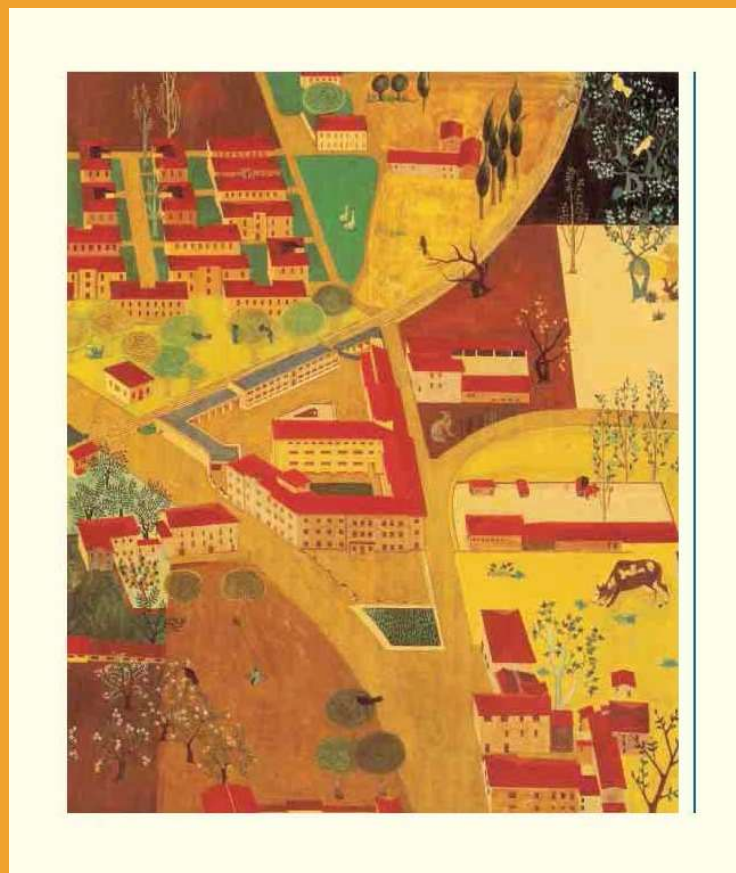




Final international conference of the COST Action ASF-STOP - Understanding and Combating African Swine Fever in Europe

29-30 January 2020,
Paul VI Center, Brescia, Italy



ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"
ENTE SANITARIO DI DIRITTO PUBBLICO



We welcome you to this final conference of the COST Action ASF-STOP- Understanding and Combating African Swine Fever in Europe, hosted in Brescia, Italy, 29-30 January 2020. Since the Launch Conference of ASF-STOP that took place in Pulawy, Poland, in December 2016, African swine fever (ASF) has continued to challenge scientists, stakeholders and animal health authorities. Within this short period ASF has further spread across Eurasia and continues advancing into south-eastern Asia causing devastating effects to the pork production and industry. Staying at the cutting edge to combat ASF requires close collaboration of scientists from multiple disciplines and from a broad geographical range. ASF-STOP, with its 32 participating countries in Europe and its extended international network, provides the optimal platform for knowledge sharing on ASF.

The Final International Conference of ASF-STOP, kindly organised by our colleagues of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), seeks to disseminate and exchange scientific knowledge on ASF and to show some of the main achievements of the Action. The two-days scientific programme covers topics related to ASF virology, vaccinology, molecular biology, epidemiology, surveillance and diagnostics, as well as contingency planning, wild boar ecology, biosecurity and policy making. The venue is Centro Pastorale Paolo VI, in the historic center of Brescia.

Welcome to Brescia and to our final international conference.

Silvia Bellini, chair Scientific Committee

Dolores Gavier-Widén, chair ASF-STOP

Local organising committee

Silvia Bellini

Marco Tamba

Luisa Garau

Gabriele Casadei

Scientific committee

Silvia Bellini (chair)- Italy

Dolores Gavier-Widén – Sweden

Francisco Ruiz-Fons – Spain

Laura Iacolina – Croatia

Ferran Jori – France

Maria Montoya – Spain

Erika Chenais – Sweden

Final international conference of the COST Action ASF-STOP- Understanding and Combating African Swine Fever in Europe

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Programme

29th January 2020

8:00-9:00 Registration

9:00-9:30 Opening Session

9:35–10:20 **Plenary talk, invited speaker Dr. Nguyen Van Long**

Session I: Virus, Diagnosis and Vaccinology

Chairs: Maria Montoya and Marie-Frédérique Le Potier

10:25–10:55: **Key-note speaker Germán Andrés:** Structure and composition of African Swine Fever virus

10:55-11:10 Maria Montoya - Serum-Derived Extracellular Vesicles from African Swine Fever Virus-Infected Pigs Selectively Recruit Viral and Porcine Proteins

11:10-11:25 Linda Dixon - The African swine fever virus A179L Bcl-2 family member is required for efficient replication in porcine macrophages.

11:25-11:40 Fernando Ferreira - Studies on viral DNA replication-related genes towards the ASFV control

11:40-11:55 Vlad Petrovan - Investigating the role of African swine fever virus Gene EP402R in virus persistence in blood in vivo

11:55-12:10 Pedro José Sanchez-Cordón - Neuropathology and viral antigen distribution in the central nervous system of domestic pigs experimentally infected with African swine fever virus

12:10–13:10 Lunch break

Session II: ASF in wild boar and Control Strategies

Chairs: Ferran Jori and Carolina Probst

13:15-13:45 **Key-note speaker Vittorio Guberti** - Surveillance of ASF in wild boar: from early detection to long lasting endemic situations

13:45-14:00 Sofie Dhollander - -Seasonality of African swine fever incidence in wild boar in the Baltic States and Poland

14:00-14:15 Vladimir Grosbois - Mapping the detectability of wild boar carcass with the SIG MCDA approach

14:15-14:30 Tomasz Podgórski - Spread by the dead: role of live and dead wild boar in spatio-temporal dynamics of African swine fever

14:30-14:45 Andrzej Jarynowski - Evaluation of mitigation strategies (border fencing and blocking animals corridors on motorway) for ASF in Poland

14:45-15:00 Xander O'Neill - Modelling the transmission and persistence of African swine fever in wild boar in contrasting European scenarios

15:00–15:25 Coffee break

Chairs: Edvins Oļševskis and Sofie Dhollander

15:30-15:45 Arnoldas Pautienius - Change in prevalence and spatial distribution of African swine fever in Lithuanian wild boar population

15:45-16:00 Kerli Mõtus - A participatory approach to support the control of African swine fever in wild boar

16:00-16:15 Ömer Orkun - Initial results of the study investigating the presence of ASF virus in wild boars and their ticks in Turkey

16:15-16:30 Rémi Pereira De Oliveira - Vector competence: a co-evolution story between African Swine Fever Virus and soft ticks Ornithodoros?

16:30-16:45 Claude Saegerman - Assessment of the impact of forestry and leisure activities on wild boar spatial disturbance and the associated risk of spreading African swine fever virus

16:45-17:00 Carolina Probst - Decomposition of wild boar carcasses

17:00-17:15: Annick Linden - ASF-WB in Belgium, one year after the emergence

19:00 Conference dinner

30th January

Session III: ASF in domestic pigs and Biosecurity

Chairs: Silvia Bellini and Marco Tamba

9:00-9:30 **Key-note speaker Anette Boklund** - Risk factor in Romanian backyard farms

9:30-9:45 Ana de la Torre - Flyers on ASF preventive measures for pig farms

9:45-10:00 Timothée Vergne - Modelling the role of stable flies in the transmission of African swine fever virus in outdoor pig farms

10:00-10:15 Jasna Prodanov Radulović - African swine fever: a biosecurity challenge for pig production in Serbia

10:15-10:30 Branko Angjelovski - Biosecurity assessment of Macedonian commercial pig farms using an online scoring system

10:30-10:45 Arvo Viltrop - Risk factors for introduction of African swine fever to domestic pig herds with emphasis to external biosecurity measures - a case-control study in Estonia

Session IV: Infection Dynamics and Control

Chairs: Erika Chenais and Fernando Boinas

10:50-11:20 **Key-note speaker Karl Ståhl** - Lack of evidence for long term carriers of African swine fever virus - a systematic review

11:20-11:35 Ludek Broz - Towards Veterinary Anthropology: Manifesto of an Emerging Field

11:35-11:50 Alvydas Malakauskas - Why me? Patterns in African swine fever outbreak farms in Lithuania

11:50-12:05 Imbi Nurmoja - Five years of African swine fever in Estonia: How close we are to freedom?

12:10–13:10 Lunch break

Chairs: Imbi Nurmoja and Karl Ståhl

13:15-13:45 **Invited speaker Andrei Blokhin** - Spatio-temporal analysis of the spread of ASF in the Russian Federation in 2017-2019

13:45-14:00 Anton Gerilovych, African swine fever and its way through Asia and towards Europe (Ukraine 2016-2019: lessons learned)

14:00-14:15 Kathryn Gowan - Housing ASF pigs in high containment

14:15-14:30 Kristīne Lambergā - Can we improve ASF control by learning from outbreaks?

14:30-14:45 Claude Saegerman - Ranking of blood feeding arthropods in Metropolitan France based on their putative vector capacity to transmit African Swine Fever virus: a first expert knowledge elicitation

14:45-15:10 Coffee break

Session V: Knowledge Communication

Chairs: Laura Iacolina and Tomasz Podgórski

15:15-15:25 **Key-note speaker Dolores Gavier-Widén** - Four years of advances in African swine fever in Europe by the ASF-STOP COST Action

15.25-15.30 Marco De Nardi. Pitch presentation of SAFOSO (<https://www.safoso.ch/>)

15.30-15.45 Alberto Laddomada - African swine fever eradication programme in Sardinia: an update

15:45-16:00 Laura González Villeta - Research gap analysis on African swine fever

16:00-16:15 Jan Hendrik Forth - Eight years wasting money - do we need ASFV whole-genome sequencing?

16.15-16.30: Marco De Nardi - Are we replacing African Swine Fever (ASF) with Avian Influenza (AI)?

16:30–17:00 Poster presentations

-Bojan Adžić, Surveillance of African Swine Fever in wild boars and domestic pigs in Montenegro

-Giorgia De Lorenzi, African swine fever: pig farms cleaning and disinfection procedures

-Kastriot Korro, Could African Swine Fever be spread in wild boars of Albania

-Branislav Kureljušić, The first occurrence of African Swine Fever in Serbia – epidemiological, clinical, pathological and molecular investigation

-Jonna Kyyrö, African swine fever surveillance in Finland 2010-2018

-Emil Wikström Lassa, Improvements in pathology capacity and early detection of African swine fever in Sweden by Short-Term Scientific Missions

-Jovan Mirčeta, Preventive measures in wild boar population in the Republic of Serbia

-Andrius Petrašiūnas, Possible transmission of ASFV by insects: studies in Lithuania

-Tamas Petrovic, Surveillance of ASF in domestic pigs after the first introduction of disease in Serbia

-Claude Saegerman, African Swine Fever virus in illegal pork meat imported in Belgium by travellers from Cameroun, August 2017

-Patricia Sastre, Diagnostic tools for the surveillance and control of African swine fever in domestic pigs and wild boar

-Alessandra Scaburri, Analysis of the introduction of pigs in Lombardy region as a tool for assessing the potential risk of introducing pathogens and to plan control activities

-Marina Štukelj, Short Term Scientific Mission in Lithuania: African Swine Fever Management and Control

-Marco Tamba, A method to identify areas at risk of African Swine Fever diffusion where planning a preventive wild boar population control program

-Ivan Toplak, The preventive measures for incursion of African swine fever in Slovenia

-Ina Toppari, Improving biosecurity on Finnish pig farms by Biocheck.UGent® evaluations

17:00 Closing remarks

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ORAL PRESENTATIONS

African Swine Fever in Vietnam

Van Long, N.¹, Quang Minh, P.¹, Duc Huy, C.¹, Manh Tien, H.¹, Pham Quan, P.¹, Dinh Chuong, V.¹ & Thanh Long, P.¹

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African Swine Fever (ASF) was firstly detected at a small farm with 33 pigs in the North of Vietnam in early February 2019. Molecular analysis indicates that ASF virus belongs to genotype II and was 100% identical to ASF virus strains detected in China. Outbreak investigations indicate that panic selling sick pigs, movement of infected pigs and pork (especially after Tet as the biggest holiday event), improper disinfection of infected farms, swill feeding and under reporting were key factors that resulted the disease rapidly spread to other locations. Vietnam has also faced difficulty in application of biosecurity practices because the country has about 3 million pig-raising households. As results, the disease peaked in May 2019 with more than 1.2 million pig culled, and then the disease has decreased gradually. By 10 December 2019, a total of more than 5.9 million pigs (approximately 22% of total pigs or 9 percentage of total pork volume in Vietnam) were culled at more than 8,500 communes (75% of total communes) of all 63 provinces of Vietnam, but more than 65% of these infected communes have not detected any further cases for at least 30 days. Currently, key control measures include culling infected and dead pigs; strictly application of biosecurity measures, compartmentalization and zoning; strict control of pig movements and carefully re-stock pigs in infected and high risk areas.

Molecular architecture of African swine fever virus

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We have addressed two relevant gaps in African swine fever virus research that concern the composition and the architecture of the infectious virus particle. Using a combination of proteomic and electron microscopy (EM) approaches we have identified the set of viral and host proteins that compose the virion and have solved its native three-dimensional multi-layered structure. Mass spectrometry identified about 70 viral proteins, including more than 40 newly identified virus-packaged polypeptides, half of which have an unknown function. Cryo-EM analysis of the mature ASFV particle unravelled a unique architecture consisting of a genome-containing nucleoid surrounded by two distinct icosahedral protein capsids and two lipoprotein membranes. The outer capsid forms a hexagonal lattice composed by 2,760 trimers of the major capsid protein p72, and 12 pentamers of a penton protein at the vertices. The inner capsid is a core shell made of mature products derived from the ASFV polyproteins pp220 and pp62. An icosahedral inner membrane, which lies between the two protein layers, contains most of the viral transmembrane polypeptides known so far. Intriguingly, only one viral protein, a homologue of the T-lymphocyte surface antigen CD2, has been located on the pleomorphic outer envelope. Finally, proteomic data indicates that the viral nucleoid encloses a complete transcriptional machinery as well as various enzymes implicated in the maintenance of genome integrity. Altogether, these results provide a comprehensive model of the ASFV architecture that integrates the available compositional, structural and functional information.

Serum-Derived Extracellular Vesicles from African Swine Fever Virus-Infected Pigs Selectively Recruit Viral and Porcine Proteins

Montaner-Tarbes, S.^{1,2}, Pujol, M.³, Jabbar, T.⁴, Hawes, P.⁴, Chapman, D.⁴, del Portillo, H.¹, Fraile, L.^{1,2}, Sánchez-Cordón, P.J.⁴, Dixon, L.⁴ & **Montoya, M.**^{1,4,5}

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African swine fever is a devastating hemorrhagic infectious disease, which affects domestic and wild swines (*Sus scrofa*) of all breeds and ages, with a high lethality of up to 90–100% in naïve animals. The causative agent, African swine fever virus (ASFV), is a large and complex double-stranded DNA arbovirus which is currently spreading worldwide, with serious socioeconomic consequences. There is no treatment or effective vaccine commercially available, and most of the current research is focused on attenuated viral models, with limited success so far. Thus, new strategies are under investigation. Extracellular vesicles (EVs) have proven to be a promising new vaccination platform for veterinary diseases in situations in which conventional approaches have not been completely successful. Here, serum extracellular vesicles from infected pigs using two different ASFV viruses (OURT 88/3 and Benin Δ MGF), corresponding to a naturally attenuated virus and a deletion mutant, respectively, were characterized in order to determine possible differences in the content of swine and viral proteins in EV-enriched fractions. Firstly, EVs were characterized by their CD5, CD63, CD81 and CD163 surface expression. Secondly, ASFV proteins were detected on the surface of EVs from ASFV-infected pig serum. Finally, proteomic analysis revealed few specific proteins from ASFV in the EVs, but 942 swine proteins were detected in all EV preparations (negative controls, and OURT 88/3 and Benin Δ MGF-infected preparations). However, in samples from OURT 88/3-infected animals, only a small number of proteins were differentially identified compared to control uninfected animals. Fifty-six swine proteins (Group Benin) and seven proteins (Group OURT 88/3) were differentially detected on EVs when compared to the EV control group. Most of these were related to coagulation cascades. The results presented here could contribute to a better understanding of ASFV pathogenesis and immune/protective responses in the host.

The African swine fever virus A179L Bcl-2 family member is required for efficient replication in porcine macrophages.

Reis, A ¹., Claire Barber, C ¹., Rathakrishnan, A. ¹, Goatley, L. ¹, Portugal, R. ¹, Goulding, L. ¹, Netherton, C. ¹, Banjari, S. ², Kvensakul, M. ² & **Dixon, L.** ¹

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The African swine fever virus encoded anti-apoptotic Bcl-2 family member A179L binds to pro-apoptotic members of the cellular Bcl-2 family thus exerting an anti-apoptotic effect and additionally binds to beclin-1 thus inhibiting autophagy. Structural analysis and binding dynamics of A179L bound to BH3 domain peptides revealed A179L is the first anti-apoptotic Bcl-2 family member to bind all core death inducing mammalian Bcl-2 proteins. Additionally, we demonstrated that A179L inhibits stress-induced apoptosis mediated through the ATF4/CHOP pathway. We demonstrated that deletion of A179L gene from the genome of a tissue-culture adapted ASFV strain BA71V did not reduce ability of the virus to replicate in Vero cells. In contrast deletion of the A179L gene from the genome of the virulent ASFV isolate Benin97/1 dramatically reduced the ability to replicate in primary macrophages. Activation of the pathways targeted by A179L in macrophages infected with the Benin A179L deletion mutant is under investigation to determine which is particularly important for virus replication in macrophages.

Studies on viral DNA replication-related genes towards the ASFV control

Freitas, F.B.¹, Frouco, G.¹, Simões, M.¹, Martins, C.¹ & **Ferreira, F.¹**

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African swine fever, a contagious and fatal disease of domestic pigs and wild boars, currently spreading in different countries in Africa, Europe, Asia and Pacific, is a major danger for global swine industry. The absence of effective vaccines and treatments to control the disease, implies the use of strict sanitary and stamping out measures bearing heavy social and economical consequences at global scale. The current worldwide epidemic scenario of ASF impels for further studies on strategies to prevent and combat the disease. In this work we review results on studies developed in our group, on functional characterization of several viral DNA replication-related proteins (A104R, I215 L, QP509 L and Q706 L), in order to underpin rational strategies for the improvement of vaccine candidates, namely, on the development of DISC viral particles for safe delivery of specific viral antigens towards the activation of prompt and effective immune mechanisms of swine, (Frouco G, et al., *J Virol.* 2017, 26;91(12). pii: e02498-16; Freitas FB, et al., *Sci Rep.* 2018, 22;8(1):3471; Freitas FB, et al., 2019, *Emerg Microbes Infect.* 8(1):291; Freitas FB, et al., 2019, *Vaccines* 7:3). Alongside, several antiviral strategies were also identified, namely regarding the possibility to use inhibitors (caffeine and wortmannin) specific for DDR-related enzymes (Simões M, et al., *Viruses.* 2015;7(9):4978), HDAC inhibitors (NaPB, SAHA) (Frouco G, et al., *Virus Res.* 2017, 15;242:24), and poisons and inhibitors (fluoroquinolones and isoflavones) against ASFV- topoisomerase II (Mottola C, et al., *Vet Microbiol.* 2013 26;165(1-2):86; Freitas FB, et al., *Antiviral Res.* 2016;134:34; Hakobyan A, et al., *Antiviral Res.* 2019 167:78; Arabyan E, et al., *Antiviral Res.* 2018;156:128). The innovative approaches herein reviewed, open new insights towards the development of new strategies on the use of pharmacological approaches and new vaccines, overall compassing effective ways to tackle and impair ASFV infection, thus disabling the fast spread of this dreadful swine disease.

Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (Projects PTDC/CVT/105630/2008 and UID/CVT/00276/2019) and by the European Union's Seventh Framework Programme (FP7/2007-2013, 311931, ASFORCE) and by the COST Action - OC-2015-1-19550.

Investigating the role of African swine fever virus Gene EP402R in virus persistence in blood *in vivo*

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Work presented by Petrovan, V.

African swine fever is a devastating viral haemorrhagic disease of domestic pigs caused by African swine fever virus (ASFV). ASFV is the only member of the family *Asfviridae*. Currently, no vaccines are available. The hypothesis of this project is based on observations from experimental infections of pigs with a genetically attenuated strain of ASFV (Benin97/1 Δ DP148R). The DP148R gene is not essential for virus replication but its deletion reduced the virulence of the virus *in vivo* and provided a high degree of protection of pigs against challenge with the virulent Benin97/1 isolate. However, adverse clinical signs were observed following immunisation with Benin97/1 Δ DP148R due to the high level of viral replication in blood. Infectious virus was present in blood and this persisted over an extended period. ASFV contains an ORF EP402R which encodes a CD2 like protein (CD2v) involved in virus persistence and dissemination. CD2v is expressed as a transmembrane protein on the surface of ASFV infected cells and having an extracellular N-terminal domain involved in haemadsorption of red blood cells to infected cells (HAD). The virus acquires CD2v upon egress from the infected cells and the protein mediates the attachment of RBC to extracellular virion. We investigated if expression of the CD2v protein contributed to the virus dissemination and prolonged persistence of virus in blood. Here we showed that deletion of EP402R from the genome of attenuated strain of ASFV (Benin97/1 Δ DP148R) dramatically reduced the virus persistence in blood, resulting in a delay and decrease in viremia and reduced clinical signs.

Neuropathology and viral antigen distribution in the central nervous system of domestic pigs experimentally infected with African swine fever virus

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Traditionally, the central nervous system (CNS) has been poorly studied in pigs infected with African swine fever virus (ASFV). The aim of the present study was to characterize the histopathological lesions and the viral antigen distribution, detected by immunohistochemistry, in the CNS of domestic pigs experimentally infected by different routes with high and moderate virulent isolates belonging to genotype I (Benin 97/1), genotype II (Georgia 2007/1) and genotype X (Ken05/Tk1).

Pigs inoculated intramuscularly with the highly virulent ASFV isolates (Benin 97/1 and Georgia 2007/1) were euthanized after reaching a humane-end point between day 5 and 6 post infection (pi), while pigs inoculated intranasally with the moderately virulent ASFV isolate Ken05/Tk1 were euthanized between day 9 and 12 pi.

All pigs were viremic. No nervous clinical signs were observed apart from head tilt. Gross examination of the CNS revealed a moderate meningeal congestion in some animals. Histopathological evaluations in different areas of brain and cervical spinal cord revealed a non-suppurative meningitis and encephalomyelitis characterised by the presence of variable sized lymphohistiocytic perivascular cuffs and endothelial fibrinoid necrosis that affected predominantly small and medium sized blood vessels. Associated neuroparenchymal lesions characterised by astrocytosis, gliosis, spongiosis and neuronal necrosis were also observed. Viral antigen was mainly detected in macrophages, endothelial cells and lymphoplasmacytic cells. A spatio-temporal association between the amount of cells immunolabeled against the viral antigen and severity of histopathological lesions was observed. However, occasionally positive endothelial cells and monocytes were also observed without associated lesions. Interestingly, viral antigen was also detected in circulating monocytes, astrocytes, ependymal cells and infiltrating macrophages within the cerebrospinal fluid (CSF).

Acknowledgements

Thank you to ASU staff (APHA, UK) and Isolation Unit staff (The Pirbright Institute, UK). Study financially supported by DEFRA, the Scottish Government and the Welsh Government.

Surveillance of ASF in wild boar: from early detection to long lasting endemic situations

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Surveillance of ASF in wild boar populations plays a pivotal role since its efficacy will affect early detection and the whole management of the infection. An efficient surveillance strategy is essential also in long lasting endemic areas. When the infection becomes endemic, a typical scenario is observed: very few (if any) positive dead animals are found; a limited number of virus positive animals together with a progressive, although limited, increased number of seropositive individuals are detected. A SIR model has been developed to simulate surveillance. A standard Hunting Ground sized 10000 ha inhabited by a 100–400–1000 wild boars (1-2-10 WB/km²) was set as the epidemiological unit. The model runs under a homogeneous mixing and is forced to obtain 2% virus-prevalence in hunted animals; lethality is 95%. The model runs for 8 years (epidemic to endemic status) assuming the continuous presence of the virus. Passive surveillance (10% and 50% of found carcasses) and active surveillance (virus and sero testing each hunted animal) were simulated setting the hunting effort 40%/year. As expected, passive surveillance is irreplaceable for both the early detection and to follow the short-term evolution of the virus in any free or recently infected area. The efficiency of both passive and active surveillance tends to converge when lowering wild boar densities till reaching a threshold (defined by the percentage of found carcasses) at which the sole active surveillance reveals the presence of the virus. At a very low wild boar density, seropositive animals are more likely to be detected than virus positive whereas infected carcasses are rarely found. The model scenario fits the epidemiological picture observed in large endemic areas where the virus can be detected in almost any hunting ground but following an unpredictable pattern.

Seasonality of African swine fever incidence in wild boar in the Baltic States and Poland

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The possible seasonality of the African swine fever incidence in wild boar in the Baltic States and Poland was evaluated. First a visual inspection of the data reported through the data collection framework of the European Food Safety Authority was carried out using LOESS smoothing to describe the global trends. The starting time was considered as the date on which a PCR positive sample was reported for the first time in a specific region since 2014. An apparent peak in the summer for the proportion of wild boar found dead testing positive was observed in Latvia and Estonia which could not be recognised visually in Poland and Lithuania. For the wild boar that were hunted there seemed to be an apparent decline in spring incidence with approximately the same proportion of positive samples over the sampling taken during the rest of the year. A comparison of seasonal incidence using a Tukey pairwise comparison between each pair of seasons proved that the incidence was not equally distributed across the year with some seasons showing a lower or higher probability of ASF occurrence than others, which statistically confirms seasonality, though it was difficult to observe a clear overall trend across the 4 countries in wild boar found dead. In hunted wild boar, however, a decline of the ASF incidence in spring was observed. There are several hypotheses for the decline of ASF in spring in hunted wild boar related to wild boar ecology which are discussed, hereby considering the ASF transmission dynamics in wild boar populations in northern Europe.

Mapping the detectability of wild boar carcass with the SIG MCDA approach

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Detectability of wild animal carcasses in natural environments is impaired by factors such as low accessibility, lack of visibility and short persistence due to rapid decomposition and scavenging. As a consequence, passive or active surveillance of wild animal mortality most of the time faces issues of low detectability. Considering situations where a disease outbreak occurs in a wild animal population, the most obvious consequence of low carcass detectability is that mortality can be dramatically underestimated. A second likely consequence is that, because the factors influencing detectability are most often not spatially and temporally homogeneous, the spatiotemporal distribution of detected carcasses does not reflect the actual distribution of carcasses. Evaluating detectability and characterizing variation in detectability over space and time is thus necessary in order to draw sound epidemiological interpretations of the distribution of wild animal carcass detections.

We report an investigation of detectability of wild boar (*Sus scrofa*) carcasses during an oedema disease (a disease caused by enterotoxigenic *Escherichia coli* strains) epidemic in the French department of Ardèche. The investigation relied on the Geographic Information System Multicriteria Decision Analysis method. With this method, a detectability index combining landscape factors identified through expert opinion as influencing detectability is first derived for two different seasons (the fall and winter hunting season and the summer season). Rasters for the identified landscape features are then used to map the resulting detectability index. As a final step, the spatial location of wild boar carcasses detected is overlaid on the detectability maps to address the importance of detectability in the spatial and temporal detected carcass distribution pattern.

This innovative method could be applied to other populations of wild boars or of other wild animal species affected by outbreaks of diseases such as PPA that generate high mortality rates.

Spread by the dead: role of live and dead wild boar in spatio-temporal dynamics of African swine fever

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This paper summarizes the research on the role of wild boar ecology and behaviour on spatio-temporal dynamics of African swine fever in a wild boar-habitat epidemiological cycle. We used multiple data sources (animal telemetry, population monitoring, surveillance data, habitat composition) and various analytical approaches to make inferences based on epidemiological situation in Poland 2014-2016.

ASF spread among wild boars was slow and stable in time and space. Wild boar movements had little effect on the spread of the disease, which gradually spread through infections between closely neighbouring social groups rather than as a result of long-distance wild boar movements. Consequently, the probability of ASF case decreased sharply with the distance to previous infections and this relationship was stronger at high population densities. Additionally, probability of ASF occurrence, disease spread rate and persistence time increased at higher population densities. The chances of ASF occurrence were also higher in areas of large forest complexes compared to the forest-field mosaic and open areas.

The main mechanism determining long-term persistence of the ASF virus among wild boars was the infection of healthy animals through contact with infected carcass, which accounted for approximately 60% of all transmission. This pathway of infection was particularly important when the number of wild boars was low, while the role of direct infections between healthy and sick wild boars increased at high population numbers. Dead wild boars infected with ASF were most often found in moist environments, near water courses and in shady places.

Our results highlight the importance of carcass-based transmission, wild boar population density and spatial patterns of disease spread in predicting ASF risk. Intensity of disease control efforts, such as carcass removal and population reduction, should thus follow a zonal design targeting high density areas, favourable wild boar habitats and matching predicted disease occurrence over the landscape.

Evaluation of mitigation strategies (border fencing and blocking animal's corridors on motorway) for ASF in Poland

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ASF expansion in Europe is according to current observation ongoing and forecast for future arrival time can be proposed with mathematical modelling and machine learning approaches, where for example most likely our arrival time estimates for Polish counties (*poviats*) are publicly available <http://interdisciplinaryresearch.eu/index.php/asf>.

We try to verify effectiveness of mitigation strategies including: i) building a fence on the Polish EU border and ii) blocking animals corridors on the A1 motorway. We use a pseudo gravity model to construct the effective propagation network and simulate the future projection of the disease spread, taking into account: 1) domestic pig abundance, 2) disease vectors (wild boar) abundance, 3) human mobility. We have reconstructed the most probable infection paths for all Polish *poviats* as well as estimated most likely disease arrival times by simulation. To compare the mitigation scenarios, we measure the difference to the baseline case of various incoming centralities (proxies for arrival times) for a “swine hot spot” in Greater Poland in infection path's network.

i) To verify a possible effect of the fence, we test a scenario in which border *poviats* are disconnected on wild boar network layer. Our results indicate that there is only a small difference in the arrival time to “Polish swine hot spot”, because most of the dynamics is currently happening to the West of the border *poviats*.

ii) To verify a possible effect of blocking animal corridors, we test a scenario in which all *poviats* on A1 motorway are disconnected on the wild boar layer. We find an important difference in the arrival time to “Polish swine hot spot” as compared to the baseline. This estimation seems to be in agreement with the observed propagation in the Baltic States.

Modelling the transmission and persistence of African swine fever in wild boar in contrasting European scenarios

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We present a mathematical model of the wild boar and African swine fever (ASF) system to explore the key mechanisms that drive infection transmission and disease persistence. Model results show that direct environmental transmission is a key mechanism that determines the severity of an infectious outbreak and that direct frequency dependent transmission and a chronic infective stage are key for the long-term persistence of the virus. We consider scenarios representative of Estonia and Spain and show that the increased degradation rate of carcasses in Spain, due to elevated temperature and abundant obligate scavengers, may reduce the severity of the infectious outbreak. The model is also used to assess disease control measures and suggests that a combination of culling and infected carcass removal can lead to the eradication of the virus without also eradicating the host population. Furthermore, early implementation of these control measures will reduce infection levels and, in some situations, prevent ASF from establishing in a population.

Change in prevalence and spatial distribution of African swine fever in Lithuanian wild boar population

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African swine fever (ASF) is fatal viral disease infectious to wild boars and domestic pigs and has devastating economical effect. Lithuania was the first country which was faced out with first-ever outbreak of ASF in European Union in 2014 which was linked to infected wild boar movement and close contact with the carcasses of other infected wild boars.

The overall results obtained from tested samples ($n=109637$) in 2014 – 2018 period confirm the relatively high prevalence of ASF virus in wild boar with 37/51 Lithuanian municipalities being affected by ASF.

However, prevalence and spatial distribution were changing over the years. Results show that ASF virus prevalence in hunted wild boars increased from 0.83% (95% CI 0.69–0.98) to 2.27% (95% CI 2.05–2.48) from 2014 to 2016 respectively. In 2017 nonetheless, there was a dramatic jump in the number of ASF positive wild boar cases resulting in prevalence of 12.39% (95% CI 11.91–12.86) ($p < 0.05$).

The central changes of ASF epidemiology was indicated in 2018 when significant differences of ASF prevalence in wild boars, including 3.7-fold increment of ASF-specific antibodies and 9,7-fold decrease of ASF virus DNA by active surveillance were detected. In the same year significant differences in the prevalence of ASF virus tested by passive surveillance at municipality level in three adjacent pairs of districts were observed.

Furthermore, in the framework of active surveillance in 2018 for the first time ASF-specific antibodies and ASF virus DNA were detected simultaneously in the same samples. Changes of recent year with compliance of other available published data suggest transformation of high lethality ASF infection course observed in Lithuania in 2017 to chronic manifestation of the disease with growing potential to further spread the disease.

A participatory approach to support the control of African swine fever in wild boar

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African swine fever (ASF) reached Poland and the Baltic states in 2014. The disease has been circulating there and was transmitted to several other countries. The spread seems not to decrease. It is known that controlling the disease in wild boar populations is inevitable to protect domestic pig holdings. However, the effect of most control measures is uncertain. Although the importance of passive surveillance is known, its implementation may not always be as consequent as needed.

Hunters are key players in the control and surveillance of ASF in wild boar. By using participatory methods, we aimed to determine the acceptability of control measures for ASF and ways to increase the motivation of hunters to support passive surveillance.

We recruited hunters from different regions of Estonia for focus group discussions. Diagrams and different visualisation tools were used to investigate the acceptability of control measures and motivation options to study the acceptability of passive surveillance. By assigning the tools to defined ranks, the mean or the median could be calculated. The results of group discussions were recorded and included in the reports.

In total, 10 focus group discussions were conducted, in which 46 hunters took part. Selective hunting of wild boar and baiting were well accepted, whereas ban of hunting or including armed forces in hunting was poorly accepted as a control measure. Regarding options for motivating hunters to support passive surveillance, the results indicated that hunters preferred to report detected dead wild boar only rather than being expected to sample and dispose of the carcass. Moreover, an increase of the currently paid incentives raises the motivation of hunters.

Generally, the study demonstrated the high value of participatory epidemiology. Without including the perceptions and thoughts of hunters, it may be difficult to control ASF in the wild boar population.

Preliminary results of the study investigating the presence of ASF virus in wild boars and their ticks in Turkey

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Although the presence of African Swine Fever (ASF) has been reported from the neighbouring countries, the current status of the disease is unknown in Turkey. This study aimed to investigate the presence of ASF virus in wild boars (*Sus scrofa*) and their ticks in different parts of Turkey. As a beginning, blood and tick samples obtained from wild boars, which were previously hunted in Ankara province (the central part of Turkey) between 2013 and 2016, were included in the study. Genomic DNA samples obtained from blood of wild boars ($n = 30$) and from their ticks ($n = 215$) were subjected to ASF virus PCRs. Tick species were identified as *Dermacentor marginatus* ($n = 62$), *Haemaphysalis parva* ($n = 57$), *Hyalomma marginatum* ($n = 27$), *Hyalomma excavatum* ($n = 5$), and *Rhipicephalus turanicus* ($n = 24$). Additionally, one of the collected *D. marginatus* was a fully engorged female and it was incubated under suitable conditions for egg production. After hatching, 40 unfed larvae were randomly selected and included in the study. All samples were screened for the presence of ASF viral DNA using conventional and nested PCRs, which are amplifying the partial *p72* gene of ASF virus. As a result of the analyses, ASF virus DNA was detected in neither blood nor tick samples collected from wild boars in Ankara. This preliminary result indicates that ASF virus may not be actively circulated in wild boars and their ixodid ticks in the investigated area of Turkey. However, we are continuing to investigate and widen the coverage of the study (sampling animals from places bordering Georgia and Armenia) in order to screen large number of samples.

Vector competence: a co-evolution story between African Swine Fever Virus and soft ticks *Ornithodoros*?

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African swine fever virus (ASFV) is the causative agent of African swine fever (ASF), a lethal viral disease of *Suidae*. Some soft tick species from the *Ornithodoros* genus are competent vector for ASFV. The strain introduced into Georgia in 2007 has been reported to originate from East Africa, where the sylvatic cycle between soft ticks and warthogs is present. This sylvatic cycle allows the virus to be maintained in the wild, making it difficult to eradicate ASF. However, the role of soft ticks in transmission and maintenance of ASFV strains circulating in Europe remains to be determined.

Two *Ornithodoros* species were selected for this study: *O. moubata* (known vector in Africa) and *O. erraticus* (reported vector in Portugal) and then infected by the ASFV Georgia2007/1 strain, giving two tick-virus pairs: *O. moubata*-Georgia2007/1 (OmG) and *O. erraticus*-Georgia2007/1 (OeG). Tick infection was attempted by feeding them on viraemic pigs then fed again 2 months later on naïve pigs to test their vector competence. Infected ticks were also used for studying the viral replication kinetics, titration and localization in the ticks.

OmG was able to transmit the virus but not OeG. Viral replication kinetics showed that replication in OeG decreased up to 3 months post infection while virus load was maintained in OmG. Interestingly, ASFV disseminated in OmG and in OeG but with a different efficiency. Infectious virus has also been isolated from ticks.

For the first time, the vector competence of *O. erraticus* for European strain was tested under experimental conditions. The ability of this tick to keep the virus infectious after feeding on viraemic pigs was proved even if it does not transmit to pigs, contrary to *O. moubata*. These results showed that co-evolution between ASFV and soft ticks is probably an important factor for the vector competence.

Assessment of the impact of forestry and leisure activities on wild boar spatial disturbance and the associate risk of spreading African swine fever virus

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#Member of the ad hoc ASF Emergency collective expert assessment group (GECU) of the French Agency for Food, Environmental and Occupational Health & Safety (Anses)

In Europe, African swine fever virus (ASFV) is one of the most threatening infectious transboundary diseases of domestic pigs and wild boar. In September 2018, ASF was detected in wild boar in the South of Belgium. France, as a bordering country, is extremely concerned about the ASF situation in Belgium and an active preparedness is ongoing in the country. One of the questions raised by this situation is related to disturbing activities that may affect wild boar movements and their possible impact on the spread of ASFV. Despite evidence of disturbance related to hunting practices, there is a paucity of information on the impact of forestry and human leisure activities. To assess this impact on wild boar movements, a systematic review was first conducted but very few useful data were obtained. For this reason, an expert elicitation was carried out by the French Agency for Food, Environmental and Occupational Health & Safety in order to deal with this knowledge gap. A total of 30 experts originating from France and adjacent neighbouring countries (North of Spain, Belgium and Switzerland) were elicited about the relative importance of six factors of spatial disturbance of wild boar (noise, smell, invasion of space, modification of the environment, duration, and frequency of the activity). Then, for each factor of disturbance, they were asked

about the impact of 16 different commercial forestry and human leisure activities. A global weighted score was estimated in order to capture the variability of a wide range of territorial conditions and the uncertainty of expert elicitation. This estimate permitted ranking all 16 activities and aggregating them in three groups according to their potential for disturbance of wild boar, using a regression tree analysis. The results of this expert elicitation provide a methodological approach that may be useful for French and other European decision makers and stakeholders involved in the crisis management of ASF.

Decomposition of wild boar carcasses

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When African swine fever (ASF) is introduced into a wild boar population, it is crucial to understand the timeline of events, especially the time of death of a specimen and the duration of carcass presence in the environment. Studies dealing with this question from a forensic point of view by describing and analysing the decomposition and necrophagous insect fauna of a carcass often use the domestic pig as a model organism. The question arises to what extent these data can be applied to the decomposition process of a wild boar.

We analyse the decomposition of seven wild boar carcasses exposed in cages to exclude the influence of scavengers (1) vs. one domestic pig and (2) in different microenvironments (sunlight, shade, water).

(1) Wild boars seem to decompose slower than domestic pigs, perhaps due to their thick rind, that may retain moisture for a longer time and might slow down the rate at which maggots metabolize carcass material. In addition, the species community and development of necrophagous insects differed between the wild boar and the domestic pig.

(2) The persistence time of wild boar carcasses in different microenvironments varied substantially. Sunlight seems to accelerate decomposition, while standing water may slow it down. Clearly, the loss of body mass of the carcasses underlies a seasonal influence, mainly triggered by temperature.

It must be expected that wild boar carcasses (infected with ASF virus) may remain present in the environment for several months.

ASF-WB in Belgium, one year after the emergence

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On September 13, 2018, ASF was confirmed in wild boar (WB) by the Belgian NRL. The two first positive cases were detected in south-east of Wallonia, province of Luxembourg. After confirmation, zoning as well as preventive and control measures were immediately implemented.

From the ASF-WB outbreak day in September 2018 to end of October 2019, 4064 WB were analysed. Among them, 3858 were sampled inside the ASF zone (1,106.62 km² area, including European zones II and I). All of the 828 ASFV-positive animals were from the zone II. Most of the positive cases were found dead (96.1 %), the remaining were killed for sanitary reasons (1.9%), culled (1.6 %) or road injured (0.4 %). In the zone II, the disease is moving from east to west within large and continuous forests.

Control strategies, including the combination of different measures, have so far proved effective to maintain ASFV inside the zone II. Since March 2019, date of the last zoning adaptation, no infected WB has been detected outside this zone. The network of fencing limited WB movements and facilitated depopulation measures (trapping, night shooting, culling and hunting) in ASF zone. Active (analysis of culled WB) and passive (active search of dead WB, carcass removal and analysis) surveillances are maintained in ASF zone. Passive surveillance is a key point in the control strategy, this activity is ongoing with strict respect of biosecurity procedures. With regard to the objective of total depopulation in ASF zone, destruction activities also need to be maintained with strong motivation of the hunters. Outside ASF zone (the rest of Wallonia), passive surveillance is also carried out. Communication networks with EU authorities, neighbouring countries as well as Belgian stakeholders (hunters, forest rangers, farmers, veterinarians, tourists, forestry workers, etc) are essential.

In conclusion, about 1 yr after the emergence, there are no cases among domestic pigs and current results in WB are hopeful. The regional and federal authorities, however, are determined to keep pressure on all actors to manage a second possible epidemic wave in the infected zone after the dispersal of piglets born in 2019 and/or accidental spill-overs of infected animals across fences.

Risk factor in Romanian backyard farms

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To investigate potential risk factors for ASF in backyard farms, a case-control study was set up in Romania. Hereto, outbreak farms from 23 counties were included as case-farms in the study. For each case-farm, two control-farms were randomly selected in the same county. Official veterinarians visited all case-and control-farms once and interviewed the herd owners. The official veterinarians completed a questionnaire containing 42 questions regarding, for instance, herd size, observations of wild boar around farms, feed and water supply and observations of ticks and flying insects on the farm. Information from these questionnaires was combined with other co-variables such as distances to nearest outbreaks in wild boar and in domestic farms, the numbers of outbreaks within certain distances around the farm and density of wild boar and domestic pigs/farms in the neighbourhood of the selected farm.

All data were analysed in a logistic regression model, with disease as outcome and the co-variables described above as explanatory variables, in a matched design. The model was run in R, version 3.5.2 "Eggshell Igloo" (R Core Team, 2018), using the survival (logistic regression) and the geosphere (distance calculations) packages.

In total, 177 case farms and 353 control farms were included in the study. Case farms had an average herd size of 10.9 pigs, while control farms had an average herd size of 3.8 pigs. The herd size was a clear significant risk factor. Other significant risk factors were: the distance to the nearest outbreak in domestic pig farms, the distance to the nearest outbreak in wild boar, the numbers of domestic outbreaks within 10km, introduction of pigs to the farm in the last three weeks, seeing wild boar around the farm and using forage from areas with ASF.

Flyers on ASF preventive measures for pig farms

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Some basic biosecurity principles apply to all farming systems and all diseases, but the main practical biosecurity measures need to be targeted to the disease and to the farming systems in which they are to be implemented. In fact, biosecurity measures are rather standardized in commercial pig holdings, whilst they are not well defined and of easy implementation in backyards.

WG3 has reviewed the measures regarding best practices and harmonized measures to protect the pig industry from ASF in Europe, specifically for highly industrialized farms, non-commercial farms, and outdoor farms. As a result, 6 technical reports and 1 scientific publication have been produced containing the most relevant measures for each of the production systems. Biosecurity measures are based on the latest working document elaborated by the Directorate General for Health and Food Safety (http://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_asf_wrk-doc-sante2015-7113.pdf), scientific literature systematic review and expert opinion conducted under the e-cost action ASFSTOP (EU One Health EJP).

Here, we present a new outcome to facilitate dissemination of the biosecurity measures: three handy flyers on ASF preventive measures for pig farms.

Modelling the role of stable flies in the transmission of African swine fever virus in outdoor pig farms

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In the European regions where the African swine fever virus (ASFV) circulates, the biological vector of the virus, soft ticks of the genus *Ornithodoros*, is considered absent. Given the epidemic seasonal variations, there are growing questions about the involvement of other potential arthropods in ASFV transmission within and between populations of domestic pigs and wild boar. As the result of an expert elicitation aiming at prioritising these potential vectors in France, stable flies *Stomoxys calcitrans* have been identified as having one of the highest probabilities of playing a role in ASFV transmission. The objective of this study was to assess quantitatively the relative contribution of stable flies to the within-farm spread of ASFV. For that purpose, a vector-borne transmission model was developed and adjusted to the context of an outdoor pig farm with varying densities of stable flies. The model was calibrated using data from a literature review and an expert elicitation. The influence of critical parameters on the model outcomes was assessed via a thorough sensitivity analysis. Results suggest that, in a context of moderate infestation (< 20 stomoxes per pig), vector-borne transmission is likely to contribute to less than 5% of ASFV transmission events. However, with higher densities (50 to 100 stomoxes per pig), vector-borne transmission could be responsible for between 6 and 22% of transmission events, leading to a most-likely within-farm epidemic peak occurring between two and seven days earlier than in the absence of stable flies. The sensitivity analysis highlighted that these results are robust against changes of most model parameters except the infective dose and the blood volume that can be regurgitated by stable flies. This work suggests that stable flies could play a non-negligible role in the within-farm spread of ASFV and emphasises the need to consider vector control in ASF control policies.

African swine fever: a biosecurity challenge for pig production in Serbia

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African swine fever (ASF) continues to spread in Europe, and in 2019 was detected in domestic swine population in Serbia, Western Balkans. Although the Veterinary Directorate of Serbia two years ago ordered the implementation a set of control and the preventive measures were applied in the country and at border crossings, the first case was detected in the central region of Serbia. However, for the experts who studied the organizational structure of pig production and the level of existed biosecurity, this was expected. According to the level of biosecurity, five different pig production types can be distinguished: commercial farm, family farm types A and B, backyards and free-range. Backyards are common practice in villages and a quite large percentage of the population is raised this way (82.7%). Even today, despite of veterinary regulation, this production is often characterized by swill feeding and almost no biosecurity. From the other side, the old types of commercial pig holdings in the past were owned by the state and in the 1990s were privatized. However, the biosecurity measures that are recognized today as the essential for sustainable pig production are not possible to implement in the old systems comparing to newly built holdings. The biosecurity measures are not officially required by veterinary regulation and are only given in a form of recommendations. Based on the results of the questionnaire conducted in 2018, it can be concluded that the most significant biosecurity risks for the commercial farms are related to different transport vehicles that enter the farm perimeter, lack of the adequate sanitary facilities for workers and visitors and the problem with the workers who are in contact with backyards in the village.

Acknowledgments

This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia, grants TR 31084.

Biosecurity assessment of Macedonian commercial pig farms using an online scoring system

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Nowadays implementation of biosecurity measures on commercial pig holding is of paramount importance regarding prevention of exotic disease such as African swine fever (ASF). The objective of this study was to assess the biosecurity status in 13 Macedonian farrow-to-finish pig herds by using Biocheck.UGent™ online scoring tool and to compare implemented biosecurity measures between large scale and small-scale farms. Descriptive statistical analysis was used to examine obtained data.

The mean score for total biosecurity was 55.4, while average scores for external and internal biosecurity were 63.7 and 46.8 respectively. Regarding the subcategories for external and internal biosecurity, purchase of animal and semen had the highest score of 79.7 for external, while fattening unit with 69.3 received the highest scores for internal biosecurity. Feed, water and equipment supply scored lowest score (37.6) for external biosecurity, whereas lowest score for internal biosecurity was recorded for farrowing and suckling period (31.6). Large-scale farms had significantly higher scores in contrast to small-scale farms for total biosecurity (65.8 vs.46.4), internal biosecurity (59.7 vs. 35.9) and external biosecurity (71.7 vs. 56.9). For internal biosecurity, large farms had higher scores for measurements between compartments and the use of equipment ($p=0.003$), for cleaning and disinfection ($p=0.009$), nursery unit ($p=0.027$) and for disease management ($p=0.029$). Additionally, large farms had higher scores than small ones for external biosecurity for feed, water and equipment supply ($p=0.020$) and for personnel and visitors ($p=0.007$).

This study reveals that biosecurity practices in Macedonian commercial pig farms are poorly implemented regarding global average scores. Although large-scale farms showed higher biosecurity level than the small farms, there is a need for further enhancement as well. Therefore, it is highly recommended for Macedonian commercial pig farms to improve both external and internal biosecurity in order to prevent entering and spreading infectious diseases.

Risk factors for introduction of African swine fever to domestic pig herds with emphasis to external biosecurity measures - a case-control study in Estonia

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External biosecurity breaches have been considered as most important risk factors for introduction of African swine fever (ASF) into domestic pig herds. However, the scientific knowledge on herd level risk factors (except the most evident like swill feeding and uncontrolled animal movements) for introduction of ASF into domestic pig herds is scarce, particularly from Europe. The aim of this study was to compare the external biosecurity level and management practices of ASF outbreak and control herds and to identify risk factors for ASF introduction. A case-control study was arranged using the data collected from outbreak herds during outbreak investigations and from a randomly selected negative herds in Estonia. Logistic regression and multiple correspondence analysis (MCA) were used to identify associations between biosecurity and management parameters and herds' case status. The average biosecurity score in ASF case herds was significantly lower compared to control herds. However, the effect of intensified official controls to general improvement in the implication of biosecurity measures in pig farms cannot be excluded on the result as the data on control herds were collected during the last year of occurrence of ASF outbreaks in Estonia (2017). Larger herds were more at risk of being cases compared to smaller ones. The biosecurity parameters significantly associated with the case status were mostly related to indirect contacts with outside farm environment or lack of proper disinfection measures at entrance to the farm.

Lack of evidence for long term carriers of African swine fever virus - a systematic review

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Although African swine fever is typically associated with high case fatality rates, a proportion of infected animals will recover and survive. Early on it was speculated that such survivors may act as virus carriers. Whereas the epidemiological importance of such carriers has almost become an established truth, the scientific basis may be questioned. With this in mind, we reviewed the literature in a systematic way. The selection of publications was based on a database search, followed by a stepwise screening and exclusion process. By this process the number of publications finally included was reduced from 3664 initial hits to 38. Based on extraction and analysis of data from these publications, it was clear that a definition of an ASFV carrier is lacking, and that any survivor or seropositive animal often has been referred to as carrier. It was also clear that evidence of any significant role of such a carrier is absent. Two types of “survivors” could be defined: 1) chronically infected pigs which eventually will succumb to the disease, and which may excrete virus in association with reactivation of the infection; 2) pigs which clear the infection, and which will not present with prolonged virus excretion. No evidence was found that suggests that these survivors can be considered as “healthy” carriers. Localized virus persistence may occur in survivors, which may cause infection after oral uptake by susceptible animals. To what extent this is relevant in reality, however, can be discussed given the virus dose generally needed for oral infection.

Towards Veterinary Anthropology: Manifesto of an Emerging Field

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One of the lessons to be learnt from the unfolding epizootic of African Swine Fever is the necessity to rethink the way human behaviour, arguably the most significant factor in disease transmission, features in considerations of veterinary epidemiology. Understanding this realization as an invitation for social sciences and humanities to join forces with veterinary expertise we note that while medical anthropology is well constituted, veterinary anthropology still has to earn its existence by offering rich studies of relations between humans and animals in various societies. Our aim in this paper is to introduce the emerging field of veterinary anthropology and suggest the possible avenues of its future development. We argue for a field that would pursue a twofold mission of producing analytical understanding of social ramifications of veterinary expertise while simultaneously facilitating interdisciplinary shift towards efficient use of anthropology's methodological and conceptual tools for the ultimate goal of veterinary medicine: animal and human health.

Why me? Patterns in African swine fever outbreak farms in Lithuania.

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African swine fever (ASF) has been circulating in Lithuania since 2014. Several ASF outbreaks in domestic pig farms were recorded, primarily during summer. In contrast to neighbouring countries, the number of outbreaks increased over time. In the last 5 years, 137 domestic pig outbreaks were reported.

In the present study, we aimed to identify risk-factors for ASF outbreaks in domestic pig holdings, thereby offering the opportunity to prevent potential future outbreaks effectively.

A prospective case-control-study was performed using data of ASF outbreaks in summer 2019. Due to the clear dominance of outbreaks in small pig holdings in the past, only farms with 100 pigs or less were included in the study. For each outbreak farm, two control farms were selected. Cases and controls were matched by region and the approximate herd size. Data were collected for 20 variables using a questionnaire that has previously been established for epidemiological outbreak investigations in Lithuania. For the univariable analyses, non-parametric tests were used. Multivariable analysis was performed by logistic regression.

In total, 19 outbreaks occurred in the time from June 2019 – October 2019. None of the affected farms had more than 60 pigs. Therefore, data from all outbreak and from 38 control farms were included in the analyses.

Only in three farms, kitchen waste was fed. In none of the holdings, the farmers were also hunters. However, all affected farms had recordings from ASF cases in wild boar in close vicinity to the holding (12 km maximum distance).

The results of the study emphasize the high risk of an ASF introduction, which is associated with the presence of infected wild boar near pig farms. Therefore, to combat ASF, the control of ASF in wild boar populations and high biosecurity measures remain the main tasks that should be focused on.

Five years of African swine fever in Estonia: How close we are to freedom?

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In Estonia, the first African swine fever (ASF) diagnosis was confirmed in September 2014, in a dead wild boar found close to the southern border of the country. Shortly after that, ASF cases in wild boar were detected over 200 km north-east of the first cases, not far from the border with the Russian Federation. In total, 73 infected wild boar were found in 2014. During 2015-2017 the virus spread through the entire wild boar population in Estonia, leaving only one county out of 15 (the island Hiiumaa) free of the infection. 3 531 infected wild boar was detected over this period, 10% of all investigated wild boar. In the same period 27 outbreaks in domestic pig farms was confirmed: 18 in 2015, six in 2016 and three in 2017. Since 2018, a decreasing trend in ASF cases in wild boar could be observed. In 2018, in total 284 ASF-positive wild boar (in 5.5% of tested) was detected, and only 58 of them were PCR-positive. In 2019 (until 30 of September), ASF has been confirmed in 67 wild boar (in 2.1% of tested) and six of them has been PCR-positive. Moreover, the last PCR-positive wild boar was found in February 2019. In the period from April 2018 to September 2019, PCR-positive wild boar has been found only in five counties out of 14 affected. In 2019, the prevalence of ASF antibody-positive wild boar has decreased compared to 2018 (5.8% of all investigated wild boar were positive in 2018 and 2.0% in 2019). Since September 2017, there has been no outbreaks in domestic pigs in Estonia. This confirms the finding of our previous study that the outbreaks in domestic pigs in Estonia were associated with the spread of ASF virus in wild boar population. In conclusion, our current surveillance data confirm that the ASF epidemic in Estonia is subsiding.

Spatio-temporal analysis of the spread of ASF in the Russian Federation in 2017-2019

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This report presents a descriptive analysis of African swine fever (ASF) epidemics in the Russian Federation (RF) in 2017-2019 based on the statistical assessment of its distribution. This study aims to describe spatial and temporal patterns of spread of ASF in wild boar and domestic pigs for the last three years. Methods of Spatio-temporal scanning statistics of Kulldorff (SatScan) and Mann-Kendell statistics (space-time cube) were used to identify potential clusters of outbreaks and presence of hot spots (areas of active flare clusters) respectively. The results showed that ASF in the country has a local epidemic pattern of spread (12 explicit clusters in wild boar and 15 epizootic clusters were detected in the domestic pig population: 10 in the European part and 5 in the Asian part), and only six of them are overlapped suggesting that ASF epidemics in domestic pigs and wild boar are two separate and spillover processes. In the Nizhny Novgorod, Vladimir, Ivanovo, Novgorod, Pskov, Leningrad regions, the clusters identified are characterized as sporadic epidemics clusters, while in the Ulyanovsk region, Primorsky Territory, the Jewish Autonomous Region the clusters are consistent. Considering low biosecurity level of pig holdings on the far east and its close economic and cultural connectedness with China as well as others potential risk factors, it can be expected that the epidemic will be present in the region for a long time. The disease has spread in the country since 2007, and now it is reoccurring in some of the previously affected regions. Outbreaks in domestic pig sector can be localized easily (no pattern detected), while the presence of the virus in wildlife (several consecutive hot spots detected) hampers its complete eradication. The results indicate that despite all efforts taken since 2007 the policy of eradication of the disease needs to be reviewed, especially measures in wildlife.

African swine fever and its way through Asia and towards Europe (Ukraine 2016-2019: lessons learned)

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African swine fever (ASF) is the biggest threat for European pig farming of nowadays. This is highly contagious disease could be transmitted as by the contacts of the domestic and wild pigs. The first outbreak of African swine fever was reported in Ukraine (the human-factor associated introduction from Russian Federation) in 2012. About 5000 disease outbreaks were detected in last years on the Eurasian nooareal of the disease.

502 outbreaks (46 – in 2019) of ASF were described in Ukraine since 2012. Most “domestic” cases were associated with weak level of the biosecurity in small farms and backyards, but also several industrial farms were involved as well. Now there exist around 10 compartments of the disease potential zones in the North-central, Northeastern and Eastern parts of Ukraine are associated with very high risk range (7-9 balls of the risk). Till 2015-2019 ASF drift within whole territory of Ukraine was associated with illegal trade with meat, corn, animals and animal products, and migration in wild boar population. Now ASF is the endemic problem in whole territory of Ukraine, mostly associated with human factor and illegal trade, as well as internal populations of wild boar. The “human factor” role study demonstrates its significance (within the DTRA-funded project UP-10). Effective disease management was started to be implemented after development and setting up of the compensation program and depopulation of pigs in private sector.

ASF represents high risks for EU member states associated with international trade, wild boar migration (7-9 balls), and moderate (2-3 balls) risk levels associated with soft ticks areal enlargement to the North. The disease control requires implementation of the effective farm insurance and private sector compensation mechanisms.

Housing ASF pigs in high containment

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The large animal team at The Pirbright Institute carries out research looking into the development of vaccines for ASF using conventional pigs as a model. The pigs are housed in a high containment unit which poses biosecurity and welfare challenges when trying to meet their natural behaviours/environment. Pigs are housed in a 20m² room which is biologically sealed enabling high pathogens to be housed and acts as primary containment.

On average 5 vaccine trials are carried out per year, each lasting up to 8 weeks with various group sizes. Previous studies have enabled refinement of practices to meet the challenges faced. This poster summarises how the pigs are housed within the containment facility and how we strive to combat the biosecurity, welfare and scientific challenges. Biosecurity challenges include waste flow management, working in a negative pressure environment, working up to SAPO 4 regulations and disinfection routines. Welfare challenges have been improved by provision of a deep straw bed, enrichment items for the individuals to express their natural behaviour and a settling in period allowing habituation to the unit, routines and staff in turn reducing stress. The animal technicians and scientists have a close working relationship where many subjects are discussed to help improve the environment and science which has led to the development of robust and reliable clinical scoring assessment system of ASF, leading to refined humane end point across various severities.

It is perceived that these challenges are hard to address but by working alongside the biosecurity team and adapting ways things are done to fit in with the limitations that we face means methods can be fixed or even improved. By addressing these challenges and mimicking the pig's natural environment as much as possible subsequently produces robust and reliable scientific results and increases animal welfare.

Can we improve ASF control by learning from outbreaks?

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African swine fever (ASF) was detected first in Latvia in wild boar at the Eastern border in June 2014. Since then ASF continued its spread in wild boar populations covering nowadays almost all country. Sporadic outbreaks in domestic pig holdings located in wild boar infected areas occurred. Most of these outbreaks were in small farms, however, few large, commercial farms were also affected thus giving opportunity for disease managers and researchers to study each case in depth to better understand the disease and to improve control measures. The epidemiological investigations in two large outbreak farms with different courses of disease and different approaches for early detection are presented. In both farms several important disease control parameters were analysed to determine the high risk period and to understand how ASF virus entered and spread within the farm until notification. The measures for early disease detection, particularly the enhanced passive surveillance was analysed and discussed. Additionally, the clinical data, mortality rates and laboratory results were analysed for a better understanding of the disease pathways within the farms. Mortality rates proved not to be the main indicators of ASF presence within a farm in respect to early detection. Enhanced passive surveillance is a key issue to detect disease introduction at early stage. The study also describes how slow ASF virus spreads within a large farm and mainly depends on direct contacts between pigs and level of internal biosecurity. Daily maintenance of high level biosecurity remains to be the main issue for virus introduction especially with regards to vehicles entering the farm for providing daily services. The anthropogenic factor still has a leading role for ASF virus introduction into the farms.

Ranking of blood feeding arthropods in Metropolitan France based on their putative vector capacity to transmit African Swine Fever virus: a first expert knowledge elicitation

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To deal with the lack of literature data on the putative vector capacity of blood feeding arthropods (BFA) and their role in the transmission of African Swine Fever virus (ASFV) in Metropolitan France, a dedicated working group of Anses performed an expert knowledge elicitation. In total, 15 different BFA (at genus or family level) were selected by the ad hoc working group as potential vectors. Ten vectors criteria were then considered: vector competence, current abundance, expected temporal abundance, spatial distribution, longevity, biting rate, active dispersal capacity, trophic preferences for *Suidae*, probability of contact with domestic pigs and probability of contact with wild *Suidae*. Among the 27 elicited experts, 14 of them participated to the elicitation. For each BFA, all experts gave a score (between 0 and 3) for each of the above criteria with an index of uncertainty (between 1 and 4). Overall, all experts gave a weight for all criteria using the Las Vegas technique (Gore, 1987). A weighted sum of score by BFA was calculated permitting to rank the different BFA in decreasing order. Finally, using a regression tree analysis, the BFA selected were grouped (classified in groups with lesser variance). Out of the ten considered criteria, the experts indicated vector competence as the most important factor. The current lack of information on this factor makes however that almost all considered BFA received approximately the same score and that potential vectors have thus mainly been prioritized only on the basis of other factors related to vector capacity for ASFV. These other factors were classified in descending order of importance as follows: abundance, biting rate, trophic preferences for *Suidae*, and probability of contact with the target host (domestic pig and/or wild boar). In the context of Metropolitan France, the stable fly (*Stomoxys calcitrans*) was ranked first followed by the lice



(*Haematopinus suis*) and mosquitoes (*Aedes*, *Culex*, and *Anopheles*). More studies are however requested to investigate the potential vectorial role of some BFA, starting with *Stomoxys calcitrans*.

Four years of advances in African swine fever in Europe by the ASF-STOP COST Action

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The ASF-STOP COST Action was launched in 2016 with the aim of creating synergy among experts on African swine fever (ASF) from different and complementary disciplines. The final goal was stopping the spread of ASF in Europe, and beyond.

This 4-year networking project has gathered more than 260 participants from over 32 COST Countries, and additional countries and organisations internationally. ASF-STOP has liaised with several international associations and institutions, e.g. Global African swine fever Research Alliance, EPIZONE, European Food Safety Authority and Wildlife Disease Association, among several others.

ASF-STOP organised two International Conferences (Pulawy - Poland, 2016 - and Brescia - Italy, 2020) and 13 scientific meetings of the 4 working groups in 9 different EU countries. It also supported exchange of scientific knowledge across Member Countries by funding over 20 Short Term Scientific Missions and 4 Training Schools. Over 10 Inclusiveness Target Countries (ITC) Grants contributed to dissemination activities. ASF-STOP has also shared information on activities, research advances and publications with participants, researchers, stakeholders and the general public through its website (<https://www.asf-stop.com/>) and social media. Further dissemination took place by active participation in scientific conferences, organization of workshops and activities targeting the pig industry. Additionally, collaboration among participants from multiple disciplines led to 5 international peer-reviewed scientific publications, funded by the Action.

This 4-year Action has significantly contributed to the establishment of a wide platform for knowledge and know-how exchange on ASF. The Action has achieved its main goals of promoting effective management and control of wild boar populations for ASF spread prevention, improvement of surveillance methods for early detection of ASF, better understanding of ASF epidemiology and development of improved management and diagnostic tools for ASF. In general terms, ASF-STOP has played an important role within the ASF European and global scientific community.

African swine fever eradication programme in Sardinia: an update

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After more than four years of hard work, final eradication of African swine fever from Sardinia is very close. The implementation of the planned actions started in 2015, under the coordination of the “Project Unit”, in the context of a very comprehensive strategy. These actions have been very effective leading to a gradual stop of ASFV transmission in the three populations of pigs (domestic pigs, free ranging pigs and wild boars) of Sardinia.

The assessment of the epidemiological data gathered in the framework of the eradication plan was carried out making use of innovative tools. These tools have enabled to evaluate the importance of the social aspects associated with ASF occurrence, and to modulate disease control measures on the bases of a detailed risk analysis. This approach made it possible to consider at least 88 % of the territory of Sardinia to be free from any ASF-risk significant indicator and that the goal of complete eradication can be achieved within a short time.

In particular, the results of the controls and laboratory checks carried out on the three population of pigs are provided, showing that the free ranging pigs population acted as ASFV carrier and that its removal was and is the key measure to achieve eradication. Conversely, a large amount of data suggests that in the areas where free ranging pigs were not present or were eliminated wild boar-to-wild boar ASFV transmission tends to fade out spontaneously.

Research gap analysis on African swine fever

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African swine fever (ASF) is a major disease of global concern due to its economic impact on pig breeding. A new approach different from previous gap analysis was carried out looking into the current breaches hampering effective management of the disease. In this report, Veterinary Services together with other stakeholders from differently affected areas in the European Union, closely involved in pig production and wild boar management were asked to identify the most significant knowledge gaps in the prevention and control of ASF through an online survey. The answers were grouped into 10 main categories according to the topic. There was a good agreement in the identification of the most relevant issues from both groups of respondents, related to the categories 'wild boar', 'African swine fever virus (ASFV) survival and transmission', 'biosecurity' and 'surveillance'. In addition, the need for improved knowledge of potential sources and pathways of ASFV introduction into unaffected areas, disinfection methods and protocols, communication methods to increase awareness among all players involved in the epidemiology of ASF (including truck drivers, hunters and tourists) to increase compliance with existing control measures were also topics mentioned by all stakeholders. Improvement of the diagnostic sensitivity of tests and a better understanding of the virulence of the circulating ASFV were also identified gaps. The purpose of the report is to facilitate evidence-informed decision making on prevention and spread of ASF based on real field concerns and to set the agenda for basic research activities.

Eight years wasting money - do we need ASFV whole-genome sequencing?

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Since 1957, African swine fever virus (ASFV), the most devastating viral pathogen of swine in the world, has spread multiple times from the endemic regions of sub-Saharan Africa across continents into Europe. Due to the large and complex double stranded DNA genome of up to 194 kbp and the lack of knowledge about any related viruses, characterization of this extraordinary virus has been limited to the analysis of partial sequences from the ASFV genotype (GT) I for decades. In 1995, the first whole-genome sequence of the Spanish cell-culture adapted ASFV-BA71v isolate (GTI) was published representing the reference sequence for ASFV until today.

Since the re-introduction of ASFV (this time GTII) into Europe in 2007 and the subsequent dramatic spread through Europe and Asia, ASFV research thrives. Supported by the technical advances in genome sequencing, novel ASFV whole genome sequences from this outbreak have become available after 2010. However, the 43 publicly available sequences cover only ten of the twenty-four known ASFV GTs and mostly represent the two pandemic GTs I (nine sequences) and II (eighteen sequences). With new GT II sequences being published with increasing frequency, issues concerning quality and value of these sequences emerge and give rise to the question about the sense of ASFV whole-genome sequencing. Especially in Europe and Asia, where the strains show over 99.9 % nucleotide sequence identity to each other, the added value is low while the sequencing costs, due to the high complexity of the ASFV genome, are high. Therefore, the current strategy of random high coverage ASFV whole-genome sequencing needs to be revised towards more targeted approaches using new and affordable methods for whole-genome screening in combination with highly sensitive deep sequencing approaches to identify changes and investigate the underlying mechanisms and virus evolution.

Are we replacing African Swine Fever (ASF) with Avian Influenza (AI)?

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East Asia is currently the hotspot of ASF. The major part of the global pig population is represented in this region and this population and farmers is under tremendous pressure. It is estimated that the regional pig population has already decreased by 40-50% due to ASF and it is predicted that up to 70% of the pig population may be lost until the end of 2020 if the virus keeps spreading at current pace.

With the devastating effect of ASF on the global pig industry, causing a shortage of animals, prices for pork and piglets increased significantly. High prices for piglets, combined with the fear that their farm may be re-infected with ASF after restocking, has made many pig farmers, especially small-scale producers, either close their business or switch from pig production to alternative proteins, especially poultry. Small-scale pig producers account for about 30 percent of the Chinese national pig meat production and even more in other Asian countries. When small-scale farms, which often lack appropriate biosecurity measures, replace pig production with poultry production, several concerns arise, especially with AI being present in the region. Changing the production system might not only exchange pigs for poultry, but also African swine fever for avian influenza. There is an increased risk to generate favourable conditions for occurrence and spread of AI, if the switch from pig production to poultry production is not accompanied by an assessment and revision of the biosecurity measures. This is of major concern due to the zoonotic threat of AI, its pandemic potential and its capability to spread globally faster and harder to control than ASF. Establishing appropriate biosecurity measures and especially pointing out differences in biosecurity needs for poultry compared to pig production will be essential to avoid jumping out of the frying pan into the fire.

POSTERS

Improvements in pathology capacity and early detection of African swine fever in Sweden by Short-Term Scientific Missions

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African swine fever (ASF) is currently the biggest threat to global pig production and early detection and vaccine development are two key tools to help combat the disease. Both require a multidisciplinary approach in which veterinary pathology plays an important role. Early detection of ASF, critical for fast implementation of control measures, is based to a large extent on passive surveillance involving post-mortem examination of animals and although Sweden is free of ASF, disease occurs in nearby countries. Studies on pathology and pathogenesis of ASF provide key basic knowledge for vaccine development. Use of standardized methods to describe pathology is needed to share and compare results from both experimental infections and lesions in naturally infected animals.

The goals of this short-term scientific mission (STSM) were to learn how to recognize and score macroscopic ASF lesions to enhance our diagnostic preparedness for early detection in Sweden, establish contacts with researchers working with ASF and gain basic knowledge in performing experimental infections in high biosecurity facilities. We therefore participated in post-mortem examinations of pigs infected with ASF virus (ASFV) at the Pirbright Institute and received practical training in recognizing and grading ASF lesions using a standardized scoring system based on a previously published system. Additionally, material and protocols have been provided through this STSM to help develop immunohistochemical techniques against ASFV to improve our diagnostic capabilities in Sweden.

This STSM also resulted in continued collaboration between our institutes. Veterinary pathologists at SVA have received additional training on identifying and scoring histopathological lesions characteristic of ASF. This histopathological scoring system, currently in use at the Pirbright Institute and Animal and Plant Health Agency (UK), which is also being applied in another on-going collaboration to study natural ASF infections in wild boar, constitutes another step towards harmonization of interpretation of ASF pathology and pathogenesis.

Surveillance of African Swine Fever in wild boars and domestic pigs in Montenegro

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Seromonitoring on the presence of antibodies against African Swine Fever (ASF) virus in wild boars in Montenegro started five years ago. After reporting of the first case of ASF in the neighbouring Serbia, Montenegro has expanded its surveillance so that, in addition to seromonitoring, PCR has been in place since autumn of 2019.

Surveillance was conducted in cooperation with the Hunting federation of Montenegro and private veterinary practices. As part of the monitoring of the wild boar population in Montenegro in 2018, a total of 303 wild boar body fluid samples were delivered to the Diagnostic Veterinary Laboratory (DVL) in Podgorica. All samples were tested in the DVL. The tests were performed using the Ab ELISA method. After examination, antibodies against ASFV (positive reaction) were not recorded in any sample.

In addition to ASF monitoring of the wild boar population, passive surveillance was carried out in domestic pigs in Montenegro. A total of nine samples of internal organs (spleen, kidney) were examined in the Diagnostic Veterinary Laboratory in 2018. DNA sequence of ASF virus was not detected in any tested sample.

Notably, after reporting of ASF cases in Serbia African Swine Fever has posed a danger not only to wild boars, but also to the population of domestic pigs in Montenegro. Only the timely diagnosis and adequate implementation of biosecurity measures can prevent the introduction and spreading of this disease in Montenegro.

African Swine Fever virus in illegal pork meat imported in Belgium by travellers from Cameroun, August 2017

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Illegal meat imported by travellers represents risks, including pathogen pollution. Out of 66 meat samples originating from sub-Saharan Africa and seized at Zaventem airport (years 2017-2018), 61 were of sufficient quality to realise a metagenomics analysis. These samples were seized either within the framework of the leaking luggage searches or during systematic controls organised by a multidisciplinary team. A de novo exploration of pathogen agents present in the samples was realised by metagenomics analysis after enrichment of viral, bacterial and parasitic particles allowing a better detection of the pathogens. This screening step was implemented on pools of samples. After a bio-informatic analysis of the results, a pool containing genomic sequences of the African swine fever virus (ASFV) was identified. Individual screening by real-time PCR permitted to determine the positive sample and to estimate the viral load (Ct). A phylogenetic analysis was performed based on a partial sequence of the gene coding for the p72 protein in order to specify the virus genotype.

This pilot study demonstrated the presence of nucleotide sequences of the ASFV (Ct value=28) in a meat sample identified as *Sus scrofa* by metabarcoding and imported illegally by a passenger from Cameroun heading to Paris Charles de Gaulle (France) in August 2017. The phylogenetic tree confirmed this origin and linked it to a viral strain of genotype I, which is frequent in sub-Saharan Africa. Since its spread worldwide, the ASFV was detected several times in illegally imported meat (e.g. Kim et al., 2019). This discovery is of great concern due to the high resistance of this virus in meat products.

African swine fever surveillance in Finland 2010-2018

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Finland is free from African swine fever (ASF). The active surveillance in hunted wild boars and domestic pigs was initiated in 2010 and extended to farmed wild boars in 2011 and includes the whole territory of Finland.

The number of hunted wild boar tested each year is relatively small due to the small wild boar population in Finland (estimated 1500–2650 heads, median 1950, January 2019). Natural Resources Institute Finland has developed the stock assessment since 2017 and the catch reporting obligation has been in force since 3 August 2017, so the historical data is not fully comparable (table 1). The number of domestic pigs 2018 was 1 041 100 animals in 1000 holdings. Wild boars were farmed in 180 farms in Finland 2017.

Finnish Food Authority is the NRL for ASF and has diagnostic preparedness to diagnose both ASF virus and antibodies. Finnish Food Authority has participated in reference samples test trials since 2004.

Table 1. Tested wild boars, farmed wild boars and domestic pigs for ASF and wild boars hunting bag and percentage of wild boars in hunting bag tested years 2010-2018, all with negative results.

Year	2010	2011	2012	2013	2014	2015	2016	2017	2018
Wild boars PCR^{1,2}	10	8	8	9	138	171	366	527	715
Wild boars ELISA^{1,2}				9	37	31			
Wild boars hunting bag	100	100	90	160	386	486	502	571	882
% of tested wild boars	10%	8%	9%	11%	45%	42%	73%	92%	81%
Farmed wild boars PCR^{1,2}		42	33	1	2	130	60	44	54
Farmed wild boars ELISA^{1,2}				43	55	107			
Domestic pigs PCR¹	4	78	45	100	40	91	128	57	55
Domestic pigs ELISA¹			39	73	73	42	24		

¹passive surveillance ²active surveillance

Surveillance of ASF in domestic pigs after the first introduction of disease in Serbia

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African swine fever virus (ASFV) was for the first time detected in Republic of Serbia in domestic pig population on 30th July 2019. The first outbreak was detected in central part of country, in backyards population. In total 17 outbreaks were recorded in backyards in the next 3 weeks. Additionally, an outbreak was detected in village near the Romanian border, 6 weeks later. Although ASFV passive surveillance in domestic pigs and wild boar as well as active surveillance among shot wild boars on the country territory was already in place, the Veterinary Directorate prepared the surveillance plan for ASF in domestic pigs. The surveillance will last 10 weeks, and different types of pig holdings will be checked. The characteristic of pig production in Serbia is existence of high number of backyards and small family farms in addition to the larger commercial holdings. Most of the farms are “farrow to finish” type and smaller number are only fattening. The surveillance is based on the weekly visits of field veterinarians to holdings with more than 10 sows or 150 fatteners and one visit in 7 to 14 days to farms with less than 10 sows and 150 fatteners. Field vets need to make a clinical observation and fill the questionnaire about the observed possible suspicious signs. They are obliged to do sampling from all dead/ill sows, gilts and boars, or up to 2 pigs older than 2 months, and send the samples to the Veterinary Institutes. The collected samples (blood swab or tissue on swab) are tested by molecular (PCR) method on daily basis. Reporting to the Veterinary Directorate are done on weekly basis except in the case of positive ASFV result that is reported immediately. After one month and more than 3000 tested samples no ASFV positive result is detected.

Acknowledgments

This work was supported by the Veterinary Directorate, Ministry of Agriculture, Forestry and Water Management and by project grant TR 31084 of Ministry of Education, Science and Technological Development of the Republic of Serbia.

Short Term Scientific Mission in Lithuania: African Swine Fever Management and Control

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In 2019 African swine fever (ASF) virus continues to spread around Europe (EU) and currently the last outbreak was confirmed in Western Balkan region. In Europe, there are numerous differences between EU and non-EU countries in terms of control measures adopted, prevention, biosecurity, control, diagnostic procedure and implementation of immediate measures (outbreak eradication) after the ASF confirmation in the country. Currently it is six years from the first ASF notification in the EU and some countries already have a proved first-hand experience in measures applied in ASF management. Lithuania has the longest experience in ASF management both in wild boar and domestic pigs and is actively involved in ASF control since the beginning of 2014. Currently, ASF is still detected in populations of domestic and wild pigs. Having in mind that practical experience is of utmost importance for non-infected, but at high risk, and consequently non-experienced countries, our colleagues from Lithuania, as a host in STSM (Short Term Scientific Mission) program, gave us an opportunity to gain knowledge in ASF management. The STSM program included training in preventive and control measures for the domestic pig population applied in an ASF affected country, assessment of farm biosecurity, including field investigation of ASF outbreaks (sampling procedure, detection of ASF virus, culling procedure, handling the cadavers, disinfection procedure and data collection as well as analysis). Also, among others, preventive and control measures implemented in the domestic pig population in Lithuania since 2014, field experience and possible epidemiological links to the wild boar population as a source of ASF infection were analysed. Furthermore, a visit to an infected area and meeting hunters of the local hunting club to share the experience was organised in order to get familiar with implementation of biosecurity before, during and after the hunt, hunting management and disease detection.

Analysis of the introduction of pigs in Lombardy region as a tool for assessing the potential risk of introducing pathogens and to plan control activities

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Lombardy is a region of the Northern part of Italy with intensive livestock husbandry. The pig sector is of particular economic relevance also in view of the processing industry, suited to the production of high quality pork products. Annually about 2 million pigs are introduced into the region also to satisfy the request of the industry for pork products' production.

The movement of livestock and vehicles used for their transport is one of the main risk factors in the spread of diseases and this is particularly relevant in areas with a higher animal density. Indeed, the development of densely populated areas increases the risk of spread of epidemic diseases.

Social Network Analysis (SNA) is used in Veterinary Epidemiology to study animal's movements and to identify the holdings that are central in the flow of animals in the population. In this study, SNA was used to analyse the flows of pigs to Lombardy to identify the holdings and areas that are more involved in the trading network and that could play a role in the transmission of pathogens into the region.

For the analysis of swine movements, the data of the Livestock National Database (BDN) referring to the year 2016 were used. The analysis was carried out considering certain factors related to the movement of animals, which may be relevant in the transmission of pathogens, such as number of commercial partners, number of exchanged animals and number of shipments.

In 2016, around 1,800,000 pigs were introduced into Lombardy, both of national and foreign origin. Based on the analysis of their movements the areas (provinces, municipalities) and the holdings more exposed to the considered risk factors were identified. This information is relevant for competent authorities to plan appropriate control activities in order to accelerate the detection of diseases and for prevention purpose.

A method to identify areas at risk of African Swine Fever diffusion where planning a preventive wild boar population control program

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African swine fever (ASF) is a devastating disease for the pig industry, gradually spreading in Europe. In some European countries, ASF induced by genotype II quickly became endemic after being introduced into the wild boar population where disease control measures are difficult to apply when the disease is spread in large territorial areas. In such scenario we need a method to identify in advance the areas at higher risk of disease diffusion due to the presence of both wild boars and domestic pigs in order to plan preventive measures. The aim of our study is the identification of such areas among the municipalities of the Emilia-Romagna Region in Northern Italy, where about 1.5 million pigs are reared each year.

The considered risk factors were: (a) presence/absence of wild boars, (b) density of non-commercial pig holdings, (c) pig density related to open-air farms, (d) pig density related to commercial farms, (e) density of introduced pigs in the last year (Oct. 2018-Sep. 2019).

For each municipality we calculated: the density values for each one of the 4 risk factors (b-e) and their relative z-scores. We then weighed each z-score as follows: $z < 2$, weight = 0; $2 < z < 3$, weight = 1; $z > 3$, weight = 2. Based on the different weights sum, the municipalities were ranked into three risk classes: high (total weight > 1), medium (total weight = 1) and low (total weight = 0).

Our results show that in Emilia-Romagna of the total 328 municipalities, the wild boar population spans over 224 which ranked as follows: 196 at low risk; 15 at medium and 13 at high risk of ASF diffusion. Our model allowed the identification of a limited number of municipalities (28/328) in which would be useful to apply preventive measures for the control of the wild boar population.

Preventive measures in wild boar population in the Republic of Serbia

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In the Republic of Serbia, Western Balkan country, there is in total 7,359,341 ha of hunting grounds. Part of them are fenced hunting grounds (86,612 ha), with 82,000 hunters and 1,200 hunting game warden. The wild boar population is estimated at about 23,700 individuals. In the last year a hunting bag was 11,179 hunted wild boars. At present, Serbia detected ASF positive cases only in domestic pigs. Preventive measures implemented in hunting grounds have been determined on the basis of zoning of the territory into the area of a low and high risk for ASF occurrence in the wild boar population. The preventing measures implemented in hunting ground are: continuous monitoring of the wild boar health status, application of biosecurity measures, field search for carcasses of dead wild boars, sampling of hunted and dead wild boars, restriction/prohibition of additional feeding, ban of swill feeding, prohibition of entry into the hunting ground of pig/wild boar meat and not heat-treated meat products without product declaration, population reduction, etc. In the risk zone, preventative measures can be different in open and fenced hunting grounds, especially considering the implementation of measures of population reduction and additional feeding. The population in the high-risk zone has been reduced to the level of 50% of the population size at the beginning of the hunting year along the border with Romania and to the level of 70% along Bulgarian border. However, a measure of density reduction is set at 0.5 individuals per 1km² (100 ha) hunting productive areas in open hunting ground in a high-risk zone. Particular attention was dedicated to biosafety questionnaires, recommending of biosafety measures, hunters education, as well as education of veterinarians and their introduction with hunting terms. These measures helped to overcome the "noise" in communication between veterinarians, hunters and decision-makers.

Could African Swine Fever be spread in wild boars of Albania?

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This presentation describes arguments why Albania could be the second state after Serbia in West Balkans which is risked by the presence of African swine fever. The presentation defines key scientific data and estimating studies in field regarding the population of wild boars, as well as commuting of the species among states of West Balkan. Strong scientific arguments to prevent the risk of African Swine Fever in wild boars consist of the presence of a moratorium to avoid hunting and the estimation of demographic / social border between Serbia, Kosovo and Albania. This presentation gives clear recommendations for the veterinary service of Albania and the need for preparing an experienced staff for the identification and management of the disease. New estimation methods of wild bear population and the comparison of this number during last 10 years give a clear view that the spread of ASF from Serbia to Albania could be rapid. The presentation also emphasizes data of the population and epidemiologic ways of ASF spread based on information taken by state institutions and organizations.

The first occurrence of African Swine Fever in Serbia – epidemiological, clinical, pathological and molecular investigation

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African swine fever (ASF) is a severe, contagious viral disease of domestic pigs and wild boar. In Europe both domestic and wild pigs, ASF usually has the acute course with high mortality. After the occurrence in 2007 in Georgia, the disease has progressively been spreading throughout European countries.

The first case of ASF in Serbia was confirmed on the 30th of July 2019. Until now, total 18 outbreaks were reported, in 4 villages: Rabrovac and Velika Krsna (municipality Mladenovac), Kusadak (municipality Smederevska Palanka) and Srpski Itebej (municipality Žitište). In total, ASF was confirmed in 31 out of 290 susceptible pigs (10.69%). Mortality rate was 6.89% and 270 pigs were killed and disposed.

All pigs in those cases were kept in backyard farming system. Clinical signs such as fever (40.2 to 40.8 °C), anorexia, ataxia, vomiting, reddening of the skin predominantly on the distal part of legs were observed.

Detected gross lesions at necropsy were: splenomegaly, serous edema of the wall of the gallbladder, hemorrhages in the enlarged lymph nodes, petechial hemorrhages on the kidney cortex and hemorrhages in the kidney pelvis, petechial and echymotic hemorrhages on the mucosa of the urinary bladder, petechial hemorrhages on the epicardium, and massive hemorrhages on the endocardium.

Results of the real-time PCR demonstrated positive result for the presence of African Swine Fever Virus (ASFV) genome. EURL for ASF confirmed that Serbian ASFV from domestic pigs based on p72 belongs to genotype II, CVR-1 variant, IGR I73R-I329L variant 2 (IGR-2) and subtype MGF variant 1 (MFG-1).

Immediately after the confirmation, surveillance followed by control and eradication measures was undertaken by the Serbian Veterinary Directorate in the infected and surrounding zones.

The preventive measures for incursion of African swine fever in Slovenia

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African swine fever (ASF) is a complex and lethal disease of swine with major negative impact on regional, national and international trade. Recently ASF has been confirmed for the first time in several countries (Hungary, Romania, Bulgaria, Belgium, Slovakia and Serbia) and further spreading of the disease in neighbouring regions is highly likely. In ASF affected countries wild boars are main source of infection for domestic pig population and indicator of ASF virus persistence in infected areas. Raising awareness, early detection, fast and reliable diagnostics, quick response and good communications are main point for minimising the spread and cost of ASF. Since 2014 National disease control centre in Slovenia, working within the Administration of the Republic of Slovenia for food safety, veterinary and plant protection (AFSVPP) has been implementing several ASF preventive actions and mandatory instructions for hunters, farmers and visitors. Since May 2018 enhanced passive surveillance, including laboratory testing of all harvested sick or dead wild boars is obligatory. Results should be available within 48 hours. All found carcasses of wild boar are reported through emergency information system number 112, collected and transported by Veterinary hygiene service. Sampling and laboratory diagnostics of ASF are performed within National veterinary institute. Real-time PCR method for the detection of ASF viral genome and ELISA test for detection of ASF antibodies are implemented within the accreditation scope, according to ISO/IEC 17025/2017. Within an annual order about 5% of harvested wild boar population in Slovenia is tested for ASF antibodies and 1% of dead domestic pigs are tested for detection of ASF virus. Within passive and active monitoring, all tested samples have been detected ASF negative since 2014. In Slovenia the ASF has never been notified.

Improving biosecurity on Finnish pig farms by Biocheck.UGent® evaluations

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A long border with Russia and abundant passengers from Baltic countries makes ASF a serious threat to Finnish pig production. The wild boar population in Finland is relatively low but has been growing until 2019.

Sikava is a voluntary swine health classification register which is run by Animal Health ETT. Sikava covers over 95 % of pig production in Finland. Cooperation exists between Animal Health ETT, authorities and stakeholders. Finnish pig producers run a two-year project 2017-2018 with ETT to introduce the Biocheck.UGent® system into common use in Finland. In the same project vets, producers and stakeholders were informed about current ASF situation in Europe and wild boar hunting was promoted. Biocheck.UGent® system is a risk-based questionnaire about most important biosecurity measures. Each question and subcategory have a weighed scoring. Farm biosecurity is divided into external and internal biosecurity.

Herd veterinarians visit farms several times per year. Several observations of swine health, welfare and biosecurity are made and recorded in the database. Every year a herd-specific health plan is updated. The Biocheck is integrated into the Sikava register by a licence and from 2019 annual Biocheck -evaluations were added to health management scheme in order to improve biosecurity in swine herds. Evaluations are made and registered by herd vets or advisors. Auditing persons are trained to do Biocheck audits in order to get unbiased evaluations.

184 biosecurity evaluations were recorded in the Sikava by September 2019. The average for external biosecurity was 70% (SD=9) and for internal biosecurity 58% (SD= 15). The average in total was 64% (SD=10). The global averages are the same.

The goal of biosecurity evaluation results should be above the global averages. Every herd can improve in some measures. Biocheck.UGent® questionnaire is a good tool to emphasize important biosecurity measures. However, it's not made only for keeping ASF out of a swine herd but helps in maintaining good health status on the farm.

African swine fever: pig farms cleaning and disinfection procedures

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The recent expansion of African swine fever (ASF) throughout several regions of the world has placed the majority of the world's swine population under threat. ASFV has a remarkable ability to survive for long periods in a protein rich environment and it remains stable at pH 4-10: this implies that it can persist for long period in the environment. Currently, farm biosecurity and good farming practice are the most effective tool available for preventing ASF virus (ASFV) introduction into pig holdings. In fact, at present there is no safe vaccine available to provide adequate protection to pigs. Considering ASFV's characteristics, cleaning and disinfection (C&D) procedures are of crucial importance to inactivate the pathogen at the premise, to prevent the off-site spread of the disease during outbreaks and to facilitate the repopulation after an outbreak. Worth to mention that regular and thorough C&D is also an important routine for eradicating endemic diseases and to reduce the risk of introducing new pathogens into the holding.

In order to identify effective C&D practices to apply against ASFV, a research using PubMed and CAB Abstracts databases was carried out. In total, 72 papers were identified. After applying exclusion criteria, 9 articles were considered for this study. A further search performed using a common browser provided 7 technical guidelines, 1 scientific opinion, 2 books, 1 conference proceedings published in English and 1 thesis and 3 technical guidelines published in Italian. At the end of the reviewing process, 24 bibliographic entries were considered relevant for the purpose of this guidance. Based on the selected publications, C&D procedures to apply in pig holdings were produced.

Diagnostic tools for the surveillance and control of African swine fever in domestic pigs and wild boar

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Numerous outbreaks of African swine fever virus (ASFV) have been reported in the last months, in wild boar and domestic pigs in Europe, Africa and Asia. Since there is no vaccine available, diagnostic tools are essential for the control of the disease. INGENASA offers several serological diagnostic tools, for different scenarios: a double antibody sandwich enzyme-linked immunosorbent assay (INgezim PPA DAS) and a lateral flow assay, LFA (INgezim ASF CROM Ag) for antigen detection; a competition ELISA (INgezim PPA Compac) and a LFA (INgezim PPA CROM) for antibody detection. Within the frame of the ASF-STOP project an indirect ELISA (iELISA) for the detection of ASF-specific antibodies and a multiplex platform for simultaneous detection of antibodies to ASFV and CSFV, have been developed. A prototype of iELISA, based on a mix of recombinant viral proteins, to determine the presence of antibodies in serum and blood samples has been optimized. Seven hundred and fifty four negative and 254 positive samples of domestic pigs and wild boar have been assayed. The results showed a specificity of 100% and sensitivity of 87.8%. This assay showed to be especially valuable when used with haemolyzed serum samples from wild boar.

On the other hand, a multiplex assay, based on the Luminex technology, has been developed for the simultaneous detection of antibodies to ASFV and CSFV. The following antigens were selected as targets: VP72 and VP30 of ASFV and the E2 protein of CSFV. A panel of 352 sera from experimentally infected animals with either ASFV or CSFV and 253 field negative sera were included in the study. The data obtained showed values of 97.3% sensitivity and 98.3% specificity for detection of antibodies to ASFV and 95.7% of sensitivity and 99.8% specificity for detection of antibodies to CSFV. This method provides a valuable tool for surveillance studies.

Possible transmission of ASFV by insects: studies in Lithuania

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Since 2014 African Swine Fever virus has spread among wild boar populations and domestic pigs in Lithuania. It was introduced into the farms with high biosecurity and the pathways for entry sometimes remain unclear. Outbreaks of ASF in pig farms in Lithuania begin in spring and are detected until autumn, suggesting that insects might be involved. It is known that *Stomoxys calcitrans* can transmit the virus between domestic pigs in laboratory conditions, but no data is available on the possibility of insects to transmit ASFV from wild boars to domestic pigs. The aim of our investigation was to check if bloodsucking insects (Tabanidae, Muscidae) and insects, developing in decaying organic matter (Calliphoridae, Muscidae) can take part in transmission of ASFV. All the mentioned insects are usually found in and around pig farms and can interact both with the wild boars and domestic pigs.

Monitoring of the proximities of farms using 5 NZI traps placed 5-20m away from the farm was performed in 2018–2019. Insects were also collected by entomological net during the outbreaks of ASF inside the domestic pig farms and outside, on the walls and several meters away. Collected insects were tested by PCR using ASFV specific primers for detecting ASFV DNA.

The most abundant insects caught using traps were horse flies (Tabanidae), stable flies (*S. calcitrans*) and blowflies (Calliphoridae). Out of 99 *S. calcitrans*, 6 were positive for ASFV DNA. No horse flies and blowflies were found to be positive.

The most abundant insects caught during the ASF outbreaks in farms were Calliphoridae and Muscidae species. Several specimens of Fanniidae, Sarcophagidae, Stratiomyidae, Syrphidae, Tabanidae flies and one Staphylinidae beetle were also collected. The virus DNA was detected in 7 insects (*Eristalis*, *Musca*, *Stomoxys*) out of 47 collected inside and 6 insects (*Fannia*, *Lucilia*, *Musca*) out of 31 collected outside the farms during the outbreaks of ASF.

Our data shows that insects could be considered as mechanical transmitters of ASFV in the wild, but further studies are needed to clarify their role in transmission.

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