Global African Swine Fever
Research Alliance

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Proceedings

3rd Annual GARA Scientific Workshop

anses
French agency for food, environmental and occupational health & safety

Investigate, evaluate, protect

Global African Swine Fever
Research Alliance

3rd Annual GARA Scientific Workshop

Mission of GARA

To establish and sustain global research partnerships that will generate scientific knowledge and tools to contribute to the successful prevention, control and where feasible eradication of African Swine Fever (ASF).

Vision of GARA

A coordinated global research alliance enabling the progressive control and eradication of ASF.

Strategic Goals of GARA

Goal 1. Identify research opportunities and facilitate collaborations within the Alliance
Goal 2. Conduct strategic and multi-disciplinary research to better understand ASF
Goal 3. Determine social and economic drivers and impact of ASF
Goal 4. Develop novel and improved tools to support the prevention and control of ASF
Goal 5. Determine the impact of ASF prevention and control tools
Goal 6. Serve as a communication and technology sharing gateway for the global ASF research community and stakeholders

Purpose of the Workshop

The main objective of the scientific workshop will be to review accomplishments made towards the gaps identified during the previous workshops on Plum Island and Pretoria. The topics will focus on virology and pathogenesis, epidemiology (Africa / Europe), vaccines and immune responses, molecular epidemiology and diagnosis, legislation and reporting in different countries , use of genome editing to understand virus and host interaction.

Participants

Target audience is approximately 100 participants from previous GARA workshops on Plum Island and South Africa as well as GARA partners and collaborators and other potential research partners. Invitees include scientific experts from European Union, Eastern Europe, Africa, Russia, Ukraine, Armenia and the Republic of Georgia, Australia, China, USA, Canada and representatives from international organizations, such as FAO, OIE, ILRI,…

Outputs of the workshop

The GARA will make use of the opportunities to identify research needs and establish collaborations and partnerships, update the gap analysis and identify potential funding sources to address those gaps. The Organizing Committee of the 3rd Annual GARA Scientific Workshop, together with the Scientific Committee, look forward to welcoming you in Ploufragan in September 2016 and assure that your participation will be fruitful and productive!

Organizing Committee

Anses, Ploufragan-Plouzané Laboratory, France

Marie-Frédérique LE POTIER

Olivier BOURRY

Nicolas ROSE

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Virginie LOISELIER

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Armanda BASTOS, *University of Pretoria, South-Africa*

Carmina GALLARDO, *INIA-CISA-Centro de Investigacion en Sanidad Animal, Spain*

Global African Swine Fever Research Alliance

AGENDA AND SCIENTIFIC PROGRAMME

Tuesday, 6th September 2016

8:15 **Shuttle departure** *(Saint-Brieuc ⇨ Zoopole Ploufragan)*

8:15-8:45 **Registration**

8:45-9:00 **Welcome Address**
***Gilles SALVAT & Marie-Frédérique LE POTIER**, France*

9:00-9:45 **General introduction: GARA presentation, main objectives and future**
***Cyril GAY**, USA*

Scientific Session 1: "Virology & Pathogenesis"

Chairs: Covadonga ALONSO, Spain – Douglas GLADUE, USA

9:45-12:45 **Selected abstracts *"Virology & Pathogenesis"***

9:45 ***Natasha GAUDREAULT,** USA*
Pigs lacking CD163 expression show no resistance to infection with African Swine Fever Virus.

10:00 ***Crystal JAING**, USA*
Whole Transcriptome Analysis of Pigs Infected with African Swine Fever Virus.

10:15 ***Douglas GLADUE**, USA*
The Ep152R ORF of African Swine Fever Virus strain Georgia encodes for an essential gene that interacts with host protein BAG6.

10:30-11:15 **Coffee Break & Poster Session**

11:15 ***Covadonga ALONSO**, Spain*
A new significance for African Swine fever cell targets at early infection.

11:30 ***Willie LOEFFEN,** Netherlands*
Effect of inoculation dose and age of the pigs on clinical, virological and serological parameters of an African Swine Fever infection.

11:45 ***Laura ZANI**, Germany*
First evidence of an attenuated phenotype of genotype II African swine fever virus in Estonia.

12:00 ***Catharina KEßLER**, Germany*
Purification and Proteome analysis of African Swine Fever Virus Particles.

12:15 ***Günther KEIL**, Germany*
Adaptation of African Swine Fever Virus field strains to productively replicate in WSL cells is not necessarily associated with larger deletions within the viral genomes.

12:30 -14:00 **Lunch**

Scientific Session 2: "Vaccines and immune response"

Chairs: Linda DIXON, United Kingdom - Marie-Frédérique LE POTIER, France

14:00-15:30 **Selected abstracts *"Vaccines and immune response"***

14:00 ***Giulia FRANZONI**, Italy*
Response of monocyte-derived dendritic cells to virulent and attenuated ASFV strains.

14:15 ***Michael PARKHOUSE**, Portugal*
Identification and utility of interferon evasion by African Swine Fever Virus.

14:30 ***Linda DIXON**, United Kingdom*
Effect of deleting African Swine Fever Virus interferon inhibitory genes on virus pathogenesis and induction of protective responses in pigs.

14:45 ***Manuel BORCA**, USA*
Use of a double viral gene deletion virus as a rational approach to develop ASFV live attenuated vaccines protecting against Georgia 2007 isolate.

15:00 ***Yolanda REVILLA-NOVELLA**, Spain*
Heterologous prime-boost vaccine strategy for ASF.

15:15 ***Fernando RODRIGUEZ**, Spain*
Could live-attenuated African Swine Fever Viruses become a vaccine reality?

15:30-16:00 **Coffee Break & Poster Session**

16:00-17:00 **Breakout Groups**

17:00-18:00 **Conclusion of the day – State of the Art**

18:10 **Shuttle *(Zoopole Ploufragan ⇨ Saint-Brieuc)***

Wednesday, 7th September 2016

8:30 **Shuttle departure *(Saint-Brieuc ⇨ Zoopole Ploufragan)***

8:30-9:00 **Registration**

Scientific Session ASF-STOP: "Epidemiology"

Chairs: Willie LOEFFEN, Netherlands – Dolores GAVIER-WIDEN, Sweden

9:00-9:20 **Introduction ASF-STOP: objectives, working groups**
***Dolores GAVIER-WIDEN,** Sweden*

9:20-9:50 **Invited speaker presentation: *Armanda BASTOS,** South Africa*
The African Swine Fever sylvatic cycle: using molecules and maths to unravel the role of the Ornithodoros tick vector.

9:50-10:20 **Keynote lecture: *Daniel BELTRAN-ALCRUDO,** FAO*
The 1996-2002 African Swine Fever (ASF) pandemic in West Africa: Lessons learnt.

10:20- 10:40 **Keynote lecture: *Klaus DEPNER,** Germany*
Wild boar as ASF driver.

10:40-11:10 **Coffee Break & Poster Session**

11:10-11:30 **Keynote lecture:** ***Krzysztof ŚMIETANKA****, Poland*

African Swine Fever in Poland - current situation and control measures

Scientific Session 3: "Epidemiology focusing in Europe"

Chairs: Willie LOEFFEN, Netherlands – Dolores GAVIER-WIDEN, Sweden

11:30-12:45 **Selected abstracts** ***"Epidemiology focusing in Europe"***

11:30 ***Imbi NURMOJA****, Estonia*
African Swine Fever Virus spread in Estonia: the preliminary results of epidemiological analysis.

11:42 ***Jan Hendrik FORTH,*** *Germany*
Evaluation of insect larvae as possible mechanical vectors for transmission of ASFV in wild boar populations.

11:54 ***Willie LOEFFEN****, Netherlands*
Quantification of transmission of African Swine Fever Virus by carriers and through indirect environmental contact.

12:06 ***Hans-Hermann THULKE****, Germany*
Active management of ASF spread in wild boar: strategic options and plausible solutions.

12:18 ***Martin LANGE****, Germany*
Towards quantitative understanding of the role of carcasses in the transmission of ASF in wild boar or feral swine.

12:30 ***Serhii FILATOV****, Ukraine*
Research on African Swine Fever Threat Reduction Through Surveillance in the Ukraine: An Update.

12:42 -14:00 **Lunch**

12:42 -14:00 **Working lunch for ASF-STOP Core Group members** *(Core group meeting)*

Scientific Session 4: "Epidemiology focusing in Africa"

Chairs: Eric ETTER, South Africa – Charles MASEMBE, Uganda

14:00-14:30 **Keynote Lecture:** ***Charles MASEMBE,*** *Uganda*
Wild life and domestic livestock interactions in endemic situation.

14:30-15:30 **Selected abstracts** ***"Epidemiology focusing in Africa"***

14:30 ***Mary-Louise PENRITH****, South Africa*
Epidemiology of African Swine Fever in Africa today: Sylvatic cycle versus socio-economic imperatives – filling the gaps.

14:45 ***Erika CHENAIS****, Sweden*
Quantitative assessment of socio-economic impacts of African Swine Fever outbreaks in northern Uganda.

15:00 ***Peter OGWENG,*** *Uganda*
A molecular and ecological investigation of the role of the bushpig in the epidemiology of African Swine Fever at the wildlife-livestock interface in Uganda.

15:15-16:00 **Coffee Break & Poster Session**

15:30-17:00 **Parallel Meeting ASF-STOP Core Group members** *(Core group meeting-continuation)*

16:00 ***Laurence VIAL**, France*
Experimental versus natural determinants influencing the vector competence of Ornithodoros soft ticks for African Swine Fever Virus.

16:15 ***Ariane PAYNE**, Uganda*
The potential role of the bushpig (Potamochoerus larvatus) in the spread and maintenance of African Swine Fever.

16:30 ***Edward OKOTH-ABWORO**, Kenya*
Integrated study of African Swine Fever in East Africa identify the disease drivers and provides insights for targeted surveillance and control.

17:00-17:40 **Breakout Groups / Breakout Group ASF-STOP**

17:40-18:00 **Conclusion of the day-State of the Art**

18:10 **Shuttle** ***(Zoopole Ploufragan ⇨ Gala dinner)***

19:00 **GALA DINNER** ***(best poster prize)***

Thursday, 8th September 2016

8:30 **Shuttle departure** ***(Saint-Brieuc ⇨ Zoopole Ploufragan)***

9:00-9:30 **GARA future perspectives : *Cyril GAY**, USA*
Election new committee

Scientific Session 5: "Molecular Epidemiology & Diagnosis"

Chairs: Carmina GALLARDO, Spain – Armanda BASTOS, South Africa

9:30-10:30 **Selected abstracts** ***"Molecular Epidemiology & Diagnosis"***

9:30 ***Silvia DEI GIUDICI**, Italy*
Molecular characterization of Sardinian African Swine Fever Viruses based on analysis of p30, CD2V and I73R/I329L variable region.

9:45 ***Juanita VAN HEERDEN**, South Africa*
African Swine Fever (ASF) genotype II in Zimbabwe during 2015.

10:00 ***Pam LUKA**, Nigeria*
Detection of antibodies to Ornithodoros saliva antigen in domestic pigs from Cross River and Taraba.

10:15 ***Clara YONA,** Tanzania*
Epidemiological study and socio-economic impact associated with persistence of African swine fever in Southern highlands of Tanzania.

10:30-11:15 **Coffee Break & Poster Session**

11:15 ***Emmanuel AWOSANYA**, Nigeria*
A new African Swine Fever Virus strain of genotype I circulates among pig herds in southwest Nigeria.

11:30 ***David WILLIAMS**, Australia*
Rapid detection and epidemiological surveillance of African Swine Fever using oral fluid.

11:45 ***Stella ATIM**, Uganda*
Evaluation of a portable real-time PCR platform (TCOR-8) for ASF during outbreaks in an endemically infected population in Uganda.

12:00-12:45 **Conclusion of the day -State of the Art**

12:45 -14:00 **Lunch**

Scientific Session 6: "Legislation and reporting in different countries"

Chairs: Ana DE LA TORRE, Spain – Denis KOLBASOV, Russia

14:00-15:30 **Introduction of discussion: FAO/EFSA/EC/OIE**
Round table discussion with experts from different regions all over the world

15:30-16:00 **Coffee Break & Poster Session**

16:00-17:00 **Breakout Groups**

17:10 **Shuttle** ***(Zoopole Ploufragan ⇨ Saint-Brieuc)***
18:10 **Shuttle** ***(Zoopole Ploufragan ⇨ Saint-Brieuc)***

Global African Swine Fever Research Alliance

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Poster Session

Welcome Address

Dear colleagues

On behalf of the French Agency ANSES and the GARA executive committee, it is my great pleasure to welcome you in PLOUFRAGAN for the **3rd GARA workshop.**
As you know this initiative was launched the 1st time in Plum-Island by Cyril Gay and the second workshop took place in Pretoria. So we can say that GARA has already visited three continents.

Today, all continents are represented. We are 135 scientists coming from 30 countries strive for the same goal: to control the African swine fever!

68 abstracts were submitted from which 39 oral presentations and 29 poster exhibitions were accepted.
I would like to thank Anses that supported financially the organization of this workshop; DTRA-CBEP (Defense Threat Reduction Agency – Cooperative Biological) & CDRF Global for their travel grants for scientist coming from affected countries (Africa or East Europe); and ASF-STOP, an e- COST action that I invited to join this workshop as we have many common objectives between GARA and ASF-STOP. Her coordinator Dolores Gavier-Widen helped in its preparation and by giving also grant travels for invited speakers.

And finally I would like to thank the **Scientific and Local Organizing Committees** for their commitment and activities to set up this event, who helped me a lot in the organization of this workshop with a special dedication to Virginie, Melanie and Cyril!
Cyril Gay will explain longer the main objectives of GARA, but I would like to remember you that we are all together here to identify the research gaps on the path to the control of the disease and propose future orientations. This can be achieved during the workshop because of your highly active participation to the breakout groups that will be organized every day by the chairs.

Every day, we'll have two different scientific sessions and time reserved for the poster session. One poster will be rewarded during the gala dinner. On the last day, we'll have a round table on ASF reporting and legislation. Representatives from international bodies as OIE, FAO and EFSA accepted to participate with representatives of affected countries. This workshop has been organized by Ana de la Torre and Denis Kolbasov, and I am sure that will be of high interest for all of us.

Finally, I wish for everybody, a successful and productive congress, a good stay in our beautiful area and some opportunities to visit a highly attractive region.
Enjoy the congress, enjoy Brittany, and enjoy our lifestyle!

Marie-Frédérique Le Potier
Chair Scientific and Organizing Committees

Scientific Session 1

"Virology & Pathogenesis"

Chairs:

Covadonga ALONSO, Spain

Douglas GLADUE, USA

Pigs lacking CD163 expression show no resistance to infection with African Swine Fever Virus

Natasha Gaudreault[1], Kristin M. Whitworth[2], Luca Popescu[1], Maria Murgia[1], Jerome Nietfeld[1], Alan Mileham[3], Melissa Samuel[2], Kevin D. Wells[2], Randall S. Prather[2] and Raymond R.R. Rowland[1]

[1]Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, U.S.A.; [2]Division of Animal Science, College of Food Agriculture and Natural Resources, University of Missouri, Columbia, MO 65211, U.S.A.; [3]Genus, plc, DeForest, Wisconsin, U.S.A.

Keywords: CD163, African swine fever virus, Georgia 2007/1

The macrophage surface protein, CD163, has been implicated as a receptor for the African swine fever virus (ASFV). Yet support for that model is based on in vitro laboratory experiments. We recently evaluated the response of pigs, genetically edited to knockout CD163, to infection with ASFV. Following infection with the virulent Georgia 2007/1 isolate, a cohort of wild type and CD163 knockout pigs showed no differences in clinical signs, pathology or viremia. Infection of macrophages in vitro exhibited similar results. This data clearly demonstrate that CD163 is not required for infection with the Georgia isolate, and that future research should focus on other possible receptors and entry pathways. Understanding these mechanisms and virus-host interactions will be important for designing better antiviral strategies against this disease.

Whole transcriptome analysis of pigs infected with African Swine Fever Virus

Crystal Jaing[1], Jonathan Allen[2], James B. Thissen[1], Raymond R.R. Rowland[3], David Williams[4]

[1]Physical & Life Sciences Directorate - [2]Computations Directorate, Lawrence Livermore National Laboratory, Livermore, California, USA - [3]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas, USA - [4]Australian Animal Health Laboratory, Geelong, Australia

Keywords: RNAseq, immune response, vaccine, diagnostic, pathogenesis

There is a significant need to better understand the mechanisms of African swine fever virus (ASFV) infection in pigs to aid the development of countermeasures and diagnostics. We pursued a genome-wide approach to characterize the pig immune responses after ASFV infection. Pigs were infected via the oronasal route with either the low pathogenic OURT88/3 strain, or the highly pathogenic GRG2007 strain. Blood samples were collected at day 0 and at euthanasia day (ED) for infections with both strains, and on 3, 7, 14 and 28 days post-infection (dpi) with OURT88/3 strain. No clinical signs or lesions at post-mortem were observed in pigs infected with OURT88/3, whereas signs and lesions in pigs infected with the GRG2007 were characteristic of highly pathogenic ASFV. Four of six pigs infected with OURT88/3 seroconverted, while none of the GRG2007-infected pigs were antibody positive, consistent with the acute disease course. High levels of virus genome and antigen were detected in multiple organs of the GRG2007-infected pigs, with highest levels in the lymphoid tissues. RNAseq analysis of whole blood samples identified 395 genes most differently expressed at ED (7-10 dpi) in the GRG2007 group, and 181 genes at 7 dpi in the OURT88/3 group. We also identified a set of common genes that are most significantly changed with both the OURT88/3 and the GRG2007 group including macrophage markers, natural killer cell markers, chemokines and other important immune response markers. Viral pathogenesis, RNAseq, gene expression and pathway analyses from this study will be presented.

The Ep152R ORF of African Swine Fever Virus strain Georgia encodes for an essential gene that interacts with host protein BAG6

Manuel V. Borca, Vivian O'Donnell, Lauren G. Holinka, Devendra K. Rai, Brenton Sanford, Marialexia Alfano, Jolene Carlson, Paul A. Azzinaro and Douglas P. Gladue

Agricultural Research Service and Department of Homeland Security, Plum Island Animal Disease Center, Greenport, NY 11944, USA Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT 06269, USA Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831 Biosecurity Research Institute and Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan

Keywords: ASFV, Viral-Host protein interaction, EP152R

African swine fever virus (ASFV) is the etiological agent of a contagious and often lethal disease of domestic pigs that has significant economic consequences for the swine industry. The viral genome encodes for more than 150 genes, and only a select few have been studied in some detail. Here we report the characterization of open reading frame Ep152R predicted to have a complement control module/SCR domain which similar to Vaccinia virus proteins involved in blocking the immune response during viral infection. A recombinant ASFV harboring a HA tagged version of the EP152R protein was developed (ASFV-G-EP152R-HA) and used to demonstrate that EP152R is an early virus protein. Attempts to construct recombinant viruses having a deleted EP152R gene, were consistently unsuccessful indicating that EP152R is an essential gene. Interestingly, analysis of host-protein interactions between EP152R with using a yeast two-hybrid screen, identified BAG6, a protein previously identified as being required for ASFV replication. Furthermore, fluorescent microscopy analysis confirms that EP152R-BAG6 interaction actually occurs in cells infected with ASFV.

A new significance for African Swine Fever cell targets at early infection

Cuesta-Geijo, Miguel Angel, Raquel Muñoz-Moreno, Lucía Barrado-Gil, Inmaculada Galindo, Sergio Viedma, Covadonga Alonso

Dpt. Biotecnología (INIA)

Keywords: ASFV entry, ASFV endosomal entry, Viral fusion, Cholesterol efflux, Interferon-induced proteins

We searched for African swine fever virus cell targets at early infection stages in order to find potential restriction factors at the endosomal entry pathway. These celular restriction factors might be used to boost innate immunity and to develop intervention strategies against the virus. ASFV traffics through the endocytic pathway where uncoating takes place at the acidi c pH of the multivesicular endosomes. After uncoating, ASFV internal membrane fuses with the endosomal membrane to allow cytoplasmic exit of virions to start replication. Cholesterol plays an essential role in the establishment of ASFV infection and is required for this viral fusion step. In fact, viral fusion of ASFV, requires the integrity of cholesterol endosomal efflux, in contrast to other DNA viruses, such as vaccinia virus or adenovirus 5 which are independent of this flux. In ASFV, the accumulation of cholesterol impairs viral exit to the cytoplasm, resulting in retention of virions inside endosomes. This mechanism might be targeted by the innate immunity restriction proteins, called InterFeron-Induced TransMembrane proteins (IFITMs). IFITMs act at the endosomal membrane impairing viral fusion. This highlights the role of cholesterol at the start of viral replication. ASFV remodels intracelular cholester ol by increasing its cellular uptake and redistributes free cholesterol to viral replication sites. Our analysis reveals that ASFV manipulates cholesterol dynamics to surpass innate immunity in order to ensure an appropriate lipid flux to establish productive infectio n.

Effect of inoculation dose and age of the pigs on clinical, virological and serological parameters of an African Swine Fever infection

E. Weesendorp, P.L. Eblé, H.W.M. Moonen-Leusen, S. Quak, W.L.A. Loeffen

Central Veterinary Institute of Wageningen UR (CVI), Lelystad, The Netherlands

Keywords: ASF, virus, age, dose, infection

Experimental infections with ASFV are mostly carried out in young piglets only. Under field conditions all different age categories are at risk and individual pigs may be exposed to very different doses of virus. The aim of this study was to investigate the possible effect of ASFV inoculation dose and age of the pigs on clinical, virological and serological parameters. Four groups of five pigs each were used in this study. Two groups (A and B) were 12 weeks old. The other two groups (C and D) were 18 weeks old. All pigs were inoculated with ASFV (Netherlands '86, a moderately virulent strain) intranasally. Groups A and C were inoculated with a relatively low dose (2ml of 10^3.5TCID50/ml) while groups B and D were inoculated with a relatively high dose (2ml of 10^6 TCID50/ml). Pigs were subsequently observed for 55 days (body temperatures, clinical signs, EDTA and serum blood samples, OPF and air samples). Age and infection dose had no or only a very limited effect on the investigated parameters. However, more of the older piglets survived the ASF infection and became carriers (five old pigs, vs. one young pig) with a lower maximum clinical score in the survivors. Increased survival times for older pigs will likely result in shedding more virus into the environment. Older pigs may therefore play a more important role in transmission of the virus. The carriers still shed virus and showed a viraemia after 48-55 dpi, without showing clinical signs of ASF. The extent to which these carriers were able to transmit the virus was investigated in a separate study.

First evidence of an attenuated phenotype of genotype II African Swine Fever Virus in Estonia

Zani Laura[1]; Nurmoja Imbi[2]; Breidenstein Christiane[1]; Leidenberger Simone[1]; Beer Martin[1]; Blome Sandra[1]

[1]Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany - [2]Estonian Veterinary and Food Laboratory, Tartu, Estonia

Keywords: African swine fever virus, attenuation, pathogenesis, characterization

African swine fever (ASF) is a severe, multi-systemic disease of pigs that is caused by the eponymous double-stranded DNA virus (ASFV) of the genus Asfivirus within the Asfarviridae family. In 2014, ASF was re-introduced into the European Union, affecting domestic pigs and wild boar in the Baltic States and Poland. Up to now, new cases have been reported especially from wild boar every week. However, the geographic region remains rather stable. This epidemiological behaviour was rather unexpected based on the experimental findings that the virus strains involved showed exceptionally high virulence. It was anticipated that the virus would either spread rapidly or die out due to self-limitation. Factors leading to the observed long-term outbreak are far from being understood. One explanation could be virus attenuation. In fact, wild boar hunted in North-Eastern Estonia were tested ASFV antibody positive without showing obvious clinical or pathological signs. To explore the reason for this phenomenon, a re-isolated Estonian virus was biologically tested in different experiments. In a first experiment, ten sub-adult wild boar were inoculated. Here, the virus showed high virulence, and nine out of ten animals succumbed to infection showing typical lesions. Subsequently, a virus re-isolated from the recovered animal was utilized in two trials with different pig breeds. Trial A comprised 12 minipigs, trial B five domestic fattening pigs. In both trials, oronasal inoculation was carried out using a blood suspension containing at least 10 4.5 hemadsorbing units. Upon infection, clinical signs were recorded daily. Furthermore, blood and swab samples were taken at regular intervals. Necropsy was carried out on all animals and organ samples were collected. Blood, swab and organ samples were subjected to PCR analyses and virus isolation, serum samples were analyzed using commercial ELISA kits and indirect immunoperoxidase test. In trial A, all animals developed fever and unspecific clinical signs within the first week post inoculation. However, nine out of twelve minipigs survived the acute phase and were slaughtered in good health status at 36 days post inoculation (DPI). One animal was found dead after blood sampling and two others were euthanized due to severe respiratory

distress. These deaths were not clearly linked to the disease course. Necropsy revealed that two of the inoculated minipig sows were pregnant. Organ pools of the fetuses were tested by PCR and were found negative. Antibodies were detected in all convalescent animals by the end of the trial. Animals of trial B displayed a similar disease course with all animals surviving till the end of the trial at 36 DPI. Apart from a severe fibrinous pericarditis in one of the animals, necropsy did not reveal any signs indicative for ASF or any other disease. While clinical signs were completely absent from 19 DPI, high ASFV genome loads were still detectable by PCR till the end of the trial. Summarizing, an apparently attenuated virus strain was re-isolated from the initial trial. Animals of different pig breeds, i.e. minipigs and domestic fattening hybrids, showed similar disease courses that were, in terms of virus detection, still comparable to the highly virulent ancestors. However, clinical signs and mortality were drastically reduced. Under field conditions, these unspecific clinical signs could easily go unnoticed and thus complicate disease control tremendously. For the wild boar situation in Estonia, circulation of attenuated strains is likely and should be further investigated.

Purification and Proteome analysis of African Swine Fever Virus Particles

Keßler Catharina[1]; Keil Günther[1]; Blome Sandra[2]; Karger Axel[1]

[1]Friedrich Loeffler Institut Federal Research Institute for Animal Health, Institute of molecular Virology and Cell Biology [2]Institute of Virus Diagnostics, Greifswald-Island of Riems-Germany;

Keywords: ASFV particles, proteome analysis, mass spectrometry

African swine fever (ASF) is a viral disease that affects members of the Suidae family such as bush pigs, warthogs, domestic pigs, and wild boars and is transmitted by direct contact with infected animals or by soft ticks of the Ornithodoros genus. Warthogs and bush pigs are generally asymptomatic and are wildlife reservoirs for the virus in Afrika. While ASF had long been endemic in sub-Saharan Africa and in Sardinia, the recent spread into Trans-Caucasian countries has raised concern as ASF severely threatens the European pig industry. In spite of this fact, literature on virus-host interactions that could be used as targets for antiviral strategies is scarce. As the morphogenesis of ASFV is very complex and leads to mature enveloped intracellular virus particles and extracellular virions that carry an additional envelope, the preparation of pure extracellular virions is challenging. To date, highly purified ASF virion preparations have not been analyzed systematically using modern mass spectrometric approaches. The intention of this study is to define the proteome of the ASFV particle and to identify virus-host interactions that potentially play a role in virus morphogenesis. This work describes the purification of ASFV particles and the characterization of the proteome of mature extracellular ASF virions using shotgun nLC-MALDI-TOF/TOF mass spectrometry. A convenient and efficient workflow for the purification of ASFV particles has been established. ASFV OUR T88/3 was propagated on WSL-HP cells which originate from a natural host (wild boar) using high-density cell culture flasks with 10 layers (HYPERFlask, Corning) to achieve high virus yields. Particles were purified by a combination of differential sedimentation and gradient density ultracentrifugation steps using sucrose and Iodixanol (OptiPrep) as gradient media. Separation was based on particle density as well as on particle dynamics resulting in preparations with high specific infectivity. Use of Iodixanol facilitated the purification of virus, probably due to its low viscosity. Total virus recovery was between 35-70 % after the gradient step. The final virion preparation was characterized by electron microscopy, western blot analysis using virus-specific proteins, and finally by shotgun nLC-MALDI-TOF/TOF mass spectrometry. We present a comprehensive catalog of viral and host-derived structural ASFV proteins.

Adaptation of African Swine Fever Virus field strains to productively replicate in WSL cells is not necessarily associated with larger deletions within the viral genomes.

Keil Günther M.[1]; Goller Katja[1]; Pollin Reiko[1]; Bishop Richard[2]; Höper Dirk[1]; Blome Sandra[1]; Portugal Raquel[1]

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Keywords: ASFV field isolates, adaptation to replicate in WSL cells, NGS sequencing

The genomes of ASFV isolates are double stranded DNA and vary in size from 170 - 193 kbp. The genomes sizes vary, strain dependent, between 170 and 190 kilo base pairs. The encompass 151 - 167 open reading frames in the range from 151 to 167. Although significance and functions for many genes have been studied so far, much more still need elucidation. For doing that, cell culture adaptation appears to be a prerequisite since the natural host cells, monocytes and macrophages, are difficult to work with as primary cultures. In the past, this process was frequently associated with considerable genome modifications including large deletions. We adapted field isolates Armenia 2007, Benin 97, Kenya1033, and Sardinia 2015) to productively grow in WSL cells. Full protein coding genome sequences were so far determined for Benin 97, Kenya1033, and Sardinia by NGS. For the present, sequence comparisons revealed that the genomes of the respective WSL-adapted virus do not contain larger deletions by comparison to respective or related field strains or to other virus adaptations to Vero cells. Detailed analyses will be presented.

Scientific Session 2

"Vaccines and immune response"

Chairs:

Linda DIXON, United Kingdom

Marie-Frédérique LE POTIER, France

Response of monocyte-derived dendritic cells to virulent and attenuated ASFV strains

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Keywords: ASFV, dendritic cells, flow cytometry, cytokines

African swine fever virus (ASFV) primarily infects cells of the myeloid lineage and despite the important role of dendritic cells (DCs) in initiating adaptive immune response to pathogens, few studies have analysed their interactions with this virus. Our study therefore aimed to conduct a detailed characterization of the interactions between DCs and ASFV strains of differing virulence. Isolated porcine peripheral blood monocytes were cultured in the presence of GM-CSF and IL-4 to induce differentiation into immature DCs (MoDC). MoDC were left untreated or matured with TNF-α and/or IFN-α. Cells were infected with an attenuated strain (BA71V) or a virulent Sardinian isolate (22653/14) of ASFV or were mock-infected. ASFV infection and its consequence on MoDC phenotype and function are being analysed using flow cytometry, ELISA and confocal microscopy. ASFV 22653/14 presented a greater ability to infect both immature and mature MoDC compared to the avirulent strain. Lower levels of late protein p72 but not early protein p30 were observed in BA71V-infected MoDCs pre-treated with IFN-α, but not TNF-α, while 22653/14 retained the ability to infect IFN-α matured MoDC. Cells infected with either strain displayed a lower expression of CD16 compared to uninfected bystander cells and BA71V-infected cells presented lower percentages of MHC I compared to the mock-infected control. Ongoing experiments investigating the effects of ASFV on other surface markers expression and cytokine responses by MoDCs will be presented. It is hoped that data generated by this study will aid our understanding of the immunomodulation of host cell responses by ASFV.

Identification and utility of interferon evasion by ASFV

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Keywords: Interferon, non-homologous virus evasion genes

The interferon (IFN) system is an early innate anti-virus host defense mechanism that takes place shortly after entry of the pathogen and long before the onset of adaptive immunity. Thus, African swine fever virus (ASFV), as an acute and persistent virus in pigs, is predicted to have evolved multiple genes for the manipulation and evasion of interferon. Although, ASFV is known to interfere with signaling pathways controlling the transcription of cytokines, surprisingly no individual virus gene manipulating the induction or impact of IFN has been described. Since an initial bioinformatics search of the ASFV genome failed to identify potential antagonists of the IFN response, our strategy was to functionally screen "unassigned" ASFV genes without existing homologies, particularly from MGFs 360 and 530, in luciferase reporter assays for their inhibition of the induction and impact of IFN. Specifically, we used reporter plasmids containing the luciferase gene under the control of: 1) The IFN-β promoter, to screen for inhibition of induction of type I IFN stimulated by the addition of Poly(I:C); 2) The ISRE DNA elements, to screen for the inhibition of the impact of type I IFN; and 3) The GAS DNA elements to screen for the inhibition of the impact of type II IFN. These experiments revealed four ASFV genes inhibiting one or more of the three luciferase assays and, as will be presented, targeting different intracellular signaling intermediates. Their deletion from wild type virus may strengthen the host interferon response and so provide an attenuated form with more restricted virus spread after the initial infection, perhaps "buying" sufficient time to allow the development of a protective adaptive immune response. The demonstration of multiple ASFV genes for the evasion of IFN responses will demand technology to construct viruses with multiple gene deletions. An alternative would be a multigene DNA vaccine.

Effect of deleting African Swine Fever Virus interferon inhibitory genes on virus pathogenesis and induction of protective responses in pigs.

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The Pirbright Institute, Surrey, UK

Keywords: type I interferon, immune evasion, pathogenesis, protection

We deleted African swine fever virus genes that encode proteins that inhibit type I interferon induction or response from the genomes of already attenuated strain OURT88/3 or virulent strain Benin 97/1. The genes deleted included several members of multigene families 360 and 505/530, I329L a homolog of Toll-like receptor 3 and DP148R. Compared to virulent isolates, the OURT88/3 isolate has a large deletion of similar MGF 360 and 505 genes as well as interruptions of other genes, including the EP402R and EP153R genes that encode CD2-like and C-type lectin proteins. The results showed that deletion of these genes did not affect virus replication in macrophages but had varying effects on the induction of IFN-beta mRNA and of interferon stimulated genes. Infection of macrophages with virulent isolates induced barely detectable levels of IFN-beta mRNA whereas varying levels were induced by different gene deleted viruses. Deletion or interruption of multiple genes from MGF 360 and MGF 505/530 or deletion of DP148R gene from virulent Benin 97/1 isolate resulted in virus attenuation and induction of goof levels of protection against challenge. In contrast deletion of the DP148R or I329L genes from OURT88/3 isolate resulted in reduced protection levels compared to parental OURT88/3 virus. The results indicate that deletion of different combinations of interferon inhibitory genes offers a promising route for construction of candidate vaccine strains.

Use of a double viral gene deletion virus as a rational approach to develop ASFV live attenuated vaccines protecting against Georgia 2007 isolate

Vivian O'Donnell[1-3], Lauren G. Holinka[1], Jolene Carlson[1-2], Marialexia Alfano[1], Paul Azzarino[1], Peter W. Krug[1], Guillermo R. Risatti[2], Douglas P. Gladue[1], and Manuel V. Borca[1]

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Keywords: experimental vaccine, recombinant virus, protection, ASFV Georgia2007 isolate

ASFV vaccine strains based in the use of recombinant viruses harboring only a single viral gene deletion that is critical for virus virulence have been under development by us in previous studies (JVirol 2015 89:6048; JVirol 2015 89:8556). These experimental vaccines harbor a single deletion where either the 9GL gene or six genes belonging to the MGF 360 and 505 families are deleted. Although these two virus strains were effective in inducing protection against the homologous virulent parental virus (Georgia2007 isolate) their use with one deletion may have some potential safety issues. In order to attempt to further attenuate our vaccines and increase the safety of a potential vaccine, we have developed two new recombinant ASFVs derived from the Georgia2007 isolate where additional genes have been deleted in our original experimental vaccines. We are reporting here the methodological development of those new mutant viruses that contain deletions in two different areas of the Georgia2007 isolate, and the results obtained using these new double gene deleted viruses in terms of their immunogenicity, and by using them as experimental vaccines that could protect against virulent isolate Georgia2007.

Heterologous prime-boost vaccine strategy for ASF

Yolanda Revilla[1], SunYoung Sunwoo[2], Lina Mur[2], Daniel Madden[2], Nick Haley[2], Igor Morozov[2], Tammy Koopman[2], Elena G. Sánchez[1], Daniel Pérez Núñez[1], Marisa Nogal[1], Carmina Gallardo[3], Marisa Arias[3], Juergen Richt[2]

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Keywords: ASFV, protein, DNA vaccine, heterologous prime-boost

Although immune responses against African Swine Fever Virus (ASFV) are not fully understood, previous studies suggest the need to activate humoral and cellular immunity to obtain a protective vaccine for ASFV. Therefore, the objective of the present study was to develop protection using a new approach based on heterologous prime-boost vaccination combining ASFV antigens encoded by DNA plasmids and recombinant ASFV proteins. For that purpose, six proteins of ASFV were expressed in Baculovirus (Sf9 cells) and six ASFV genes were cloned into pcDNA 3.1 expression plasmids. The immunogenicity of the constructs were tested in two independent experiments. In total, 72 piglets were immunized with 15 different combinations of proteins and DNA plasmids. Serum and blood samples from vaccinated animals were evaluated by ELISA, ELISPOT and virus neutralization assays. The results identified some promising combinations of plasmid DNAs and antigens. ELISA antibody titers were high with a number of proteins including p15, CD2-V, p54 and p35. Interestingly, the combination of when combined of these proteins with plasmid DNA, showed increased levels of virus neutralization capacity. Specifically, neutralizing antibodies were more efficiently induced in pigs immunized with combinations of ASFV proteins and heterologous plasmid DNAs (i.e. p15 protein and CD2v DNA). Some of these combinations reached up to 80% of ASFV infection inhibition levels. The results from this work opens a new avenue for future studies on the most appropriate combination of ASFV-specific cDNAs and proteins to develop a rationally designed, safe, efficacious and DIVA-compatible vaccine for ASF.

Could live-attenuated African Swine Fever Viruses become a vaccine reality?

Paula López-Monteagudo[1], Anna Lacasta[1], Andreas Gallei[2], Veljko Nikolin[2], Javier M Rodríguez[3], Elisabet López[1], Sonia Pina[1], Francesc Acensi[1,4], María Jesús Navas[1], Laia Bosch[1,2], María Luisa Salas*[3], Fernando Rodríguez*[1]

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Keywords: ASFV, Vaccine, Crossprotection, CD8 T-cells, residual virulence

The 3rd GARA scientific meeting represents the ideal forum to discuss the future of ASFV vaccinology. Conversely to the most skeptical ones, we really believe that obtaining a safe and efficient vaccine against ASFV is not only feasible but also necessary if willing to control the disease, at least in endemic areas where stamping out policies are not feasible. In contrast with the recurrent failure of classical inactivated vaccines, live attenuated viruses (LAV) have demonstrated to confer solid protection against experimental homologous challenges, showing limited protection, if any, against heterologous ASFV challenges. Here we will present some of the most recent advances obtained in collaboration between our laboratory at CReSA-IRTA, the laboratory of Dr. ML Salas at the CBMSO-CSIC and Boehringer Ingelheim Veterinary Research Center (Hannover, Germany). Our results demonstrated the feasibility of obtaining a recombinant live attenuated virus by gene targeting, capable to confer protection not only against the lethal homologous virus challenge (BA71; Genotype I), but also against heterologous virus strains from the same (E75) and from different genotypes, including Geogia07 (Genotype II), the virus currently circulating in continental Europe. Additional evidences regarding the mechanisms involved in the crossprotection conferred will be also presented. We will finish our presentation by discussing the risk of this and any other ASFV-LAVs, mainly due to their potential residual virulence. Even if obtaining safe, efficient and affordable crossprotective vaccines: Could live-attenuated African swine fever viruses become a vaccine reality? We hope that our talk will incentivize a multidisciplinary discussion between the present experts.

Scientific Session

"Epidemiology"

Chairs:

Dolores GRAVIER-WIDEN, Sweden

Willie LOEFFEN, Netherlands

Invited speaker presentation

Armanda BASTOS

The African Swine Fever sylvatic cycle: using molecules and maths to unravel the role of the Ornithodoros tick vector

Armanda D.S. Bastos[1], Roumen Anguelov[2], Magali Jacquier[1], Carin Boshoff[1,3,4], Livio Heath[4]

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Keywords: Ornithodoros, PCR, phylogenetics, seasonal variation, modeling

The Ornithodoros soft tick plays a central role in the epidemiology of African swine fever (ASF) in the sylvatic setting, and represents a significant threat to all regions to which the disease may be introduced. This is due to the almost worldwide distribution of Ornithodoros species, many of which are capable of maintaining and transmitting the ASF virus (ASFV) under experimental conditions. Large-scale tick surveys conducted in the 1970s and 1980s in South Africa revealed that overall ASFV infection rates in ticks were low across all populations evaluated. Although surveys conducted in the last 15 years confirm that these low overall rates of infection remain unchanged, the virus may have disappeared from at least one population within the southern-most distributional range of ticks previously identified as ASFV-positive. We explore the possibility of virus extinction in a sylvatic setting, using mathematical models, and investigate seasonal variation in tick infection rates using molecular approaches. A mathematical model that incorporates an extinction process was developed which identified key parameters essential to virus perpetuation that may be useful for eradication strategies. Although there were significant seasonal differences in adults versus nymphs and males versus female ticks in the wet and dry seasons, there was no significant difference in ASFV infection rates between seasons. The molecular results further confirmed the patchy distribution of the virus whilst nucleotide sequencing and phylogenetic analysis of positive samples revealed high levels of field heterogeneity in the Kruger National Park, South Africa for ticks sampled along a 400km longitudinal gradient.

Keynote Lecture

Daniel BELTRAN-ALCRUDO

The 1996-2002 African Swine Fever (ASF) pandemic in West Africa: Lessons learnt

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Keywords: West Africa, Retrospective study, African swine fever

An ASF pandemic was observed in previously unaffected countries in West Africa during 1996-2002. It started in Côte d'Ivoire in 1996, followed by outbreaks in Benin, Nigeria and Togo in 1997, Ghana in 1999, and eventually Burkina Faso in 2003. These newly infected countries, which had significant and fast-growing pig populations, became ASF endemic. Only Côte d'Ivoire (and Ghana for a brief period) managed to eradicate ASF. Despite the importance of the pandemic and the generation of a large amount of information as a result of various activities aimed at surveillance, outbreak control or disease eradication carried out by veterinary services and international organizations, such as FAO, throughout the region, much of the data generated have remained archived and unpublished or only partially published. As a result, the dynamics and underlying factors driving this pandemic have not been fully elucidated or exploited. This study aimed to consolidate historical information generated by working towards the control and eradication of ASF. This descriptive analysis entailed the evaluation and review of archived records and reports of outbreaks, data from veterinary services, veterinary consultants and peer-reviewed publications. The analysis of this information will allow a better understanding of the disease dynamics in a region infected for the first time and learning how the prevention and control interventions that were implemented worked or failed. This will help the development of better tailored, sustainable and locally sound interventions.

Keynote Lecture

Klaus DEPNER

Wild boar as a ASF driver

Klaus Depner[1] and Vittorio Guberti[2]

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Keywords: ASF, wild boar, persistence, contagiosity

After the genotype II of ASFV reached the eastern borders of the European Union in January 2014 two main epidemiological scenarios were forecasted. ASF would fade out spontaneously from the local wild boar population or, alternatively an epidemic wave would start moving westward very rapidly, affecting large areas of Europe. However, after almost two and a half years both epidemiological hypotheses proved to be wrong. The virus did not fade out nor assumed an epidemic wave behavior. On the contrary the infection survived locally with a steady low prevalence (below 3%). Field data as well as experimental studies on ASF indicate an overall high case-fatality rate and a rather low contagiosity and low mortality during the initial phase of infection.

Current findings indicate that in wild boar populations ASF shows a pattern of habitat bound persistence lacking a tendency of dynamic spatial spread. Therefore ASF in wild boar can be considered a habitat-borne disease where infected carcasses in combination with the tenacity of the virus and the low contagiosity (contagiosity index <0.3) play a key role in capturing the disease within particular areas. In such affected areas wild boar are becoming the drivers for ASF. Such circumstances are likely to contribute substantially to months or even years of pathogen persistence explaining the current picture of ASF spreading rather slowly and with continuing circulation in affected wild boar populations. Furthermore, the wild boar density appears to be less important concerning virus persistence whereas the number of dead animals which are highly infectious and which may remain in the fields for weeks until they are completely decomposed can play a more relevant role.

Within this context, a revision of the current understanding and approaches towards ASF control and eradication is needed.

Keynote Lecture

Krzysztof ŚMIETANKA

ASF surveillance in Poland: update on current situation

Krzysztof Śmietanka[1], Andrzej Kowalczyk[2], Grzegorz Woźniakowski[2], Edyta Kozak[2], Magdalena Łyjak[2], Małgorzata Pomorska-Mól[2], Zygmunt Pejsak[2], Krzysztof Niemczuk

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Keywords: African swine fever virus, polymerase cross-linking spiral reaction, pigs and wild boars

Since the 1st of January 2014 till 30th of April 2015, 92 cases of ASF in wild boar (WB) and 3 outbreaks of ASF in domestic pigs (DP) have been detected in Poland. These events occurred in the same area of 11 municipality in Podlaskie region. Samples of blood and internal organs were collected. The sections of tissues were processed as 10 (w/v) homogenates in PBS then submitted for DNA extraction. Next, real-time PCR was conducted with primers and probe complementary to the conserved p72 gene sequence (Fernandez-Pineiro et al. 2013). ELISA was conducted using the Ingezim PPA Compac 1.1.PPA K3 ELISA kit (Ingenasa). Immunoperoxidase test (IPT) was performed using fixed VERO infected Ba71V ASFV cells on 96-well plates. All samples from DM and WB were tested by real-time PCR. In total 33,317 examined WB, 148 were found as ASF-positive. From all 148 ASF-positive WB, 128 were positive only in PCR (including 11 from hunted WB), 7 positive by PCR and serological methods (including 3 from hunted WB), and 14 only in serological methods (all 14 from hunted WB). Prevalence of ASF in WB was the highest in found dead animals (2.65%), indicating that ASF-cases in WB in Poland are caused by high virulent virus, when infected animals supposed to die so quickly that immunological system is not able to produce antibodies. Number of survivors was found lower than 1% (0.0565 % prevalence of serologically positive hunted WB) confirming /supporting the thesis concerns a possibility of recovering small percentage of animal infected with high pathogenic type of ASFV.

Scientific Session 3

Epidemiology
"Focusing in Europe"

Chairs:

Dolores GRAVIER-WIDEN, Sweden

Willie LOEFFEN, Netherlands

African Swine Fever Virus spread in Estonia: the preliminary results of epidemiological analysis

Nurmoja Imbi; Kristian Maarja; Blome Sandra; Gallardo Carmina; Viltrop Arvo

Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, Estonia - Veterinary and Food Laboratory, Estonia - Veterinary and Food Board, Estonia - Friedrich Loeffler Institute, Institute of Diagnostic Virology, Riems, Germany - European Union Reference Laboratory for African Swine Fever, Centro de Investigacion en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain

Keywords: African swine fever, epidemiology, wild boar, domestic pig

As a result of extensive spread of the highly virulent ASFV genotype II in Eastern Europe since 2007, the first case of the disease in Estonia was confirmed in September 2014. The first infected dead wild boar in Estonia was found in the very south of Estonia, 6 km from the Latvian border. Subsequently, the virus was detected in wild boar in 3 counties bordering Latvia, over the following three weeks, but also in northeast Estonia in a location over 200 km from the other ASFV affected counties. The epidemiology of the infection in these two areas was different during the first year of the epidemic – in the southern outbreak area high mortality among wild boar was observed. However in the northeast outbreak area there was no mortality, and among hunted animals most wild boar were only antibody-positive. A challenge experiment conducted at FLI in Germany, with the virus isolated from the northeast outbreak, revealed that the virus was virulent causing 90% lethality among challenged animals. This indicates that the incursion of the virus into northeast Estonia could have happened much earlier. Starting from July 2015, the ASFV situation in wild boar changed drastically, when the virus spread fast to new previously uninfected areas. In addition, during the eight-week period in summer 2015, 18 outbreaks in domestic pigs were confirmed. In total, 1 836 ASFV positive wild boar have been found in 12 counties between September 2014 and April 2016. The ASFV strain currently circulating in Estonia, belongs to the p72 genotype II, which has been circulating in Eastern European countries since 2007. However, in July 2015, on the basis of the central variable region (CVR)-PCR product analysis, a new genetic variant of the virus was detected in wild boar in southern Estonia.

Evaluation of insect larvae as possible mechanical vectors for transmission of ASFV in wild boar populations

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Keywords: African swine fever virus, blow fly, maggots, mechanical vector

African swine fever (ASF) is a contagious viral disease of domestic pig and European wild boar. In 2007, ASF virus (ASFV) was introduced into eastern Europe, reaching the EU in 2014 (Baltic States, Poland). Considering the rapidly progres sing course of disease with case fatality rates of up to 100 %, it is unclear why the virus keeps slowly spreading through the East European wild boar populations in the absence of the only known vector (Ornithodoros soft ticks) instead of quickly eradicat ing its natural hosts and, ultimately, itself. In addition to direct contact to infected animals with high virus load, pigs and wild boar can become infected by oral ingestion of ASFV-containing organic material in which the virus remains stable for weeks or even months. Thus, transmission might also be possible through carcasses and insect larvae developing on those, which are known to belong to the diet of wild boar. To check the hypothesis of immature insect stages serving as mechanical vectors of ASFV, larvae of two commonly found necrophagous blow fly species, Lucilia sericata and Calliphora vicina, were bred in the laboratory on ASFV-infected wild boar tissue. After preset periods of development, maggots were removed and tested for replicating virus via titration on porcine macrophages and for viral DNA by qPCR, both in an unwashed status and following external cleaning. The study is meant to contribute to understanding the epidemiology of ASF in eastern Europe and to predicting the further spread of ASFV within the wild boar population.

Quantification of transmission of African Swine Fever Virus by carriers and through indirect environmental contact

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Keywords: ASF, virus, carrier, transmission

The Eastern European ASFV strain seems almost 100% lethal. After infections with less virulent strains, however, several pigs survive the infection and become carriers, defined as pigs recovering from the acute disease, no longer showing clinical signs, but virus positive and potentially being infectious. The infectivity of these carriers was determined in an experimental transmission study. Twenty pigs were inoculated with ASFV through the intranasal route with the moderately virulent strain Netherlands '86. Six pigs survived the acute phase of the infection and became carriers. These six carriers were housed in clean pens, 28 days after they were inoculated, one pig per pen. In each pen, one contact animal was added for a maximum of 13 days, or until the contact animal became infected. After 13 days, all remaining contact pigs were removed and 6 new contact pigs were added 24 hours later for another 13 days (or until they became infected). Between day 28 and 41 after the inoculation, none of the contacts became infected with ASFV. Between day 42 and 55 after inoculation, two of the six contacts became infected with ASFV. The overall transmission rate in carriers was estimates to be 0.019 (0.0027-0.048) per day. This is much lower than in the acute phase of the infection, where transmission rates for this particular virus range fr om 0.45 to 0.92 per day. Even though virus transmission from carriers is very limited, on a population level this could be a crucial factor for the virus to become endemic in wild boar.

Active management of ASF spread in wild boar: strategic options and plausible solutions

Hans-Hermann Thulke, Martin Lange

Helmholtz Centre for Environmental Research Leipzig - UFZ

Keywords: wild boar, control measures, population reduction, model

African swine fever (ASF) is an emerging viral disease in Eastern European domestic pigs and wild boar. Since its introduction into Poland and the Baltic states in early 2014, ASF spreads continuously in wild boar populations, despite its successful control in domestic pigs. The objective of this study was the investigation of alternative ad-hoc concepts proposed to halt ASF spread in wild boar. For the moment vaccination of wild boar is not an option. All expert debates regarding useful control measure target population regulation but with alternative measures and timelines. Here, the two extreme concepts are compared in a virtual wild boar population affected by ASF: 1) a drastic, active depopulation (e.g. removal of 80% of the population within four months), versus 2) "soft" passive population reduction through usual hunting but targeting any female able to reproduce for multiple hunting seasons. To quantitatively compare the strategy concepts, an individual-based epidemiological model was implemented. The model was parameterized from literature regarding the demography of wild boar, the epidemiology of ASF, any others were calibrated to the spatio-temporal dynamics of the most recent development of ASF in Eastern Europe. Applying the two candidate measures over different dimensions and variable effectivity lead to the following insights: Drastic depopulation towards less than 20% of the initial density showed a timely effect and required a control zone of about 50 km width. Targeted hunting applied as efficient, was effective in the prevention of spread after at least two years of application. This would require a control zone of about 200 km width. The practicability of such control measures can be debated. Therefore, we applied the two approaches in combination in a strutured approach. The improved, more practical dimensions of measures in space and over time are presented. The mixed strategy was useful to halt spread of ASF in the model population. The role of carcass, resp. their removal, will be addressed in the context of these measures.

Towards quantitative understanding of the role of carcasses in the transmission of ASF in wild boar or feral swine

Martin Lange, Hans-Hermann Thulke

Helmholtz Centre for Environmental Research Leipzig - UFZ

Keywords: wild boar, uncertainty, transmission, carcasses

In Eastern Europe, African swine fever was found spreading predominantly in wild boar populations. However, experimental data suggest limited transmission of ASF virus (ASFV) through direct contact between living hosts. The transmission through virus -laden blood is well acknowledged and confirms the specialisation of the virus to vector transmission (soft ticks). In Eastern Europe the missing vector link but continuous spread in wild boar populations pointed to the carcasses as possible driver in the host-to-host transmission of ASFV in wildlife. Literature as well as expert opinions are contradicting regarding the functioning of carcass mediated transmission of ASFV. The reason is the special social behavior observed with wild boar towards dead conspecifics. The knowledge gap was the accessibility of dead animals for non-mate wild boar groups (between group contacts) and the approach behaviour of wild boar in the vicinity of dead conspecifics (transmission risk given an accessible carcass). In this study we estimated the two unknowns by compiling observational data of ASF notification in wild boar with an agent-based spatial-explicit ecological model simulating spread of the disease on the landscape scale. Methodologically, thousands of ASFV epidemics were simulated on the same geography where the observational data was collected starting from the reported scene of introduction. In the simulations the two uncertain traits of ASF were varied across the complete technical range (0-1). Subsequently, virtual sampling and a set-theoretic version of the confusion matrix was used to identify those simulations that were congruent with the observational data. The data-driven procedure selected parameters in favor of hypotheses that argue about the severity of the clinical phase of the infection which would disable systematic retreat of sick animals, i.e. greater values of the carcass accessibility. Truly infective contacts to carcasses, however, should be rather sporadic, i.e. minimal chance of actual transmission. Moreover, findings definitely exclude assumptions of targeted and intensive resource use through scavenging. The findings enhance comparative assessments of control measures in wild boar, stipulate adequate field experimentations and increase awareness for the carcass issue.

Research on African Swine Fever Threat Reduction Through Surveillance in the Ukraine: An Update

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Keywords: African swine fever, soft ticks, epidemiology, wild boar, Ukraine

African swine fever (ASF) is a high-consequence viral disease that threatens the pig farming industry in Europe. ASF outbreaks have occurred in Ukraine. The factors driving the spread of ASF among domestic swine and wild boar populations in the region remain to be fully understood. Our collaborative research project was established to generate science-based knowledge that could be used to implement a system for sustainable surveillance of ASF virus (ASFV) in Ukraine. Because ASFV can replicate in, and be transmitted by certain soft tick species, research efforts focused on minimizing the cost of early ASFV detection by determining the necessary sample size of soft ticks and suids for accurate detection in a local region; perform small scale surveillance efforts in selected regions to establish methods for a routine, national program; and train personnel to perform a routine, national surveillance program. ASFV infection in wild boar populations was documented. The transboundary movement of ASFV-infected wild boars appears to be a risk factor for outbreaks in the Ukraine and the region. Soft tick surveillance was conducted in the context of global change. This is significant because the last reports of soft tick research in Ukraine were published more that 50 years ago. Soft tick collections evidence another risk factor for the emergence of ASF. Soft tick field and laboratory research capabilities in Ukraine offer the opportunity to investigate soft tick-pig/wild boar-ASFV interactions through collaboration with other science partners in the region. These efforts will help bridge the knowledge gap on the epidemiology of ASF in Ukraine, which is required to develop countermeasures that mitigate the burden of this high-consequence disease for the pig farming industry in Europe. *USDA is an equal opportunity provider and employer. This research project was funded by the U.S. Defense Threat Reduction Agency (CBEP Agreement IAA# U.S.C. 3318(b) – 15217).

Scientific Session 4

Epidemiology
"Focusing in Africa"

Chairs:

Eric ETTER, South Africa

Charles MASEMBE, Uganda

Keynote Lecture

Charles MASEMBE

How important are wildlife and domestic livestock interactions for African Swine Fever endemic situation in Africa

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Keywords: African swine fever, bushpig, livestock, warthog, wildlife

The pig industry in many sub-Saharan African (SSA) countries is on the rise and is anticipated to continue growing in the coming years. This growth is mostly happening in developing countries and 75% of this is practiced by smallholder farmers. The population of pigs in SSA has increased fourfold in the last 50 years. This industry is however faced by the lethal and devastating African swine fever (ASF) disease. ASF exists both in the wild and the domestic pigs. There is a high chance of ASF transmission between wild and domestic pigs due to the porous interface. The main wild species of concern are the warthog (Phacochoerus africanus), the bushpig (Potamochoerus larvatus), and the soft tick (Ornithodoros genus). in order to appreciate the role played by the wildlife in the maintanace, and transmission of ASF, concerted efforts are needed to investigate the existence, prevalence, and characteristics of the ASF virus from each of the above species. This keynote presentation will highlight the potential role played by the key species and point the challenges faced while conducting research on ASF in Africa.

Epidemiology of African Swine Fever in Africa today: Sylvatic cycle versus socio-economic imperatives – filling the gaps

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Keywords: ASF; warthog; domestic pig; outbreaks

The diagnosis of African swine (ASF) fever in Kenya in 1910 and its association with wild African suids (warthogs and to a lesser extent bush pigs) and the well-documented sylvatic cycle between warthogs and soft ticks of the Ornithodoros moubata complex sharing their burrows has resulted in an indelible impression that wild suids are the major player in ASF transmis sion in Africa. Available information about more than 4,900 outbreaks reported to the World Organization for Animal Health (OIE) by 33 countries in Africa in the period 1996 – 2016 has been examined. The sylvatic cycle (SC) involving warthogs is known to occur in 10 of the countries and suspected but not confirmed in 5 more (Angola, Burundi, Congo Republic, Democratic Republic of Congo and Rwanda). These SC countries accounted for 48.9% of the reported outbreaks. Therefore 51.1% of the outbreaks occurred in 15 countries where wild suid involvement could be excluded. The probable cause of 61.1% of all reported outbreaks in SC countries could not be determined from the available information. In three SC countries (Botswana, Namibia and South Africa) 90 to 100% of the outbreaks originated from warthogs/ticks. However, in the remaining SC countries, warthog involvement was suspected in on ly 7 outbreaks (6 in Zambia and 1 in Zimbabwe) (0.7%), with 939 (99.3%) outbreaks reported to have originated within domestic p ig populations, predominantly as a result of movement of live pigs, feeding of uncooked swill containing infected pork, and scav enging by free-ranging pigs. The predominance of primary domestic outbreaks makes investigation of the socio-economic issues driving risk practices in pig husbandry and trade (already identified in several countries) imperative for better management of ASF.

Quantitative assessment of socio-economic impacts of African Swine Fever outbreaks in northern Uganda

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Keywords: smallholders, social impacts, economic impacts, ASF outbreaks

Outbreaks of infectious animal diseases may disrupt the livelihoods of poor people in the same ways as civil unrest or other catastrophes. In poor households these impacts are aggravated as the animals represent multiple roles. Uganda is a low-income country with the largest pig population in east Africa. The majority of pigs are kept by smallholders. African swine fever (ASF) is endemic in the domestic pig population. The objective of this study was to investigate the socio-economic impacts of ASF outbreaks in northern Uganda in a longitudinal survey. Structured interviews with two hundred, randomly selected, pig-keeping households were undertaken three times with six months interval. Questions related to family- and pig herd demographics, social and economic variables, pig trade and pig business. The study showed that pigs were kept in extreme low-input-low-output farming systems involving very small monetary investments. Household ASF yearly incidence was nineteen percent. Outbreaks resulted in lower gross margin income from the pig production. This negative effect could partly be explained by the households' poverty status and the size of the pig herd. Trade and consumption of sick and dead pigs were coping strategies used to minimize losses of capital and animal protein. The results indicate that causality of economic impact in smallholder systems is complex. Pigs are mostly kept as passive piggy banks rather than active working capital, disqualifying disease control arguments based only on standard economic models and instead requiring solutions adopted to the local situation. The quantifications of the losses incurred by ASF outbreaks can serve to advice international bodies and governments towards the value of improved animal health in low-income countries.

A molecular and ecological investigation of the role of the bushpig in the epidemiology of African Swine Fever at the wildlife-livestock interface in Uganda

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Keywords: African swine fever, bushpig, Potamochoerus larvatus, epidemiology, and wildlife-livestock interface

African swine fever is a devastating hemorrhaging fever of pigs, caused by the Africa swine fever virus (ASFV). The disease causes up to 100% mortality in domestic swine, depending on the strain. The bushpig, Potamochoerus larvatus, is known to be susceptible to ASF and yet it shares a natural interface with both warthogs and domestic pigs. The bushpig's role in the epidemiology of ASFV is not adequately understood. This study used a molecular and ecological approach to investigate the role of the bushpig in the epidemiology of ASFV at the wildlife-livestock interface in Uganda. GPS/GSM tracking collars were used to monitor the movement pattern of free-ranging domestic pigs and bushpigs. Five free-ranging domestic pigs at the wildlife-livestock interface were collared for two weeks. Three bushpigs were captured and collared for two months. ASFV detection was done in domestic and wild pig samples using RT-PCR. A new-recorded home range of 8.5 square kilometers of bushpigs in Uganda was observed. Interestingly, there was an overlap between free-ranging domestic pigs and bushpig activity times, indicating the possibility of interaction between the two species. 14 free-ranging domestic pig blood samples, and two bushpig blood samples tested positive for ASFV. The bushpigs could be playing a role in ASF epidemiology at the wildlife-livestock interface and serve as a link between the sylvatic and non-sylvatic cycle.

Experimental versus natural determinants influencing the vector competence of Ornithodoros soft ticks for African Swine Fever Virus

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Keywords: Ornithodoros soft ticks, African swine fever virus, vector competence, determinants

African Swine Fever (ASF), presently occurring in Africa, Caucasus and Eastern Europe, is one of the most economically devastating viral diseases for swine. Although ASF is contagious, soft ticks of the genus Ornithodoros can be involved in the epidemiology of the disease in some countries, as vectors and natural reservoirs of African Swine Fever Virus (ASFV). As soon as ASFV is introduced in a free area where the presence of Ornithodoros soft ticks is confirmed or suspected, one main issue is the possibility of ASFV transmission through tick bites and the long-term persistence of ASFV in tick reservoirs because of the obvious difficulties to eradicate such ticks in pig buildings. The vector competence of soft ticks for ASFV has been mainly studied by mimicking natural tick-to-pig and tick-to-tick transmission cycles through experimental infections in laboratory. As a consequence, experimental design can highly impact the vector competence results, as far as experiments may be sometimes limited by technical inherent constraints. By selecting and reviewing the 32 available papers and theses published since 1969 to date on the vector competence of Ornithodoros soft ticks for ASFV, it was possible to identify the different ways to define vector competence depending on authors, and to highlight the diversity of methodologies used to test and measure ASFV vector competence. An analysis of experimental (tick infection route, ASFV titer in blood meal, tick colony status and tick development stage…) versus natural (tick species, ASFV isolate, host of ASFV isolate…) factors that may influence tick infection with ASFV was helpful to propose optimized processes to quickly and efficiently test the vector competence of Ornithodoros soft ticks for ASFV. Knowledge of the potential biases due to the methodology is also useful to be much more critical on results and be able to conclude on general patterns of ASFV vector competence.

The potential role of the bushpig (Potamochoerus larvatus) in the spread and maintenance of African Swine Fever at the wildlife-livestock interface in Uganda

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Keywords: African swine fever, Potamochoerus larvatus, wildlife-livestock interface, Uganda

In Uganda, African swine fever (ASF) is endemic in domestic pigs and causes large economic losses for rural communities. Although warthogs (Phacochoerus africanus), in association with soft ticks are considered the main wild vertebrate host of the virus in the endemic African setting, they are not the only wild African suids with a potential role in ASF epidemiology. The sylvatic cycle also involves the bushpig (Potamochoerus larvatus) but to date, little is known about the epidemiological role of this species in the maintenance and spread of ASF, although it is likely to share habitats and resources with both, warthogs and free ranging domestic pigs. A previous pilot study carried out in Uganda showed that bushpigs can be naturally infected with ASF virus and can seroconvert. The aim of this ongoing study is to increase the sample size of captured and sampled bushpigs 1) to be able to estimate ASF prevalence and seroprevalence and 2) to better evaluate their temporal and spatial interaction pattern with domestic pigs. Blood from bushpigs has been sampled and tested for ASF virus genetic material and antibody detection. We have fitted domestic pigs and bushpigs with GPS harnesses, estimate their home ranges and use of habitat to determine where and when the interspecies contact is more likely to occur. The expected results should highlight the role of the bushpig in the sylvatic cycle of ASF and the way it could be connected with the domestic cycle. Further research will then be needed to investigate what are the route of transmission and if this species is able to maintain the virus.

Integrated study of African Swine Fever in East Africa identify the disease drivers and provides insights for targeted surveillance and control

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Keywords: African swine fever, Epidemiology, Maintenance ,Transmission, Control

We conducted studies to generate insights into factors influencing African swine fever (ASF) occurrence and control. Epidemiological research linked to laboratory diagnostics, mathematical modeling, social networks study and value chain analyses were used to generate evidence for control. ASF virus in internal tissues of healthy pigs demonstrated a carrier state. A 17% prevalence of exposure of pigs to bites by the ASF virus tick vector (Ornithodoros Moubata) was shown. High burden of other super-infections including helminthes, enteric viruses and ectoparasites was demonstrated in the pigs, portending reduced to increased host resilience and thus susceptibility to ASF virus. Critical ASF transmission paths and nodes using social networks analysis showed that pig value chain actors play a critical role in spreading the disease. Molecular characterization of ASF viruses in the area showed close similarity and limited diversity consistent with historical East African viruses from a genotype IX family. The study identified drivers for virus maintenance, transmission and spread, this understanding not only important in tracking virus movement but also important for trans-boundary surveillance of ASF. Results suggest need for innovative approaches for Prevention, Detection and Response (PDR) to ASF based on the epidemiological evidence to mitigate transmission and spread locally and globally.

Scientific Session 5

"Molecular Epidemiology and Diagnosis"

Chairs:

Carmina GALLARDO, Spain

Armanda BASTOS, South Africa

Molecular characterization of Sardinian African Swine Fever Viruses based on analysis of p30, CD2V and I73R/I329L variable region

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Keywords: African swine fever virus genotyping; Sardinia; CD2v

African Swine Fever (ASF) is an highly lethal hemorrhagic disease affecting pigs. ASF is present in Africa, in several Eastern and Central European countries and in Sardinia (Italy). Previous sequencing data of genes that codify for viral protein p54, p72 and the Central Variable Region (CVR) within the B602L gene, revealed that Sardinian isolates show a very low level of variability. In this study we have chosen three different genome regions to investigate the within-genotype relationships and to provide a more accurate assessment of the origin of outbreaks. The analysis of p30 and I73R/I329L sequences obtained from ASFV collected in Sardinia over a 13–year period confirms a remarkable genetic stability. The sequence comparison of the protein encoded by the EP402R gene (CD2v), carried out on various strains from 1978 to 2014, revealed a temporal sub-division of Sardinian viruses into two sub-groups: one group includes isolates from 1978-1990 and the second one is comprised of the viruses collected in the period 1990- 2014. These data, together with those obtained from CVR within the B602L gene analysis, demonstrated that the viruses circulating in Sardinia belong to p72 genotype I, but have undergone genetic variations in two different regions of the genome since 1990. We proposed the cytoplasmic region of CD2v protein as a new genetic marker that could be use to analyze ASFVs from different locations in order to track virus spread. Our study reaffirms the need to analyze other genome regions in order to improve the molecular characterization of ASFV.

African Swine Fever (ASF) genotype II in Zimbabwe durina 2015

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Keywords: Genotype, Zimbabwe, domestic pigs

Zimbabwe borders South Africa to the south, Botswana to the west, Zambia to the northwest, and Mozambique to the east and northeast. An outbreak of a severe haemorrhagic disease in domestic pigs based on clinicat signs was reported to veterinary officiais in July 2015 in the Mashonaland Central area, Zimbabwe. During the 2015 suspected outbreak of ASF the first cases were reported in Foya and Mukumbura, Mt Darwin, Mashonaland Central, Zimbabwe on 10 July 2015 in free-ranglng swine of varying age groups. The aim of this study was to characterise the African swine fever viruses (ASFV) responsible for the 2015 outbreak in Zimbabwe. The infection, post mortem and laboratory reports confirmed the incidence of ASF. Molecular diagnosis and characterisation of ASFV outbreak stralns was performed on tissue samples collected in July 2015 from domestic pigs. Genetic characterisation of the variable 3'-end of the B646L gene and complete E183L gene as weil as the central variable region (CVR) of the 9RL ORF indicated that the 2015 outbreak of ASF viruses grouped with other genotype II viruses which caused outbreaks in Malawi, Tanzania, Mauritius as weil as Mozambique. The p72 and p54 data suggest that the disease was caused by closely related ASFVs, since they had 100% nucleotide similarity. This study constitutes the first description of genotype II in Zimbabwe.

Detection of antibodies to Ornithodoros saliva antigen in domestic pigs from Cross River and Taraba states, Nigeria: Pilot Study

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Keywords: African swine fever, Tick Salivary antigen, ELISA, Nigeria

African swine fever (ASF) is a viral disease with severe consequences and its growing distribution is a threat to the pig industry glabally. It is caused by ASF virus (ASFV), the only DNA virus transmitted by an arthopod vector, soft ticks of the genus Ornithodoros. These ticks have been reported in Europe, East and Southern Africa to be part of ASFV transmissio cycle and are responsible for genetic variations and long-term maintenance of the virus in those regions. Since 1996, ASF has continually been reported among domestic pigs in Nigeria with a few reports from wild pigs. In an attempt to assess the risk of tick-domestic pig transmission cycle in Nigeria, 728 serum samples were collected from domestic pigs in two states of Nigeria (Cross River and Taraba) and analysed for anti-tick antibodies using the recombinant rtTSGP1 indirect ELISA. Of the samples analysed, 27 showed moderate to high reactivity, which was indicative of tick bites among the sampled domestic pigs. Positive sera were found in the two sampled states, both of them having optimum climatic conditions for soft ticks as well as national parks harboring wild swine (warthogs) able to maintain the developmental cycle of the ticks. Although this study did not include the direct searching of soft ticks within the study area, it provides solid evidence of interaction between soft ticks and domestic pigs in Nigeria's ecosystem. This finding will support further studies to establish the role of ticks in the epidemiology of ASF and informed control and eradication in Nigeria.

Epidemiological study and socio-economic impact associated with persistence of African swine fever in Southern highlands of Tanzania.

[1]

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Keywords: African swine fever, Northern and Southern Tanzania, Genetic variability, Epidemiology, Genome sequencing, persistence factors, next generation sequencing

In Sub-Saharan Africa, African swine fever is the disease that has caught attention since it is bringing great loss in the Agriculture field and to the society, and Tanzania is among the countries that are most affected by this disease. ASFV is maintained in a sylvatic cycle between wild swine (warthogs and bushpigs) and argasid ticks of the genus Ornithodoros. Deaths of pigs especially in the places with high pigs' population have been experienced in the Northern and Southern parts of Tanzania where most pigs are kept due to the frequent outbreaks of African swine fever. The genetic nature of the ASF viruses involved in the outbreaks has been partially determined and has indicated persistent circulation of ASF within the pig population rather than its emergence from warthogs. The main objective of my research is to investigate the genetic variability and relatedness of ASFV and factors that lead to persistence of ASF in Tanzania. The specific objectives are to investigate the epidemiology of ASF through genome sequencing of ASFV recovered from pigs during new outbreaks and in archived samples, to investigate the factors that lead to persistence of ASF in order to enhance the implementation of simple ASF control approaches and to determine the socioeconomic impacts of ASF, and lastly to provide education to the farmers upon the implantation of simple ASF control approaches. The methodologies in this research are developed respectively to the objectives outlined; epidemiological links between different ASF outbreaks will be determined by comparing ASFV recovered from pigs, warthogs and ticks, genome sequencing of ASFV in collected and archived samples will be done using a combination of dideoxynucleotide and next generation sequencing.. For the socioeconomic impact and control options for ASF there will be the collection and analysis of quantitative and qualitative data to assess knowledge. The main outcome that is expected from my study is to bring about the total control of African swine fever virus in Tanzania and to help the African society all together in searching for better lives of people especially farmers without the presence of ASF. This study will help with the controlling of African swine fever in Tanzania that has brought about high loss and low socioeconomic status in the society.

A new African Swine Fever Virus strain of genotype I circulates among pig herds in southwest Nigeria

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Keywords: African swine fever virus, sequencing, phylogeny, Southwest Nigeria

Twenty two different genotypes of African swine fever (ASF) virus based on the p72 gene circulate on the African continent. Nigeria had experienced epizootics of ASF in pig herds between 1997 and 2005 in the southwest region of the country; with the implicating strains identified. However, ASF is believed to be currently enzootic in southwest Nigeria; and the circulating ASF virus strain in the enzootic state is unknown. This study aimed at identifying the current circulating field strain (s) of ASF virus and the evolutionary trend overtime in southwest Nigeria. DNA extraction of 144 pooled blood samples by farms was done according to the manufacturer's description. The extracted nucleic acid was amplified using the conventional PCR targeting p72 gene with expected band weight of 850bp. The amplified PCR products were sequenced by Sanger sequencing and the genomic sequence analyzed using BioEdit, BLAST and MEGA 6 software. Of the 144 pooled blood samples by farms, only 11 (7.6%) had bands at 950bp. A new field strain of ASF virus of genotype I that shared the same ancestry with ASF virus strains or isolates from Spain and Brazil was identified as circulating among pig herds in the enzootic state. The new field strain also differs in amino acids composition from previously identified ASF virus field strains in southwest Nigeria during epizootics. The current circulating field strain of ASF virus identified is suggestive of a mutational change likely responsible for the decrease in morbidity and mortality recorded in sporadic cases.

Rapid detection and epidemiological surveillance of African Swine Fever using oral fluid

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Keywords: Diagnostics, oral fluids, surveillance

In the event of an African swine fever virus (ASFV) outbreak, the key to rapid detection and control will be immediate access to accurate diagnostic tools and an efficient surveillance (sampling) strategy. The major barrier to efficient surveillance is the complexity and cost of collecting and testing statistically appropriate numbers of samples. Oral fluid-based diagnostics provide a possible solution by allowing pen-based sampling. Using specimens derived from an experimental ASFV contact-infection model, we compared the diagnostic performance of a selection of commercially available diagnostic kits and OIE-recommended tests for the detection of specific antibody and viral DNA. A comparison of diagnostic performance as a function of specimen was also performed (i.e., oral fluid vs. serum vs. fecal specimens). In three groups of pigs (n=6, n=8, n=16), 2, 2 and 4 "donor" pigs, respectively, were intramuscularly inoculated with the moderately pathogenic Malta/78 strain of ASFV and then returned to the remaining (contact) pigs in each group. Donor pigs developed severe disease between 6 and 11 dpi and were euthanased having reached predetermined humane endpoints. Contact pigs began showing clinical signs from day 5 dpi, which progressed to severe clinical signs between 14-35 dpi, at which point the majority of pigs were euthanased. Oral fluids collected with cotton rope, oral swabs, and faeces (pen) were collected daily and blood was taken every 2-3 days. Preliminary antibody results indicated that oral fluid samples were comparable with individual oral swabs (100% agreement) and were consistent with the development of serum antibodies, albeit at much lower titres. Similarly, PCR detection of viral DNA in oral fluid samples showed high levels of agreement with individual oral swabs (88%) and whole blood samples (67%). Viral DNA was not detected in any of the pen fecal samples tested, indicating that this sample type was not suitable for PCR testing. The results of additional testing, including comparison of diagnostic kits, and the implications for ASFV laboratory diagnosis will be discussed in this presentation.

Evaluation of a portable realtime PCR platform (TCOR-8) for ASF during outbreaks in an endemically infected population in Uganda.

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Keywords: African swine fever, on-site diagnosis, molecular diagnostics

The objective of this study was to evaluate a diagnostic system for the detection of ASFV on-site or in simple lab settings in remote areas. The study was carried out within an OIE twinning project between the Swedish National Veterinary Institute, and the Ugandan National Animal Disease Diagnosis and Epidemiology Center. Blood and tissue samples were collected from 56 sickly or apparently healthy domestic pigs during outbreak investigations in Uganda. Sample preparations included simple dilution in PBS or nucleic acid extraction using magnetic beads-based method. A dried-down ASFV PCR kit with internal control (Tetracore ASFV, Tetracore Inc., Rockville, Maryland) was used on a portable real-time PCR thermocycler TCOR8 (Tetracore Inc., Rockville, Maryland), performed on-site in the affected villages and/or in a simple lab setting. As a reference, the OIE recommended UPL assay was performed on a stationary instrument at NADDEC. Twenty-two samples were initially found positive for ASFV using the Tetracore ASF kit on the TCOR8. Two samples had high Ct values (38 and 39.9, respectively) and became negative when retested by the same assay, and where considered false positives. Sixteen samples were positive regardless of sample preparation methods. Contamination occurred in one diluted sample, as the nucleic acids extracted from the blood sample were negative for ASFV. For the non-extracted samples, inhibition was more evident in most 20-fold diluted compared to the 40-fold diluted blood samples, as revealed by shifting of Ct values of the IC. The results of the Tetracore ASF on TCOR8 were in very good accordance with those of the reference method. The results have important implications for molecular detection of ASFV on-site or in a simple lab setting. By testing non-extracted, simply diluted, blood samples, confirmation of an outbreak can be performed within 1.5-2 hours, and appropriate actions can be taken. The experience of performing the real-time PCR assays on-site highlighted critical factors that need to be considered, including biosafety issues, simplicity of sample preparation and turn-around time.

Posters

P01

Development of monoclonal antibodies against ASFV major structural proteins: p72, p54, and p30

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Keywords: African swine fever virus, p72, p30, p54, monoclonal antibodies

African swine fever virus is the etiologic agent of one of the most devastating diseases of swine for which there is currentl y no vaccine. The aim of our study was the development of monoclonal antibodies (mAbs) against three major ASFV structural proteins (p72, p54, and p30), and their epitope mapping. The final goal is their use as reagents in the development of diagnostic assays and research reagents. Anti-p72 and anti-p54 mAbs were produced at PIADC against the respective proteins based on ASFV Georgia/07 strain, while anti-p30 mAbs were produced at KSU against a p30 protein based on ASFV BA71V strain. The epitope mapping was conducted by indirect ELISA and western blotting using overlapping fragments expressed in E.coli as antigens. A total of 29, 12, and 3 mAbs were produced for p72, p54, and p30, respectively. The p72 mAbs reacted with the region between amino acid (aa) 110 and 171, 180 and 250, 280 and 303; 5 out of 12 p54 mAbs reacted with the region between aa 60 and 143; 1 out 3 p30 mAbs reacted with the region between aa 130 and 143. We also estimate the relative affinity and identified good candidates for diagnostic assay. The fine mapping using commercially synthesized overlapping oligomers is currently ongoing.

P02

Dynamics of protection against virulent challenge in swine vaccinated with attenuated African Swine Fever Viruses and their differences in pathogenesis and immune responses

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Keywords: ASFV, Interferon-gamma, Cytokine, Antibody, Protection

African swine fever is a lethal hemorrhagic disease of swine caused by a double-stranded DNA virus. There is no vaccine to prevent the disease and current control measures are limited to culling and restricted animal mov ement. Swine infected with attenuated strains are protected against challenge with homologous virulent virus but there is limited knowledge of the host immune mechanisms generating that protection. Swine intramuscularly infected with Pret4 virus develop cl inical disease by day 3-4 pi ending with all swine dead or euthanized by day 10 pi, while a derivative strain lacking virulence-associated gene 9GL (Pret4 delta9GL virus) is completely attenuated. Groups of swine were infected with Pret4 delta9GL virus and challenged with the virulent parental virus at 7, 10, 14, 21, and 28 dpi. ASFV-specific IFN-γ production in PBMCs, as well as anti-ASFV antibodies and cytokines in serum were assessed in each group. Challenge at 7 dpi had 40% surviving while other groups challenged between 10 and 28 dpi had 60-80% surviving challenge. This model was used to correlate the presence of host immune response and protection against the challenge. Protection increased with time between first infection and challenge, with the exception of ASFV-specific antibodies in the surviving swine challenged at 21 and 28 dpi, no solid correlation between any of the parameters assessed and the extent of protection could be established. These results underscore the complexity of the system under study where it is very plausible that protection against disease or infection relies heavily on the concurrence and/or interaction of different host immune mechanisms.

P03

Comparative study of protection in pigs immunised by different routes and doses with the naturally attenuated African Swine Fever Virus isolate OUR T88/3 and role of immunomodulatory cytokines.

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Keywords: ASFV, Vaccine, Immunity

The present study is an extension of previous works where protection induced by intramuscular administration of the attenuated OUR T88/3 genotype I isolate was demonstrated. In the work reported here, comparative assessments were utilised in order to demonstrate differences among various combinations of doses (103, 104 and 105 TCID50/ml) and routes of immunisation, including the commonly used intramuscular route along with intranasal route. The results showed that intranasal immunisation of pigs with low and moderate doses (103 and 104 TCID50/ml) provided complete protection (100%) against lethal challenge with a homologous high virulent ASFV isolate. Moreover protected pigs had either no detectable or minimal quantities of viral genome in blood at termination. In addition, only mild and transient adverse clinical reactions and mild lesions, consistent with secondary bacterial infections, were observed before and after challenge. However, in group of pigs immunised intranasally with 105 TCID50/ml and in all groups immunised intramuscularly (103, 104 and 105 TCID50/ml); the rates of protection conferred were lower ranging from 33% to 66%. Regarding immune protection mechanisms, along with the possible role of humoral immune response, our results suggested that survival of pigs after challenge was associated with a balance between pro- (TNFα and IL-1β) and anti-inflammatory cytokines (IL-10), without participation of IFNγ. In contrast, animals that died showing an acute form of ASF displayed an imbalance linked to an exacerbated increase of IL-10 along with an anomalous antibody response.

P04

Evaluation of porcine adenovirus vectored vaccines for African Swine Fever

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Keywords: Porcine adenovirus; vaccine, African swine fever

Currently, there is no available vaccine for African swine fever virus (ASFV). Inactivated vaccines as well as subunit vaccines for ASF have not been able to demonstrate protection against ASF. It has been previously demonstrated by other laboratories that it is possible to elicit protective immunity against homologous ASF challenge using attenuated live ASF vaccines. In addition, DNA vaccines which elicit cell mediated immunity have also been demonstrated to provide partial protection against homologous challenge. These experiments illustrate that strong cellular immunity is required for a vaccine to be effective against ASF. Porcine adenovirus vectored vaccines are able to elicit strong cellular immunity and have been previously demonstrated as an effective vaccine vector in swine. Bioinformatic analysis using different computational prediction tools were used to identify the potential T and B cell antigens \epitopes of Malawi genotype II ASFV. These potential antigens or chimeric antigens (containing different epitopes) were expressed using adenovirus vector systems (porcine adenovirus-3 \ human adenovirus-5) .Three recombinant adenoviruses expressing three vaccine antigens are currently being evaluated in a homologous ASF Malawi genotype II virus challenge model. Groups of six pigs will be vaccinated two times with different recombinant adenoviruses. Following vaccination, the pigs will be challenged with Malawi genotype II and then evaluated for clinical disease and viral loads in blood and swabs from mucosal surfaces.

P05

Is Ornithodoros erraticus able to transmit the Georgia2007/1 African Swine Fever Virus isolate to domestic pigs?

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Keywords: ASF, Tick, vector competence, transmission

As no vaccine or antiviral are available to fight against African swine fever (ASF), the only tools to control it are preventive measures based on early detection and actual knowledge of the epidemiological risks. In sub-Saharan countries of Africa, ASF persistence is described to be related to different and complex epidemiological scenarii involving domestic and wild suids and soft tick vectors of the genus Ornithodoros. In EU, O. erraticus, is known to be able to maintain and/or transmit some ASFV isolates classified in the genotype I and to amplify the Georgia2007/1 ASFV (genotype II), at least during 3 months. The objective of the current study was to evaluate the ability of O. erraticus ticks to become infected and to transmit Georgia 2007/1 ASFV to pigs. Ticks were engorged artificially on parafilm membrane and naturally through biting on vireamic pigs. For artificial feeding, the blood was infected by mixing with an ASFV culture or it was directly sampled on a vireamic pig. The ticks infected through natural blood feeding were then proposed to secondarily engorge on healthy pigs three and five months later, respectively, to assess their capacity to transmit the virus. Depending on the infection method, the percentage of infected ticks and the ASFV titer in ticks were different. The virus load was higher in tick when it was engorged by biting a vireamic pig than by artificial engorgement except if the blood was sampled on a vireamic pig. The preliminary trial of virus transmission using ticks previously engorged on vireamic pigs did not allow concluding on the ability of O. erraticus to transmit Georgia2007/1. Conversely, the inoculation of naturally infected tick homogenate caused typical ASF clinical signs in pigs. Further hypotheses will be discussed during the workshop before concluding on transmission.

P06

Research facilitation of pig sector and government preparedness for exotic incursions of swine fevers: the case of Scotland

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Keywords: Preparedness; Control; Modelling; Industry structure; Exotic pig diseases

The pig industry is one of the major agricultural livestock sectors for Great Britain (GB). However, insufficient knowledge currently exists on the pig sector's structure and its particular vulnerability to incursions of swine fever (SF). An extensive programme of research initiated by Scottish Government (SG), through its Centre of Expertise on Animal Disease Outbreak (EPIC) and its Strategic Research Programme improves the evidence base for rapid disease control decisions in the face of SF outbreaks. This programme aims to help SG (1) better understand pig industry structure, and (2) develop more robust surveillance and control plans against SF. Researchers therefore acted as knowledge brokers between industry stakeholders and policy-makers, allowing for critical issues and limitations to be identified and ground-truthing assumptions and research results. Overall, the emergent industry structure is more complex than the theoretical top-to-bottom structure of vertically integrated pig breeding companies. Efforts focussed on risks posed by the non-commercial (i.e. backyard) herds, which greatly differ in biosecurity and movement behaviours from commercial herds but are connected through animals' movements, potentially exacerbating spread over wide geographical areas. Integrated modelling study results demonstrate how widespread epidemics are possible throughout the year, regardless of the duration of the silent period. However, few geographical areas in GB were likely to generate epidemics from single incursions. The limited influence of behavioural factors in the uptake of mitigation measures will be presented. This ongoing applied/policy linked research programme has already generated invaluable knowledge for developing more robust biosecurity and surveillance plans for emerging/exotic swine diseases for GB. The predominant benefit, however, has been to facilitate collaborations and discussions between researchers and various actors for better uptake of coherent industry level exotic swine disease control.

P07

The role of socio-economic factors on African Swine Fever in Sardinia

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Keywords: African swine fever, Material Deprivation Index, eradication plan, risk factor

In Sardinia, African swine fever (ASF) has been present since 1978 with a specific epidemiological situation influenced by risk factors such as uncontrolled movements of animals and local breeding traditions. Although different control strategies have been adopted, some socio-cultural risk factors remain present on the island hampering the success of eradication plans. The recent research activities and the programs in action finalized to ASF control and eradication, have highlighted a strong association between outbreaks and specific geographic areas, where probably the social and cultural context plays a key role. Particularly, the illegal pigs breeding is universally recognized like principal risk factor of ASF persistence in region. To evaluate socio-epidemiological relation between ASF and social condition, number of seropositivity and virus positivity were evaluate related to Material Deprivation Index (IDM). The deprivation indices are instruments able to indirectly synthesize the possession of material and social resources connected to geographical different size areas; within these areas the proportion of people presenting a certain combination of features, indicative of a socio-economic disadvantage condition, is measured. The IDM has been made for all 377 Sardinian municipalities using last ISTAT 2011census data. The result of the analyses conducted for each municipality show a strong correlation between the number of ASF seropositivity and virus positivity and IDM value, indicating an higher concentration of cases in those municipalities of Sardinia that are more materially deprived. This work will be useful to enrich the actual eradication plan with specific socio-cultural actions.

P08

African Swine Fever Risk Assessment and Preparedness in Georgia

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Keywords: ASF, Risk, Assessment, Surveillance

African swine fever (ASF) is a highly contagious viral infection of domestic and wild pigs that is usually lethal and for which no vaccines exists. Pigs become infected mainly through the oropharyngeal route after contact with infected pigs or through feeding on contaminated products (e.g. swill and garbage) Transmission through vectors (Ornithodoros ticks) can cause long-term virus persistence. In April 2007, Georgia reported ASF, with the majority of regions being affected. Various factors associated with regions at high-risk for ASF include: the density of pig farms, associated level of trade, and/or the presence of an ASF sylvatic cycle, which could be seasonally dependent. In some areas, domestic animals are in contact with wild hosts (wild boars, hogs), increasing the risk of transmission. ASF risk assessment was conducted by epidemiologists at NFA, as part of the national early-warning system emergency diseases. Risk assessment consists of identifying threats, assessing the likelihood that disease outbreak might occur, and modifying the risks by evaluating their potential consequences. Risk assessment and analysis is continuous and regularly updated at NFA. Annually, NFA trains 120 government and 200 private veterinarians at the field level. Training focusses on ASF and the following topics: early detection of the infection; enabling legislation for declaring national emergency measures; surveillance zones and disease free zones; inspection and quarantine procedures to contain the disease, including pig movement controls and prohibitions on the sale of potentially infected pig products; epidemiological surveillance and analysis; safe burial or burning of carcasses and other infected material; cleaning and disinfection of infected premises. NFA devote an appropriate level of resources to implementing effective border and import quarantine policies that prevent the introduction of ASF diseases in country.

P09

ASF Public Outreach Program in Georgia

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Keywords: ASF, outreach campaign, awareness

Due to potential economic implications, African swine fever virus (ASFV) is a pathogen of bioterrorism and veterinary health concern. Georgia was affected by a nationwide outbreak of ASF in April 2007. ASF outbreaks were simultaneously reported in three different locations across the country. Reports indicate that the index case had occurred several weeks earlier. Epidemiological data suggests that the outbreak started in the Poti harbor region, probably as a result of direct contact between free-roaming pigs and insufficiently decontaminated/attenuated waste from ships. The main aim of the public outreach program was to raise awareness to mitigate future outbreaks of ASF in Georgia. Additionally, this project contributed to long-term sustainability by developing a framework for conducting outreach campaigns for future disease outbreaks. Representatives in Georgia were taught how to conduct and implement an outreach campaign, including how to identify target audiences (e.g. local farmers, veterinarians, and members of the pork industry) and effectively create and disseminate educational materials. Over 450 veterinarians and farmers were trained and 20,000 educational materials (i.e. flyers, guidelines, etc.) were distributed during the outreach campaign. Pre- and post-ASF knowledge tests were developed and improvements in trainee knowledge after completing training was observed, post-test scores on average were 16% higher than pre-test scores; indicating that the outreach program was successful. Regional and state veterinarians, along with government agencies will be responsible for measuring the long-term success through analysis of ASF lab results, monthly disease reports, and veterinarian updates. The number of swine disease cases, specifically the number of ASF outbreaks, will be an indicator of long-term success of the outreach program.

P10

Raising awareness of farmers and veterinarians for efficient respond to African Swine Fever in Ukraine

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Keywords: African swine fever, epidemiology, Ukraine

African swine fever (Pestis Africana suum, ASF, Montgomery's disease) is a viral disease of swine characterized by fever, hemorrhagic diathesis, significant bleeding, and dystrophic-necrotic changes in various organs. Both domestic and wild pigs are susceptible to ASF. It is one of the most dangerous diseases of pigs that becomes widespread in European countries and is mandatory for reporting to the World Organization for Animal Health (OIE). Ukraine reported 65 ASF outbreaks in 2012-2016. Aim. To increase awareness of ASF among veterinarians and farmers through a public outreach and educational activity. Methods. Workshops to train trainers on the regional level, in particular, veterinarians and epidemiologists working at the state animal hospitals and state regional veterinary administrations. Results. In 2015, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) and Institute of Veterinary Medicine (IVM) of the National Academy of Agrarian Sciences took part in the project entitled "Community outreach to support understanding of African swine fever (ASF) ecology and epidemiology in Eastern Europe (EE): Training and implementation for methods and strategies for control and prevention" that was implemented within the Defense Threat Reduction Agency (DTRA) Cooperative Biological Engagement Program (CBEP) in Ukraine. The project aimed at establishing a regional alliance between Armenia, Georgia, Kazakhstan, and Ukraine to exchange experience and raise awareness to and provide education on ASF. Specialists of SSRILDVSE and IVM were trained as trainers before implementing their own outreach program in Ukraine. Through this project, 14 training sessions were arranged in 14 Oblasts of Ukraine. Veterinarians from 307 rayons took part in these trainings. 531 epizootologists of state regional veterinary administrations and 4482 veterinary doctors of district animal hospitals were trained. Additionally, educational materials (flyer and poster) were developed. They included information about the clinical and epidemiological patterns of ASF, common sources and routes of exposure, preventative measures, how to recognize symptoms, and how to respond to suspected ASF cases. 100 000 flyers were printed and distributed among farmers and populations, as well as 1500 posters for veterinarians of 24 Oblasts. Due to these efforts, necessary information was brought to the attention of veterinary doctors of district animal hospitals who communicate directly with farmers and persons that work with swine. Conclusion. The ASF outreach and education campaign in Ukraine contributed to effective monitoring and reporting of ASF, as well as to raising awareness of farmers and population about prevention and control of ASF which in turns makes it possible to respond more efficient to ASF outbreaks. Early diagnostics and effective measures can localize the disease and prevent its further spread in the country.

P11

Quality of available habitats for wild boar as a online cartographic tool for managing African Swine Fever in Eurasia

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Keywords: African Swine Fever, surveillance, wild boar distribution, land coverage, habitat

The current African Swine Fever (ASF) epidemic in Eurasia represents a risk for the swine industry with devastating socio-economic and political consequences. Wild boar appears to be a key factor in maintaining the disease in endemic areas and spreading the disease across borders, including within the European Union. Therefore, it is crucial to know their distribution to help predict and interpret the dynamics of ASF infection. We present here the online version of the standardized distribution map based on global land-cover vegetation (GlobCover) that quantifies the quality of available habitats (QAH) for wild boar across Eurasia through an expert opinion, as an indirect index for quantifying wild boar. The QAH-map were validated using field data on wild boar locations from several databases as well as the locations of ASF notifications in wild boar and domestic pigs (WAHID, 2007-2016). This map can serve as a useful epi-tool for defining risk scenarios and identifying potential travel corridors for ASF, which could help manage populations of wild boar and domestic pig in wildlife management and epidemiological studies. This tool could help in resource allocation decisions and improve prevention, control and surveillance of ASF and potentially other diseases affecting swine and wild boar in Eurasia. This map was produced at 300-m resolution using ArcGis10.0 (ESRI©). The results are available online to the public, providing access to the data and the map for all who may be interested on it (researchers, wildlife managers, epidemiologists and administration). This work was supported by FP7/2007-2013 and AT2015-002 projects.

P12

Integrating ASF sero-prevalence data in wild boar with the decreased-virulence-hypothesis

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Keywords: ASF, serosurvey, seroprevalence, modelling, observational evidence,

Since the entry of ASFV into the Eastern European Union the infection in wild and domestic animals was associated with huge lethality of above 90%. ASFV spread in the EU was accompanied with lots of surveillance efforts and monitoring performed in wildlife populations of the affected area. In particular the temporal development of the rate of sero-positive samples from the affected area was under investigation. Increased sero-prevalence might indicate a possible change in case-fatality due to the infection in wild boar compared to observations short after introduction and in early transmission experiments. Identification of alterations in lethality of the virus infection is important because existing understanding of perpetuation and persistence of ASFV in wild boar could subsequently become questionable. Moreover, accepted control approaches in domestic pigs may also be less adequate once the dead of infected swine will be less inevitably. However, there are still debates whether one actually can observe dedining case fatality from field survey data likewise accessible from the affected area in the EU. Therefore, we have implemented a novel spatial transmission model of ASF between the animals of a regional population of wild-boar. The simulation model reflects transmission events depending on stochastic spatial proximity of free ranging animals. The model was tailored to study different sampling procedures along with the expansion of the ASFV affected area; and to confront these with alternative scenarios regarding the case fatality of the infection. When comparing the temporal dynamics of the virtual serology surveys it was evident that agreement with available field observation is most plausible without relying on the “decreased lethality hypothesis”. Finally, we conclude from the model experimentations about suitable sampling protocols to actually demonstrate increase in ASF case fatality using serological data.

P13

Evaluation of the efficiency of passive surveillance in the early detection of African Swine Fever in the wild boar

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Keywords: African swine fever, wild boar, surveillance, early detection

African swine fever (ASF) was introduced in the Baltics countries and in Poland in 2014. In such countries, ASF surveillance is performed in domestic pigs and wild boar and it is based on active and passive activities. Passive surveillance in wild boar is carried out by testing for virus and antibodies all sick or found dead individuals whilst active surveillance is performed testing hunted wild boar. To evaluate the efficiency of passive and active surveillance in early detecting ASF, the average ASF infection parameters observed in the Baltic countries and Poland were used to settle a theoretical scenario, assuming a 2% (0,02) prevalence.

- ASF Lethality rate: 95% of the infected wild boar die in 5 days (0,95/5=0,19 day-1)
- Hunting rate: 40% of the wild boar is hunted in one-year period (0,4/365=0,0011 day-1)
- Daily rate at which a wild boar found dead is ASF positive: 0,19*0,02=0,0038 day-1
- Daily rate at which ASF infected wild boar is hunted: 0,0011*0,02=0,0000219 day-1

The ratio between lethality and hunting rates is: 0,19/0.0000219=8675. It means that the rate at which ASF virus kills an infected animal is 8675 times higher in respect to the rate at which a hunter culls a virus positive individual. Under the assumption that any dead wild boar is immediately retrieved in the forest, the probability of detecting ASF in dead animals is 174 times higher in dead wild boar in respect to hunted animals (0,0038/0,0000219). Instead, it appears that only 10% of dead wild boar is found in the forests and in such instance, the ratio becomes (0,0038*0,1)/0.0000219= 17,4, but even virologically testing only 10% of the dead infected wild boar, the daily rate at which ASF is detected is 17 times higher in comparison with active surveillance. In a population of 1000 wild boar, with ASF prevalence of 2%, passive surveillance based on testing 10% of dead infected animal identifies 1 positive every 2,5 days, whereas testing hunted animals can detect 1 positive every 36 days. In case ASF diagnosis would be performed only by means of serology in hunted wild boar, the daily probability of detecting ASF would decrease to 0,00000109day-1 (0,0000219*0,05), since only 5% of the infected animals survive to the infection and develop antibodies. This means that, virologically testing 10% of dead infected animals has 348.6 (0,00038/0,00000109) times more probability of detecting ASF in respect to active surveillance, based on serology of all hunted wild boar.

P14

Serological analyses of African Swine Fever in Nigeria, 2006-2015

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Keywords: African swine fever, ELISA, Antibodies, Antigen, Nigeria, Prevalence

African swine fever (ASF) is an important trans-boundary animal disease with a significant socio-economic impact on subsistence and commercial pig production in Nigeria. Most smallholder pig farmers in Nigeria depend on this sector as their main source of livelihoods. Improving the control of ASF will ultimately enhance food security and reduce extreme poverty among smallholder farmers. In this study we present the report of serological analyses of ASF from 2006-2015. A total of 3168 sera and 92 tissue specimens were collected during outbreaks in Pigs and analysed for ASF virus antibodies and antigen detection using indirectenzyme-linked immunosorbent assay (I-ELISA), Blocking ELISA and competitive- ELISA (cELISA), respectively. An overall seroprevalence of 13.89% (95%CI: 12.72 – 15.13) was recorded within this period. The year 2010-2012 recorded the highest prevalence of 26.70% (95%CI: 23.81 – 29.74), followed by 2012-2013, with 13.10% (95%CI: 10.62 – 15.94), 2006-2007, 8.92% (95%CI: 6.54 – 11.84), 2008- 2009, 8.69% (95%CI: 6.92 – 10.76) and 2014-2015, 4.52% (95%CI: 2.83 – 6.85). ASF antigen was also detected in 31.52% (95%CI: 22.66 – 41.53) of the tissue specimens. This report reveals that ASF is still enzootic in Nigeria. Therefore it is imperative to put in place a comprehensive routine surveillance with an early and rapid diagnostic system, and also consider the option of compensation in order to achieve control and eradication of ASF in Nigeria.

P15

Biosecurity breaches and within-farm virus contamination during an African Swine Fever outbreak on a medium sized farm in Uganda

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Keywords: environmental sampling, farm level biosecurity, low income countries

Uganda is a low-income country with the largest pig population in east Africa. African swine fever (ASF) is endemic with numerous yearly outbreaks. In the prevailing smallholder subsistence farming systems, farm biosecurity is largely non-existent. The continuous circulation of ASF in smallholder settings creates biosecurity challenges also for larger farms. Outbreaks of ASF are largely under-reported and as a consequence the progression of outbreaks are seldom described, creating lacunae in the epidemiological knowledge of ASF. In this study we investigated and described an outbreak of ASF on farm level in an endemic area, including investigations of on-farm environmental virus contamination during the outbreak. The study was carried out on a medium sized pig farm which had 35 adult pigs and 103 piglets or growers at the onset of the outbreak. All pigs had died or been slaughtered within three months of the outbreak. Informal interviews with farm representatives were undertaken at the beginning, during the first month, and three months after the outbreak. Biological samples were taken on days 4 and 34 of the outbreak. ASF was confirmed by presence of ASFV nucleic material from both these samplings. Environmental samples (soil, manure, water, feed, pig hair) were taken on day 26 of the outbreak. All 35 environmental samples (soil, manure, water, feed, pig hair) were positive for ASFV nucleic material. The ASFV positive biological samples confirmed earlier epidemiological knowledge and the clinical picture. Breaches and non-compliance in biosecurity protocol that most likely lead to the propagation and within farm spread of the outbreak was revealed, and confirmed with the results of the environmental samples. These latter results are important for future formulation of advice in outbreak situations, especially pertaining to outbreaks in endemic countries.

P16

Phylogeography of the African Swine Fever ornithodoros vectors in Southern Africa

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Keywords: Ornithodoros, phylogeny, South Africa

African swine fever virus maintenance and transmission dynamics have been intensively studied in Ornithodoros ticks due to the role that they play in disease epidemiology and in the African sylvatic. However the taxonomic status of ticks of the Ornithodoros moubata complex is fraught with inconsistencies and the evolutionary history of the group is understudied. We address this for the southern African region by genetically characterising tick populations from eight game parks within the ASF control area of South Africa, and from sampling sites in neighbouring Mozambique and Swaziland and comparing these with previously characterised populations from Zimbabwe, Namibia, Tanzania and Uganda. In common with a previous study, the 16S rRNA gene phylogeny revealed the presence of three geographically discrete warthog-associated Ornithodoros lineages, but uncovered high levels of intra-lineage variation within the southern African region. Phylogenetic analyses indicated the presence of two topographically distinct haplotypes in the Kruger National Park that have some degree of overlap in central to northern region. These results suggest historical barriers to gene flow in the largest game park within the ASF control zone of South Africa, and that tick variation is largely underestimated.

P17

Socio-economic impact of African Swine Fever outbreaks in smallholder pig systems in four districts along the Uganda-Kenya border

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Keywords: African swine fever, economic impact, financial losses, Uganda, Kenya

A study was done to assess the impact of African swine fever (ASF) outbreaks on smallholder pig farmers along the Kenya - Uganda border. Data was collected using quantitative and qualitative methods. A cross-sectional questionnaire survey was undertaken among 645 pig keeping household. Two repeated cross-sectional surveys were undertaken after 3 and 6 months after the main cross-sectional survey involving 120 households. Twenty four focus group discussions were carried out in 13 villages (7 from Kenya and 6 from Uganda) involving 268 pig farmers both men and women. It was found that 8.9% (n=57) of the households had ASF outbreaks during 2012. There was no significant difference ($p>0.001$, $X2= 2.8$) in the proportion of households affected by ASF in Uganda and Kenya. On average each ASF affected household lost $190.6. The number of pigs sold during ASF outbreak was 1.33 times more than those that died due to ASF. The prices of pigs during ASF outbreaks declined by >50%. A very highly significant proportion of households (~83.1%) felt impact due to psychological fear, closure of markets, failure to restock, pigs sold early and lower sale prices. It was important that appropriate strategies be developed to ameliorate the impacts of ASF to the farmers.

P18

Consultation on a research strategy for the implementation of a regional control program for African Swine Fever in Africa

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Keywords: African swine fever, Research strategy, Disease control

As a follow up to the Consultation workshop on the Regional strategy for the control of African swine fever (ASF) in Africa held between 10-12 November 2015 in Ouagadougou, Burkina Faso and the validation and adoption of the strategy document, the BecA-ILRI Hub convened a consultative workshop to develop a research strategy to support the implementation of an ASF control program in Africa. The aim of the workshop was to identify key knowledge gaps that require research and that underpin the implementation of the continental ASF control program. The workshop documented institutional capacity of partners and on-going activities within the institutions on ASF. Participants identified three broad research pillars that include: Understanding of ASF epidemiology underpinned by molecular virus characterization, risk and socio-economics of ASF; Development of diagnostics needed for the disease surveillance and development of vaccines for its control. A key result of the workshop was the identification of synergistic activities between institutions and the establishment of new research and development partnerships. The initial consultation is expected to enhance communication between current joint research activities, initiate search for donor support and inform implementation of future collaborative research activities aligned to the identified research gaps in order to support local and global ASF control programs.

P19

Polymerase Cross-Linking Spiral Reaction (PCLSR) for detection of African Swine Fever Virus (ASFV) in pigs and wild boars

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Keywords: African swine fever virus, polymerase cross-linking spiral reaction, pigs and wild boars.

African swine fever (ASF) is considered a considerable threat, in the production of pigs worldwide. The ASF aetiological agent – ASFV, is the sole member of the Asfivirus genus, belonging to the Asfarviridae family. An effective ASF vaccine is not currently accessible, thus the only measures of ASF spread control include, reliable and fast diagnosis. Officially approved, diagnostic methods include, virus isolation, serological assays, including ELISA and immunoperoxidase assay (IPT) and different modifications of the polymerase chain reaction (PCR). The spread of the African swine fever virus (ASFV) among infected pigs and wild boars, is currently one of the most important facets of virus transmission in eastern Europe. So far, isothermal amplification methods including loop-mediated isothermal amplification (LAMP) and cross-priming amplification (CPA) have been used as an alternative among ASFV detection methods. However, the main disadvantage of LAMP and CPA is the high contamination risk and possibility to generate false positive results. Here we present for the first time the design, development and practical implementation of polymerase cross-linking spiral reaction (PCLSR) for the fast and direct detection of genetic ASFV material in the blood and sera of infected pigs and wild boars. It has been shown that PCLSR is a rapid, sensitive and specific isothermal method for the detection of ASFV DNA, in directly collected blood or sera from pigs and wild boars. The sensitivity of PCLSR was slightly below the sensitivity of the official, universal probe library (UPL) real-time PCR. The PCLSR was capable of detecting ASFV DNA in all examined blood samples, originating from pigs; n=10 and wild boars; n=78. The obtained results were also confirmed by the officially approved, real-time PCR. The developed new assay might be further used by local and county veterinary officers, hunters or pig farmers, for preliminary ASF diagnosis. The PCLSR as a novel isothermal amplification method might be also designed and used for rapid detection of other swine pathogens.

P20

Genomic analysis of Sardinian 26544/OG10 isolate of African Swine Fever Virus

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Keywords: African Swine Fever Virus, Next Generation Sequencing, Sardinia, Italy.

African swine fever (ASF) is an extremely contagious viral disease affecting domestic pigs and wild boars, caused by a large cytoplasmic DNA virus, the African swine fever virus (ASFV), the only member of the Asfarviridae family. ASF is endemic in sub-Saharan countries and in Sardinia (Italy) and has become more prevalent in Russia and the Caucasus region since its spread from eastern Africa to Georgia in 2007. Comparative genomic analysis aims to underscore genetic assortment diversification in distinct viral isolates, to identify deletions and to carry out evolutionary studies. In this work we used Next-Generation Sequence (NGS) technology to sequence the first complete genome of a Sardinian ASFV p72 genotype I strain, noted 26544/OG10, isolated in 2010 from domestic pigs. The genome was assembled as a single contig of 182906 bp long, it contains 165 ORFs and has a 99.80% nucleotide identity to the L60 strain. The complete 26544/OG10 genome sequence was submitted to GenBank with accession number KM102979. We performed a comparison analysis against the 16 ASFV genomes available in the database and found that 136 ORFs are present in nine ASFV isolates annotated to date. The most divergent ORFs codify for uncharacterized proteins such as X69R and DP96R, which have 51.3% and 70.4% nucleotide identity to the other isolates. A comparison between the Sardinian isolate and the avirulent isolates OURT88/3, NHV, BA71V was also carried out. Major variations were found within the multigene families (MGFs) located in the left and right genome regions.

P21

A universal protocol to generate consensus level genome sequence for African Swine Fever Viruses

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Keywords: Next generation sequencing (NGS), Zaire

There are twelve complete African swine fever virus (ASFV) genome sequences published. Molecular epidemiology studies on ASFV isolates have extensively used partial sequence analysis of variable genome regions to determine relationships between strains. However, whole genome sequencing (WGS) is the route to achieve a proper discrimination required and clarify the relationship between the ASFV isolates. Previous sequencing protocols had challenges such as those encountered during PCR amplification and cell culture. The aim of the present study was to test an alternative approach for the detection of ASFV using a new purification method combined with next-generation sequencing (NGS). In this study nine AFSV isolates of different genotypes have been chosen and their DNA were sequenced by NGS to obtain WGS. The study sequence data were compared to published complete genome sequences from GenBank. Whole genome NGS plays an important role in viral diagnosis, epidemiological investigation and in host-pathogen relationship.

P22

Development of a DNA/LNA optical biosensor for the rapid diagnosis of African Swine Fever

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Keywords: African swine fever virus; diagnosis; DNA/LNA optical biosensor;

African swine fever (ASF) is a highly lethal disease affecting the members of the suidae family, including domestic and feral pigs, wild boar, african wild suidae. The disease is highly infectious and is a threat to all countries where pig farming is widespread. Until a few years ago the disease was confined to the countries of sub-Saharan Africa and Sardinia, in recent years the disease has spread to some countries in the Caucasus and the Russian Federation. Given its uncontrollable diffusion, many efforts have been focused on the development of a rapid diagnostic test to contain any contagion. The purpose of this work is to evaluate the applicability of an optical biosensor for the rapid diagnosis of ASF. We immobilized, on the surface of biosensor, two different probes: a ssDNA probe and a chimeric ssDNA/LNA probe. The inclusion of LNA oligonucleotides in a DNA probe increases the affinity for complementary strands, as well as both sensitivity and specificity for target nucleotides. Furthermore the LNA oligonucleotide can bind the dsDNA in the major groove forming a dsDNA:LNA triplex. The extraction of DNA from blood of infected animals was perfomed using FTA mini cards. The extracted DNA was directly analyzed on a surface biosensor. For verification of the selectivity of the apparatus, 13 different dsDNA samples extracted from blood of experimentally infected pigs have been used. The dsDNA extract was denatured to ssDNA in order to allow the hybridization with the complementary ssDNA probe. In parallel, the same extracts dsDNA were directly tested with ssDNA/LNA probe. After each use, the surface of the biosensor derivatized with the two types of probes was regenerated by washing with PBS (ssDNA) or with PBS-T (ssDNA/LNA). As a negative control, was used an extract dsDNA of a healthy animal and no appreciable signal was detected. Our results proved that the biosensor derivatized with the probe ssDNA has poor selectivety for positive samples, 4 out of 13 samples are at the background level, probably due to low viral load and/or the low sensitivity of the probe, whereas the biosensor derivatized with the ssDNA/LNA probe was able to detect appreciable signals on the same samples. The limit of detection was of about 150 viral genomics copies. Due to the higher sensitivity of the probe ssDNA/LNA and the detection capability in real time, the biosensor can provide quick responses on the presence/absence of the virus after simple extraction procedures. In fact, the ultimate goal of this work is to perform analysis directly on the farm by connecting the biosensor to a portable PC without the amplification step of the extracted DNA.

P23

Complete genome sequencing of Sardinian African Swine Fever Virus isolates

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Keywords: African swine fever virus; next generation sequencing; full length sequence analysis

Partial sequence analysis of variable genome regions has been extensively used for molecular epidemiological studies of the African swine fever virus (AFSV) isolates. By this analysis the Sardinian isolates have been classified as members of the same p72 genotype I with minor differences in the number of tetramer repeats within the B602 gene. Such low level of intra-genotype diversity doesn't make it possible to investigate the genetic relatedness of ASFV outbreaks in Sardinia. To enable comparison at a higher resolution, nine Sardinian ASFV isolates collected from 1978 to 2012 were selected for whole genome sequencing. Six of them were cell culture isolates whereas two were organ homogenates and one contained erythrocytes enriched from blood by ultracentrifugation. All samples, except the erythrocytes, were treated with RNase and DNase to remove host material prior to DNA extraction and analysis of quality and quantity. The DNA of the nine ASFV samples were fragmented, tagged, and sequenced using an Illumina MiSeq. Moreover, the erythrocyte DNA sample had sufficient purity (ASFV comprised 67.80% of the genetic content as indicated by the MiSEQ data) and quantity (a few micrograms) to allow long-read sequencing using the Pacific Biosciences (PacBio) RSII platform. Acquired sequencing reads per sample were analyzed and assembled, performing both a de novo and a mapped assembling. Verification and gap-filling were performed by Sanger sequencing. The nearly entire genomes were obtained for all the isolates and one of them, the enriched erythrocyte sample collected in 2008, was completely sequenced. This genome sequence of 184.842 bp is rather different from the ASFV complete genomes already published in GenBank, mostly in the inverted terminal repeats. This genome was annotated by software-aided transfer of annotations. Further analysis are in progress to obtain whole genome sequences for the remaining samples and to identify biological relevant differences by comparative genomics.

P24

Molecular analysis of African Swine Fever Virus (ASFV) associated with disease outbreaks in Ukraine during 2012-2015

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Keywords: African swine fever, outbreak, PCR, Ukraine

African swine fever (ASF) is one of the most dangerous viral diseases of swine. It is characterized by high mortality and causes significant economic losses. Initiated in the Caucasus from Georgia and Armenia (2007-2008), ASF has spread rapidly in Eastern Europe (2007-2015), southern European part of Russian Federation (2008-2011), entered into Ukraine and Belarus (2012-2013), Poland and Baltic States (2014-2015) and led to the formation of permanent and resistant distribution areas of disadvantage. By May 2016, Ukraine reported 61 ASF outbreaks in 11 Oblasts. Objectives. The main goal was to study DNA of ASFV isolated from pathological material selected from domestic and wild pigs during ASF outbreaks in Zaporizhia, Luhansk, Chernigov, Sumy and Kyiv regions and to compare data on phylogenetic analysis of ASF virus (ASFV) isolated in Russian Federation, Poland, Baltic and Caucasus. Methods. Samples were collected from all locations with the reported ASF cases: Zaporizhia (2012) – 1 sample, Luhansk (2014) - 5 samples from wild boars and 1 - from a domestic pig; Chernigov (2014) - 1 sample from a wild boar and 1 sample from a domestic pig, Sumy (2015) - 1 sample from a wild boar, Kyiv (2015) - 1 sample from the domestic pig. Genomic DNA was amplified by OIE-PCR and qPCR. Genetic characterization of the viral DNA was achieved by sequencing three independent regions of ASFV genome including: C-terminal end of VP72 coding protein gene, the full genome sequence of the p54-gene, and the central variable region (CVR) within the B602L-gene. Results of this analysis were compared with phylogenetic data on ASFV detected in Russia, Poland, Baltic countries, and Caucasus. Results. All samples tested positive by PCR and qPCR assays. PCR protocols with primers p72-U/p72-D, which produce the amplicon 478 bp, primers PPA89/PPA722 – 678 pb, primers CVR1/CVR2 – 665 pb, recommended by EU and FAO Reference Laboratory for ASF, (CISA-INIA), Valdeolmos, Madrid, Spain), were used for genotyping. These three independent regions of the ASFV genome were investigated by sequencing. Phylogenetic tree was generated and analysed. Phylogenetic analysis was conducted using the Ceneious program. Conclusions. The sequence analysis of VP72, p54, and CVR regions of Ukrainian ASFV genome exhibited 100% identity to viruses registered during ASF outbreaks in Caucasus (2007), Russia (2008), Poland (2014), and the Baltic countries (2014). This genotype of ASFV is responsible for the disease outbreaks in the Eastern Europe since its introduction in Georgia (2007) and belongs to the genotype II of ASF viruses.

P25

The challenges encountered in diagnosing African Swine Fever in Uganda: are positives truly positive?

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Keywords: PCR Diagnosis, False Positive, Realtime, Conventional, Blood Direct, African swine fever

African swine fever (ASF) is a highly contagious infectious disease of pigs, with serious social and economic impacts constraining trade of swine and pig products and affecting food security. This disease is a major constraint to piggery in endemic countries. Whenever it strikes, mortality rates reach between 90-100% in the affected animals and the disease has neither treatment nor vaccine interventions. Polymerase Chain Reaction (PCR) is currently considered as a standard test for diagnosis of ASF. Using the PCR method, the sample DNA is probed for the ASF virus genome which confirms presence or absence of the virus in the host. Recently some confirmed positive samples in the laboratory with the Realtime and conventional gel based PCR method have been found negative after sequencing. The full extent of this problem has not yet been determined. In this study, domestic pig samples were collected from several ASF outbreaks reported in selected districts of Uganda. These samples were tested for ASF using different ASF diagnostic methods both PCR based and one non PCR based method: the Realtime PCR method, Conventional PCR, Blood Direct PCR method and Immunochromatographic lateral flow test. A total of 77 samples were tested. Preliminary Results indicate that the blood direct PCR method as a more sensitive method compared to the Realtime PCR with 48% of the samples testing positive for ASFV. These results highlight a gap in reliability of the diagnostic methods currently under use in an endemic setting.

P26

Molecular characterization of Malagasy African Swine Fever Virus strains from 2008 to 2014.

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Keywords: ASF, PCR, Phylogeny

African swine fever (ASF) is an economically important disease of domestic pigs in the world. ASF is occurred in Europe and endemic in most of sub-Saharan Africa, including Madagascar Island. Sporadic cases are reported with a 17.8% [95% CI, 10.26 - 25.66] viroprevalence for different ASFV isolates and different areas collected within 2008-2014 from Madagascar. Comprehensive relationship between isolates and comparative analyses were performed using public available sequences by the maximum likelihood method. Isolate genotyping was assessed by comparing concatenated amino acid sequences of proteins encoded of proteins encoded B646L, KP177R and CP204L genes from 346 (including 15 Malagasy), 140 (including 7 Malagasy), and 37 (including 14 Malagasy) isolates respectively. Analysis of the fragment of the gene B646L showed 100 % homology among isolates suggesting a single isolate of contamination until now. However, CP204L and KP177R genes showed more diversity , respectively with 0.9 % and 0.6% difference but no new spatiotemporal affiliation has been highlighted that would indicate the origin of new genotype. This stability can be explained notably by the virus circulation exclusively domestic cycle that follows the virus in the island. Analysis showed that Malagasy isolates studied always placed in the viral genotype II. However, the diversity among the isolates from Madagascar remains low at these loci despite over 18 years of virus circulation.

P27

African Swine Fever situation in Lithuania

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Keywords: African swine fever viruse, wild boar, domestic pig, PCR, ELISA, IPT

On 24th January 2014 Lithuania notified two primary cases of African swine fever (ASF) in wild boar: one male animal, 12 months old, hunted on 22 of January 5 km from the border to Belarus and one female, 3 years old found dead on 20 January 2014 about 40 km north from the border to Belarus. The distance between the two animals was about 36 km. The animals were tested positive for ASF virus (ASFV) genome by real time PCR at the National Reference Laboratory for ASF in Lithuania (NRL). The results were confirmed by the European Reference Laboratory for ASF (CISA-INIA, Madrid, Spain). Intensive wild and domestic animal monitoring programme was started. First positive ASF in a large (~20000 pigs were kept) commercial pig holding with the highest biosecurity confirmed on 23rd of July 2014 in Ignalina district. 290 pigs were sampled from infected farm, and 102 were found positive. 19 217 pigs were killed and destroyed by burying on the territory of the farm. And then ASFV outbreak territory expanded to middle of Lithuania. During this period ~24000 animals were tested for ASF (>10000 domestic pigs, >13000 wild boar). In 2015 were tested 25679 domestic pig (13 were positive) and 24188 wild boar (132 were positive) and in 2016 till 1 of June were tested 1887 domestic pig, 13547 wild boar samples (118 were positive). During 2014-2015 the Institute has received 32 food samples (meat products) confiscated on the border with Belarus, and found 4 of these to be positive for ASFV. The methods used for ASFV detection included higly specific real-time PCR, enzyme-linked immunosorbent assay (ELISA) Ab and Ag, immunoperoxidase test (IPT) (since 2015) and pathological examination.

P28

Validation of new Elisa & PCR for the diagnosis of African Swine Fever

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Keywords: ASF, diagnosis, qPCR, serology

African Swine Fever Virus (ASFV) is a highly virulent swine disease characterized by fever, hemorrhages and high mortality rates. ASF control and eradication programs require accurate and reliable diagnostic tests. IDvet offers indirect ELISAs based on three recombinant ASFV antigens (P32, P62, and P72) for antibody detection in serum, blood filter paper and meat juice samples. This ELISA demonstrates excellent specificity, especially for wild boar samples. IDvet has also developed two new tools, a competitive ELISA and a real-time qPCR, for ASF diagnosis. The ID Screen® African Swine Fever Competition ELISA allows for the detection of anti-ASFV P32 antibodies. Specificity was evaluated through the analysis of 280 disease-free sera from domestic and Iberian pigs. Measured specificity was 100.0% (CI 95%: 98.7% - 100.0%). 8 positive reference sera from the ASF European Reference Laboratory (EURL-ASF, Madrid, Spain) were correctly identified as positive. eroconversion was detected between 6 and 13 dpi. The test correctly identified sera from all genotypes tested, including genotype II. The test was also evaluated by the EURL. Results indicate a diagnostic specificity of 99.4% (n=177) and a diagnostic sensitivity of 95.8% (n=213). Perfect agreement (k=0,95) with the IPT (Immunoperoxidase test) was obtained. The ID GeneTM ASF is a TaqMan ready-to-use real-time PCR assay based on the simultaneous detection of ASFV and an endogenous internal positive control. It may be used for blood, serum, plasma, swabs and tissues samples. Results may be obtained in less than two hours (exaction in only 20 minutes, and amplification around 1hour). DNA sample panel from the EURL was tested. The ID GeneTM ASF kit correctly identified all samples (14/14, including DNAs from genotypes I, II, V, VIII, IX, X) and did not show any cross-reactions with 31 other pathogens. The detection limit of the PCR was <10 copies, indicating high sensitivity. The EURL-ASF reference panel consisting of 16 ASF lyophilised samples including experimental and clinical field samples, was also tested. DNA extraction was performed by magnetic beads (IDGeneTM Mag Fast) as per manufacturer's instructions. All samples were correctly scored positive and negative. To conclude, IDvet offers a full range of tools for the accurate and rapid diagnosis of African Swine Fever, either by serology or qPCR.

P29

Application of chicken egg yolk anti African Swine Fever viral protein vp73 IgY in a card agglutination test for sero-diagnosis of African Swine Fever

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Keywords: African swine fever, IgY, ASF viral proteins, Card agglutination test.

African swine fever (ASF) is highly pathogenic infectious viral haemorrhagic disease of domestic pigs endemic in many African countries including Uganda, where sporadic outbreaks of the disease occur annually. There is neither vaccine nor drug for ASF; therefore timely diagnosis is vital for institution of appropriate control measures. The aim of the study was to evaluate the possibility of using chicken egg yolk anti-ASF vp73 IgY in development of card agglutination test (CAT) for diagnosis of ASF. Purified ASF viral protein vp 73 from Ugandan isolates of ASF virus was used to raise antibodies in laying hens. Chicken egg yolk immunoglobulin IgY was extracted and purified using 8.8% NaCl salt precipitation technique and the purified polyclonal antibodies were passively coupled onto aldehyde/Sulfate polystyrene latex beads suspended in 2-(N-morpholino) ethanesulfonic acid (MES) buffer pH 6.6 and used to detect ASF viral antigens in serum samples. The diagnostic sensitivity and specificity of positive and negative predictive values of the developed assay were calculated to evaluate the test performance. The developed in-house CAT had diagnostic sensitivity and specificity of 77.3% and 86.9%, respectively, while the positive and negative predictive values were 74.7% and 88.4% in the same order. In conclusion, Chicken IgY- based ASF Card agglutination test could be used in conjunction with other diagnostic tests for field diagnosis of ASF and this could contribute towards control of the disease in East Africa in general. However, the developed test should be validated further before field application.

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