or inapparent infection through to severe disease and very high mortality. In field cases, many other pathogens are implicated in the disease, but the level of consistency seen in experimental infections, whilst not absolute, is sufficient to consider that there is a molecular basis for pathogenicity of PRRS virus.

The workshop agreed that there was now sufficient scientific evidence to confirm the involvement of a highly pathogenic (HP) variant of an American strain of PRRSV in the recent severe disease seen in some SE Asian countries, termed "pig high fever disease" (PHFD). Whilst there are certain molecular characteristics of isolates of this variant that can be used to distinguish it from conventional strains – specifically, a discontinuous 30 aa deletion in Nsp2 region – our understanding of the basis of this markedly increased pathogenicity is still unclear. Co-infection with other viruses, PCV-2, PPV, SIV, and CSF, and bacteria, *P. multocida, Salmonella, S. suis,* etc are also likely to play a part in the field disease and high mortalities observed. However, due to the lack of true SPF pig in Asian countries, the conditions for a co-infection study are limited. However, one report of reproduction of the disease from virus rescued from an infectious clone, provides additional compelling evidence of pathogenicity of this virus in uncomplicated infections in young pigs.

In vaccine development, a recombinant PRRSV was constructed containing structural proteins of a Chinese HP-PPRSV isolate which induced protective immunity in pigs. An adenovirus expressing shRNA directed against ORF1b of another Chinese HP-PRRSV inhibited HP-PRRSV replication providing potential strategy for preventing and control of PRRSV infection.

A putative novel PRRSV receptor on MA-104 and PAM was identified which was different from the four PRRSV receptors identified so far. Although this putative receptor has not been fully confirmed in terms of involvement of PRRSV binding and/or entry of permissive cells, blocking of this receptor protein was reported to prevent infection of both MA104 and PAM cells by conventional strain VR2332 and also the HP-PRRSV variant.

The basis of immunity to PRRS is still not understood. The mechanisms by which the virus manages to subvert the immune response, avoiding viral inhibition and clearance are not clear. Likewise, the apparent delay in the onset of sterilising immunity, the poor immunological memory exhibited by infected pigs, so that they are vulnerable to re-infection, even with similar strains and how the virus persists for long periods, are considered priority areas for future research.

The development of an effective vaccine is unlikely to be achieved via the route of conventional attenuation of a single strain. This is partly because of the nature of the virus and its ability to either not induce, or suppress immunity and also by the increasing diversity it is exhibiting.

The global nature of the disease, the rapid and increasing diversity of the causative virus and the apparent evolution of more virulent strains are of great concern to the international community of pig virologists.

Session 2. Classical swine fever (CSF) and African swine fever (ASF) diseases situation, epidemiology, diagnosis, prevention and control, on-going and future research.

Åse Uttenthal:

Global situation concerning CSF

The lecture described the (rather limited) information available on the CSFV disease status worldwide; mainly the OIE (WAHID) system is useful for this information (www.oie.int). Here detailed maps of the disease situations can be downloaded, but the maps are of limited value due to the lack of reporting the disease status, especially those countries that have the disease. CSF has been eradicated in Australia, USA, Canada and most EU countries.

However sporadic outbreaks occur in the EU, often due to infections of wild boar. In some countries of South and Central America CSF is detected occasionally and is usually eradicated. In Asia and Africa CSF is controlled by vaccination. The limitations of the reporting systems are discussed, also in connection to control measures.

After a short description of the disease and the difficulties in the clinical diagnosis the handling of CSFV in Europe was described. Experiences of the known sources of virus introduction were presented and the economic losses of an eradication campaign for CSFV described. The importance of the strain virulence for the clinical diagnosis was underlined.

Several precautions are used in CSFV free countries in order to maintain the CSFV free status. Experiences from Denmark which exports high numbers of pigs and pig products were described.

To assure the limitations in CSFV outbreaks an improved cooperation between pig-producing countries is needed. This EU-China cooperation is a large leap in the right direction.

Frank Koenen:

Controlling CSF in commercial, backyard and wild pig populations.

Dr Koenen presented EU strategies towards CSFV eradication, which has previously been based on stamping out only. Recently the use of DIVA vaccines has been taken into consideration. In the backyard pigs in some of the new EU member countries where CSFV is endemic live C-strain vaccines are used both for backyard pigs and for wild boars (wb). It is important that each batch of live vaccine is tested to assure the efficacy of the batch. In some large commercial herds which are expected at low risk for CSFV the E2 marker vaccine is used.

Early recognition of the infection in domestic pig holdings is of high importance. For wb different strategies such as increased hunting, vaccination and the actual number of wb per square kilometre are important factors for the choice of a CSFV eradication strategy. At least 40% susceptible of the pigs must be vaccinated before there is a reduction in the infection. Increased hunting often results in larger epidemics probably because the repopulation will increase the number of young susceptible pigs. If wb populations are CSFV positive it is very difficult to maintain the domestic pigs free from CSFV, as direct and indirect contact between domestic and wb occur repeatedly. In a single small population of 600 to 1000 wb the CSFV infection will die out by itself. Biosecurity is an important tool to avoid (re)-introduction of the disease. A strategy for eradication should be planned in peace-time.

Irene Greiser-Wilke:

Diagnosis and molecular epidemiology of Classical swine fever virus.

Dr Greiser-Wilke presented the diagnostic methods and recommendations from the EU Reference Laboratory (EURL) for CSFV in Hannover. RT-PCR and real-time RT-PCR are recommended to replace antigen ELISA, as this is a method with rather low sensitivity. The use of the immunofluorescence test on cryosections is a very fast method but it can only be performed by very skilled personell. Therefore it cannot be recommended. For antibody testing the ELISA test can be used for high-throughput analysis but the specificity may be low, especially in pig populations where other Pestiviruses like BVDV and BDV are likely to infect the pigs. Some commercially available ELISA kits do not discriminate between the different Pestiviruses. Still, the antibody ELISAs are indispensable for screening purposes. The alternative method is the neutralisation test, which is a highly specific test but time- and labour-consuming and not all laboratories have the facilities for handling the live CSFV. General recommendations for CSFV diagnostics are available in the OIE manual chapter 2.8.3, which could be consulted for more details.

Also the CRL database for CSFV strains, which is accessible by the WWW, was presented. The usefulness of this free database is dependant upon continuous updating of the database and if possible also of storage of new isolates in the freezer at CRL. The sequences of many isolates which are stored in the CRL database are not available at GenBank. The database includes a module for automated genetic typing. The database is helpful for epidemiological investigations to evaluate the origin of new outbreaks.

Changchun Tu:

Antigenic differentiation of classical swine fever viruses in China by monoclonal antibodies and altered expression of apoptosis genes of pigs peripheral blood leukocytes caused by classical swine fever virus infection.

Dr Tu presented data on analysis of the diversity of Chinese CSFV isolates which by genetic analysis had revealed four subtypes (1.1; 2.1; 2.2; 2.3). The first Chinese isolate (Shimen) was obtained in 1945 and in 1956 eradication campaign was proposed. Since 1990 CSFV has been seen in combination with other infections (PPV, Aujeszky's disease, PRRS). Recent Chinese strains were analysed by monoclonal antibodies obtained from VLA Weybridge, UK. The CSFV strains were all determined to be CSFV Pestiviruses but further differentiation based on monoclonal antibodies was not possible. There was not strong evidence that antigenic classification of CSFV by monoclonal antibodies coincided with phylogenetic analysis.

Dr Tu also presented microarray studies on the altered expression of apoptosis genes in peripheral blood leucocytes in healthy and CSFV infected pigs. Using microarray the function of the majority of the genes remained unknown, but 24 genes could be related to the apoptotic process. The expression of these genes showed differences in healthy and infected pigs. Further studies on these altered genes and their proteins may improve our understanding of the CSFV associated leukopenia and host response to CSFV infection.

Huaji Qiu:

Novel approaches to CSF control, using vectored vaccines.

A new vaccine approach based on vector vaccines using an Alphavirus, namely Semliki forest-virus (SFV) as a vector for the expression of the E2 protein of CSFV was presented. The vaccines were tested in a challenge study where the vaccine after 2-3 vaccinations showed an ability to reduce the severity of disease. Both clinical symptoms and the level of virus in the blood following challenge were reduced compared to a non-vaccinated control group of animals. The use of vector vaccines expressing only the E2 gene should allow for a DIVA diagnostics of the vaccine if it proves to be efficacious.

A combination of SFV vector vaccines and expression of PRV VP22 may induce stronger lymphoproliferative responses and cytokine levels. The vector vaccines may represent future vaccine candidates.

CSFV summary, conclusions recommendations and priorities

CSFV summary and conclusions

The precautions against CSFV are very different in the EU compared to Asia. Whereas the EU has eradicated CSFV in most areas and vaccine is no longer used, the disease is endemic, also with a prophylactic vaccination still widely used in several countries in Asia. EU countries do experience reintroductions of CSFV to the domestic pig population and during the last decade especially with the introduction of DIVA vaccines the use of vaccination in connection to CSFV outbreaks has come nearer. The disease does still circulate among wild boar in some countries and the presence of wild boar and backyard pigs which are kept at a lower biosecurity level and which also often are not registered poses a constant threat to the control of CSFV.

From an epidemiological point of view, the use of vaccine make registration and surveillance more difficult. The production systems need to be investigated as well as the actual distribution of CSFV and its role as a secondary agent in other infections like the HFPD need to be elucidated. As PRRS, CSFV and ASFV are immunosuppressive diseases and their clinical appearance is indistinguishable, there is a strong need for more research in the virus-host interaction of these diseases. Also the gathering of information on sequence variations on CSFV will be of common interest.

CSFV - Recommendations and priorities:

1. Live, attenuated CSFV vaccines usually give good protection even to the fetal infection. However, it is crucial that all batches of live vaccines are properly tested to assure a continuous protection.
2. DIVA vaccines and accompanying DIVA diagnostics are useful if they are effective. New live marker vaccines for oral vaccination may be used in both wild boar and in backyard pigs where vaccination can be performed by the pig owner.
3. Research into virus-host interactions is recommended, especially to assist the differential diagnosis among CSF, PRRS and ASF.
4. Exchange of information on CSF status in Asia and Europe is needed. An improved sharing of CSFV strain and sequence information will improve the cooperation.

JM. Sánchez-Vizcaíno:

Epidemiological Models of ASF virus diffusion and Control Strategies.

Prof. JM. Sánchez-Vizcaíno began his interesting talk, giving an overview of the disease, with special emphasis in epidemiology, carrier animals, biological vectors, and routes of transmission.

He emphasised the complexity of this important disease, describing the various scenarios for ASFV infection of domestic pigs and complexity of virus transmission among different susceptible hosts that may occur. He explained those epidemiological scenarios that could be found in Africa, in some cases involving a sylvatic cycle and tick vectors in eastern, central and south countries; or the wild suids without ticks in west parts of Africa. Also, he gave an approach of virus diffusion in Central and South America in the past (which directly occurred between infected domestic pigs, in a backyard system, without ticks) or even the complex diverse scenarios that were found in Europe, since 1960 to date. These different scenarios involved direct transmission, with and without ticks and indoor and outdoor production systems, as well as current scenarios, confirmed in the eastern countries of Europe, involving direct transmission between pigs in a backyard system, and/or contacts with wild boar infected animals. Therefore, there is no single recipe for the control of this complex disease and each scenario needs a specific strategy tailored to the epidemiology.

In the near future, it is very likely that ASF will be further disseminated to new regions. Therefore, he recommended that countries should review their ASF contingency plans, review their diagnostic methods, promote education on this disease, including all the different aspects of the disease, from clinical signs to laboratory diagnosis, and control strategies. He also recommended an assessment should be made of tick populations and their competence as vectors of ASF.

He finished his talk emphasizing future research in epidemiology and risk analysis of the ASFV introduction in free countries, characterization of current circulating field viruses in Africa and Europe; the development of new control strategies focused on the different epidemiological scenarios, in Africa; control of ticks; and future research on host-virus interactions with a view of a vaccine.

M Arias:

Trends and Perspectives in ASF diagnostic Research.

Dr. Marisa Arias talked about the recent developments and advances in ASF diagnosis. New procedures for ASFV genotyping has been recently adopted, as it has been described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008, and at the web site.: www.asf-referencelab.info. She gave an overview of new techniques developed in recent years and now available. The main trends have been primarily the standardization of molecular detection techniques, based on PCR tests. Thanks to this development, two highly sensitive, fast, specific and reliable conventional (Agüero et al., 2003) and real-time (King et al., 2003) PCR methods were validated and they are in use in a number of National Reference laboratories in Europe.

Recent findings have revealed that variants of ASFV exist, that widely differ genetically. One of the fears arising with this finding is that, to some extent, current diagnostic techniques might be missing some of these new variants. Antigenic variation of different ASFV proteins has

been also observed, which could lead to a decrease of sensitivity using the current diagnostic procedures. There is a need to evaluate the sensitivity of the diagnostic tests with the circulating strains.

In addition, Dr. Arias outlined the new developments, with a special emphasis on front-line and pen-side tests, which could be used in developing countries, as these represent the majority of those currently affected. Such tests will therefore assist in improving diagnostic capacity and could improve general knowledge about the epidemiology of the disease in various regions where it is currently unclear. It would include tools ranging from dipstick formats to isothermal amplification assays. The absence of the need for expensive PCR equipment and the comparative simplicity of isothermal technologies means that they could be adaptable for rapid and simple field first-line diagnosis of ASF.

A. Zaberezhny:

Current Situation and Methods of control of ASF in Eastern Europe.

Prof. Alexei Zaberezhny outlined the situation of ASF in the Caucasus region since the spring of 2007, when ASF was first confirmed in the region. ASF was reported in Georgia in June 2007, and it spread to adjacent territories, Armenia, Russia and Azerbaijan. Last evidence of spread was in Russia in new regions of Chechen Republic (May 2008) and North Ossetia (July, 2, 2008) ***<NOTE:*** *The disease has subsequently been reported in domestic pigs in Orenburg, Russia, near the border of Kazakhstan – OIE Follow-up Report No 5, 25/7/08>*

ASF was reported in domestic pigs in Georgia, and in wild boar in Russia (Chechnya). The first case in domestic pigs in Russia is in N. Ossetia. It is likely that ASF exists in wild boar in Georgia.

It remains to be seen whether the local ticks can transmit the virus. Mountain terrain, woods, backyard pig farming, large population of wild boar, and movement of people are of major risk factors of spread. A monitoring programme is under way in Russia, in all territory, with emphasis in southern Federal district.

ASF summary and conclusions:

ASF Summary and conclusions:
African swine fever is a very complex disease. The various scenarios that may occur are also complex. Therefore, there is no single recipe for the control of the disease and each scenario needs the specific ones.

The current situation of ASF in Caucasus regions is a matter of serious concern. The last outbreaks in Russia indicate continuous spreading to other regions. Mountain terrain, woods, backyard pig-farming, large populations of wild boar, movement of people are major risk factors of spread.

A variety of suitable ASF diagnostic tests are available and validated. It also includes new rapid and robust conventional, Real-time and ASFV-CSFV multiplex PCR tests. New procedures for ASF genotyping have been recently established.

ASF - Recommendations and priorities:

Information about disease, clinical and laboratory diagnosis can be found available at www.asf-referencelab.info. Protocols are also described at www.oie.int: OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* 2008 chapter 2.8.1.

At present there is no vaccine against ASFV. The disease control is dependent on good awareness of practitioners and rapid diagnosis and the enforcement of strict control sanitary measures.

a) Concerning epidemiology and control:

1. The last outbreaks in Russia indicate continuous spreading to other regions. Spread by wild boar is considered inevitable and surveillance programmes should be increased. Special emphasis must be put on the carrier animals and the study of ticks as potential vectors.
2. Since African swine fever represents a permanent threat for EU and Asia countries, efforts to support the control of the disease in Eastern European countries should be increased. Cooperation in this issue is of greatest importance. It should include education of farmers and hunters.
3. The future will be very probably the ASF dissemination to new regions. Therefore, the different countries should review their ASF contingency plans, review the diagnostic methods, and promote education on this disease (including from clinical signs to laboratory diagnosis, and control strategies); Also, studies to detect the presence of ticks in the regions should be encouraged.
4. Epidemiology: Establish new epidemiological model for the control and eradication of ASF in different scenarios
5. Vaccine: Future research should be focused with the view of a vaccine: The role of virus and host genes in infection, the characterization of ASFV virulence factors, the host response to infection, and studies to elucidate the pathways to block immune evasion are some of the priorities in this field. Past concerns surrounding vaccine development, about the lack of cross-protection with different strains are irrelevant to the problems in the Caucasus, since only one strain is involved.

b) Concerning diagnosis:

1. Further studies should be performed in order to assess the sensitivity of the ASF diagnostic techniques with the new circulating strains.
2. New ASF diagnostic techniques and procedures for ASF characterization should be roll-out to National Diagnostic Laboratories in Asia.
3. There is a certain risk of ASF spreading and so the need to be prepared at the national diagnostic laboratories. The possibility to set up a Regional Reference Laboratory for ASF in Asia should be explored.
4. Training and Annual ring-trials are offered from the OIE/CRL Reference laboratories sited in Spain (UCM and CISA-INIA). Active plans for training in place in Asia and Europe should be promoted.
5. New research focus on techniques to be used in developing countries (front-line/pen-side test) as they represent the majority of ASFV infected countries. Cost should be kept down and the developments with Russian/Asian partners should be encouraged.

Session 3. Foot-and-mouth disease and swine vesicular disease: situation, epidemiology, diagnosis, prevention and control, ongoing and future research.

Foot and Mouth Disease

Transboundary animal diseases may be described as those epidemic diseases which are highly contagious or transmissible and have the potential for very rapid spread, irrespective of national borders, causing serious socio-economic and possibly public health consequences. FMD is endemic in many regions of the world and is an important global constraint to improving market opportunities, and accordingly has been ranked highly, particularly in the mixed crop–livestock systems of Asia as „the most important constraint to international trade in animals and animal products, which restrict trade in a south-to north direction"

Dr. Zhang presented data regarding the pathogenesis of FMD. He mentioned that the disease is particularly severe in pigs and that per head they have the highest rate of respiratory excretion of the virus. Moreover, he stated that the epithelia from tongue, foot skin and pharyngeal regions are the major target of the virus where the virus is restricted to the basal cell layer. For a better understanding of the pathogenesis of FMD in pigs, Dr Zhang and his colleagues are focusing on the investigation regarding the association between cell type, cell status, virus replication and the outcome of the infection. The aim is to develop safer and more effective vaccine/antiviral agents against FMD.

Dr. Zengium referred on the current situation, epidemiology, diagnosis, control, on-going and future research on FMD in China. He presented data indicating that there is a big progress in the control of FMD in China from 2005 to 2007. This is due to an effective vaccination, disease monitoring and management programme. He mentioned that China has developed a number of molecular and antibody dependent diagnostic tools to be used for diagnosis in epidemiological investigation.

FMD and SVD Recommendations and priorities:

FMD - Recommendations and priorities:

1. Since FMD is prevalent in China and some Southeast Asian countries, research regarding socio-economic impact and epidemiology should have priority. For diagnosis, cheap and reliable rapid diagnostic methods, e.g. pen-side tools and/or lab on site techniques, should be developed for use in developing countries. Tests available at SVANOVA must be cheap.
2. Taking into account that FMD type A is prevalent in Central Asia countries and may invade China and Southeast Asian countries, it is essential to develop antigen stocks in advance that match the prevalent strain for vaccination as these countries are currently adopting vaccination strategies.
3. New types of stable DIVA vaccines, such as recombinant vaccine, live vector vaccine and epitope vaccine should be developed for use when FMD is under control.
4. True role of carriers in re-introducing disease should be assessed.

Swine Vesicular Disease

Swine Vesicular Disease
Swine vesicular disease (SVD) is a contagious viral disease, first diagnosed and probably first appearing in Italy in 1966. The clinical signs are indistinguishable from foot-and-mouth disease in pigs. The mean incubation period of SVD is between two and seven days, and following a transient fever of up to 41°C, vesicles (blisters) develop on the coronary band, typically at the junction with the heel and on the snout.

Dr Silvia Bellini gave detailed information on the disease situation in Europe and on the control measures applied for SVD. She mentioned that SVD diagnostic procedures are laid down:
- at the Community level in the Commission Decision 2000/428/EC, establishing diagnostic procedures, sampling methods and criteria for the evaluation of the results of laboratory tests for the confirmation and differential diagnosis of swine vesicular disease, and
- in the O.I.E. Manual of Diagnostic tests and vaccines for terrestrial Animals.

For this purpose, protocols and diagnostic tools are available and used for rapid diagnosis and differentiation of FMD and SVD.
Dr. Shuanghui presented results of an approach for the development of a molecular vaccine against SVD. They tested the immune response of both guinea pig and swine to this vaccine and found that both of these animals produce virus-specific neutralizing antibodies and mount a lymphoproliferative response.

SVD - Recommendations and Priorities:
1. Harmonisation of test systems and protocols should be sought.
2. Strengthening farm bio-security. Bio-security has an horizontal value and, when properly applied it represents a barrier for many diseases. It should be taken very seriously since it is the first step for a valuable strategy of prevention and disease control.
3. The prevalence of this disease in Asia is poorly understood. Therefore it needs more investigation.
4. Surveillance for SVD should be conducted in China and Southeast Asian countries.

Session 4. Aujeszky's disease, porcine circovirus, swine influenza: situation, epidemiology, dignosis, prevention and control, ongoing and future research.

Aujeszky's disease

Unfortunately Dr Mettenleiter could not attend the meeting. Therefore there was only one presentation on the subject of Aujeszky's disease (Pseudorabies).

Dr Qigai described the Chinese Pseudorabies (PrV) control program and showed some good economic benefits on large farms. One of the main problems is that some farmers refuse to cull seemingly healthy, but virus carrying pigs. Several locally produced vaccines are on the market and also locally produced diagnostic kits. He further described the construction of genetically engineered vaccines using attenuated PrV as vector in order to simplify vaccination strategies and reducing costs for farmers.

Prof Hans Nauwynck talked about the pathogenesis of Porcine Circovirus infections. In an excellent overview he showed the effects of host-pathogen interactions based on experimental infections in gnotobiotic pigs. Many syndromes are associated with PCV2 infections like PMWS, PDNS and PRDC.

Dr Norbert Stockhofe presented data on the interaction of PCV2 infections with the PDNS syndrome. This work based on a case control study was done in the framework of the EU project Control of Porcine Circovirus-associated Disease (PCVD).

Dr Liu Changming presented the diagnosis and vaccine development of PCV 2 infections in China.

Dr Francois Madec gave an overview of Swine Influenza infections in Europe. First of all, he paid attention to the interspecies transmission of Influenza A viruses. The swine influenza surveillance in Europe was described based on the work carried out in the EU project ESNIP2.

Dr Zhiyong Ma described the molecular analyses of Swine Influenza viruses in China. As the pig might play an important role in the interspecies transmission, he showed the prevailing influenza strains isolated in China with phylogenetic trees.
Recently, four H9N2 strains were isolated in China from pigs with a PRRS infection. As H9N2 is a remarkable type that can cause infections in both chicken, duck, pig and humans, these four swine strains were compared to a group of 24 H9N2 strains of avian origin.

Aujeszky's disease – Summary and conclusions:
Suid herpesvirus type 1 (SHV1) is the cause of Aujeszky's disease, also called pseudorabies. Culling of seropositive animals without vaccination in regions with a low pig density or intensive vaccination with gE-negative marker vaccines followed by culling gE-seropositive animals in regions with a high pig density allowed several European countries to become free of SHV1. SHV1 may be reintroduced accidently or may spread from feral swine. It is important that Europe has an emergency plan in order to combat SHV1 during an outbreak. It is important to use all available tools. Antivirals and antisera should be used in order to block virus replication, shedding and transmission in acutely infected

Porcine circovirus
Swine influenza

animals. Vaccination should give a long term protection. At present, there are no commercial antivirals and antisera available. Research is needed to solve this shortcoming.

At present, several strong veterinary research teams are using SHV1-infection as a model for studying the invasion of alphaherpesviruses through mucosae, along nerves and via blood. The results help us in understanding and controlling alphaherpesvirus infections in man. At present, there is no funding of this type of model work.
The experience that European authorities have developed in time may be of interest to Asian authorities to control Aujeszky's disease.

Porcine circovirus – Summary and conclusions:
After three EU projects, several vaccines became available that are able to control postweaning multisystemic wasting syndrome (PMWS) and PCVD. Although several pathogenic and immunological aspects of PMWS have been elucidated, there are still a lot of black boxes. A fully reproducible PMWS model is still not available. Further, the role of PCV2 in PCVD, porcine dermatitis and nephropathy (PDNS) and porcine high fever disease (PHFD) is still unclear. Rapid diagnostic tests for PMWS, PDNS, PCVD and PHFD are not available.
PCV2 is evolving and should be regularly characterized genetically, antigenically and pathogenetically.

Swine influenza – Summary and conclusions:
Different swine influenzaviruses (SIV) are circulating regularly in pig populations. H1N1, H3N2 and H1N2 are the main subtypes in Europe. Airborne spread causes regular infections in pig herds. It may be associated with acute respiratory disease, though subclinical infections are frequent. Vaccines should contain the three subtypes in order to be fully protective.

In China, the same subtypes are circulating. However, infections with H9N2 (avian origin) and H5N1 (single case) have also been reported. More research should be performed on the latter two SIV subtypes to have a better view on the seroprevalence and to understand better the pathogenesis and the possibility to spread to man.

General recommendations

A continuous surveillance should be done worldwide in order to define the drift within each subtype, to trace new subtypes and to examine the danger for spread to man. The SIV network should be linked with the networks that do similar work with equine influenzaviruses, avian influenzaviruses and human influenzaviruses.
It would be very interesting to have easy-to-perform, quick serological tests to screen big populations.

Additional, general recommendations emerging from the workshop:
The participants at the workshop were unanimous in their view that networks are of great value, both in sharing information and coordinating research effort. This approach is exemplified with respect to FMD and international donors should encourage the development of networks for other diseases.

The OIE Twinning initiative was applauded as an excellent example of transferring skills, developing laboratory capability and stimulating national investment and commitment. The workshop agreed that further opportunities should be sought for other diseases and other candidate countries.

The value of training visits too was highlighted. The workshop attendees endorsed the need to encourage opportunities for visiting scientists should be encouraged (both ways). Generic programmes for training should also be explored, such as are provided by ASEM-Dialog, EPIZONE, ConFluTech, Marie Curie programs etc. DAAD, DANIDA, China scholarship Council (CSC) can also support capacity building.
It was agreed that emphasis should be given to young scientists in exploring these opportunities.

Other considerations

It emerged that many scientists in academia and national laboratories in Asia were unaware of the internet resources provided by international agencies and their reference laboratories. International ref Labs were requested to ensure that national laboratories and research groups in Asia are aware of these information resources. For their part, international agencies should also disseminate this information through local offices and during international meetings. The meeting acknowledged the value of genetic information on pathogens and novel technologies to detect them. It was proposed that such information should be provided to central databases where possible, to ensure they are comprehensive and up to date

Other considerations
For collaborations, it was agreed that proposed work must be "fit for purpose" in addressing agreed needs and providing realistic solutions. Mutual benefit should arise.

In developing their capabilities, it was agreed that national laboratories and research centres should pay particular attention to issues such as genetic manipulation and biosafety, when developing their portfolio of activity. It was acknowledged that researchers ultimately carry responsibility for biosecurity of the pathogens they work with. Risks should be carefully assessed, especially for emerging viruses or strains, attenuated or engineered viruses.

For their part, international funders also place strong requirements on socio-economic impact of projects, gender issues and, for projects involving animals, the funding body's standards for ethics and welfare will also need to apply.

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.