

African swine fever- recent research advances and strategies to combat the disease in Europe

COST Action CA15116: Understanding and combating
African Swine Fever in Europe (ASF-STOP)



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Puławy, Poland

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ASF-STOP Conference programme

7 December

	Opening	Speakers	Abstract (page number)	
10.00-10.30	Minister of Agriculture of Poland, Krzysztof Jurgiel Director of PIWet-PIB, Krzysztof Niemczuk Dolores Gavier-Widén, Chair ASF- STOP		-	Chair: Krzysztof mietanka
10.30-11.00	African Swine Fever: a call for action	Mur, Lina	15	Invited speaker
11.00-11.30	Compartmentalisation: general principles and potential applications in African swine fever control	Bellini, Silvia	17	Key note speaker
11.30-12.30	Session I: ASF current situation in Europe			Chairs: Dolores Gavier- Widén, Edvins Olsevskis
11.30-12.00	The role of wild boar in the maintenance of African swine fever virus in Poland, 2014 - 2016	Smietanka, Krzysztof	19	Key note presentation
12.00-12.30	Recent epidemic of African swine fever in pigs in Poland	Pejsak, Zygmunt	21	Key note presentation
12.30-13.30	Lunch			
13.30-14.00	Experience from recent animal trials using recent genotype I and II ASFV strains– implications for epidemiology and control	Blome, Sandra	23	Key note presentation
14.00-15.00	Session II: Epidemiology, control and prevention			Chairs: Tomasz Podgorski, Karl Ståhl

14.00-14.30	African swine fever in Sardinia: lessons learned that might be useful to the rest of Europe	Laddomada, Alberto	25	Key note presentation
14.30-15.00	ASF virus contagiousity and wild boar behavior	Depner, Klaus	27	Key note presentation
15.00-16.30	Session III: ASF-virology, vaccinology and diagnostics			Chairs: Marie-Frederique Lepotier, Ivan Toplack
15.00-15.15	Cross-protective immunity and African Swine Fever virus serotype-specific proteins	Malogolovkin, Alexander	29	Key note presentation
15.15-15.30	African swine fever virus entry and uncoating	Alonso, Covadonga,	31	
15.30-16.00	Coffee break			
16.00-16.15	Interaction of porcine conventional dendritic cells with African swine fever virus	Montoya, María	32	
16.15-16.30	Comparison of three isothermal amplification methods for detection of African swine febravirus in blood from infected wild boars and pigs	Wozniakowski, Grzegorz	33	
16.30-17.00	Session IV: ASF in wild boar			Chairs: Chris Walzer, Sophie Rossi
16.30-16.45	Behaviour of free ranging wild boar towards their dead fellows –potential implications for the transmission of African swine fever virus	Probst, Carolina (ECI)	35	
16.45-17.00	Potential implications of wild boar carcass	Ruiz-Fons, Francisco	36	

	and hunting remains consumption for African Swine Fever transmission in Mediterranean areas			
17.00-18.00	Session V: ASF in domestic pigs			Chairs: Silvia Bellini,
17.00-17.15	Breeding disease and biosecurity measures on pig commercial Farm in the Republic of Serbia	Bojkovski, Jovan	37	
17.15-17.30	Risk factors for introduction of African swine fever to Slovenia	Stukelj, Marina	38	
17.30-17.45	Measuring the interactions between wild boar and domestic pigs	Vicente, Joaquín	40	
17.45-18.00	African swine fever in domestic pigs in Estonia in 2015 and 2016: epidemiological analysis of outbreak investigations	Imbi, Nurmoja	41	
18.00-19.00	ECI oral presentations			Chair: Francisco Ruiz-Fons, Antonio Lavazza
18.00-18.15	Modelling the spatial distribution of reported ASF outbreaks in the Russian Federation using national surveillance data, 2007-2014.	Vergne, Timothee (ECI)	43	
18.15-18.30	Alternative sampling strategies for African swine fever surveillance in wild boar –Assessment of African swine fever virus antibody	Carlson, Jolene (ECI)	44	

	detection from dry blood swabs			
18.30-18.45	Diagnostic testing of dead animals is the optimal surveillance tool for control of epidemics of African Swine Fever	Halasa, Tariq (ECI)	45	
18.45-19.00	Hybridization levels in European <i>Sus scrofa</i> , comparison between genetic and survey data	Iacolina, Laura (ECI)	47	
19.00-19.30	Poster session			5' per poster presenter

8th December

09.00-13.00	Session VI: ASF in wild boar and epidemiology			
				Chairs: Arvo Viltrop, Fernando Boinas
09.00-09.30	African swine fever is a global threat requiring concerted efforts: lessons from an endemic country, Uganda	Masembe, Charles	49	Invited speaker
09.30-09.45	Does wild boar behaviour predict slow spread of African Swine Fever?	Podgorski, Tomasz	51	
09.45-10.00	Moving ASF risk assessments to the local level	Dietze, Klaas	52	
10.00-10.15	The surveillance of African swine fever in wild boar population in Serbia	Petrovic, Tamas	53	
10.15-10.30	Knowledge and Attitude Related to African Swine Fever of Backyard Farmers, Hunters and Governmental	De Nardi, Marco	55	

	Veterinarians in Armenia			
10.30-11.00	Coffee break			
11.00-11.15	Non-invasive sampling of wild boar– Applicable for ASF virus detection?	Globig, Anja	57	
11.15-11.30	Participatory epidemiology – a way to support the successful design of surveillance strategies and control measures	Schulz, Katja	58	
11.15-11.30	African swine fever and its way through Asia and towards Europe (Epidemiology and risk assessment in Ukraine)	Stegniy, Borys	59	
11.30-11.45	Analysis of the threat of spread of African swine fever in Ukraine’s wild boar population: Current knowledge and future CBEP- Ukraine sponsored research	Fedorenko, Olga	60	
11.45-12.00	Development and Implementation of Public Outreach, Biosurveillance, and Policy Development Activities for African Swine Fever within the Cooperative Biological Engagement Program in Ukraine	Mykhalovskaia, Natalija	62	
12.00-12.15	Determination of sensitivity and timeliness of surveillance strategies using a simulation model	Schulz, Katja	64	

12.15-12.30	TBA			
12.30-13.00	TBA			
13.00-14.00	Lunch			

Posters

Title	Presenter	Abstract ID	Comments
Understanding the dynamics of ASF spread at the interface between wild boar and domestic swine	Costa, Joao	65	
Comparison of different sample preparation methods for rapid identification of African swine fever under field condition	Lihong, Liu	66	
Study of the dynamics of wild boar's population in Albania (<i>Sus scrofa</i>)	Korro, Kastriot	67	
The wild boars population characteristics in the Republic of Serbia	Prodanov-Radulovic, Jasna	68	
Preventive measures to minimize the risk of African swine fever spread during wild boar hunting	Bellini, Silvia	69	
ASF surveillance in Finland and the estimation of wild boar movement based on rabies antibody detection	Nokireki, Tiina	70	
Presence verification of classical swine fever in Albania	Turmalaj, Luigj	71	
Modelling the spread of African swine fever in Germany	Breidenstein, Christiane	72	

TBA: To be announced

Workshop: Advances in vaccinology

Calendar and venue

This workshop will take place in Pulawy, Poland, on the 8th December 2016 (<http://www.piwet.pulawy.pl/asf-stop/>)

Bakground and objectives

The workshop will make a summary of the actual efforts in developing a vaccine against ASFV by analysing the data discussed in the recent GARA meeting (Sept 2016).

The workshop aims at describing the actual efforts in ASFV vaccinology, the gaps that are identified and challenges faced by each strategy. Also, discussions will be encouraged to make plans for overcoming such challenges.

Workshop Program

Session 1 9:00-10:30

09:00-09:10 Opening and introduction. Maria Montoya and Marie-Frédérique Le Potier

9:10-9:40 Invited speaker: Shawn Babiuk: Development of capripoxvirus vaccines and potential to transfer knowledge to the development of African swine fever vaccines

9:40-10:00 Linda Dixon: Global Alliance for research on African swine fever virus (GARA): Criteria for ASFV vaccine development

10:00-10:20 Discussion between acceptable vs. ideal vaccine, biosafety and what we need to do to make a vaccine from the lab to the field.

10:20-10:50 - (30') Coffee break

Session 2 10:50-13:00

10:50-11:10 Covadonga Alonso: What do we have to explore in ASFV-cell interactions to develop a vaccine?

11:10-11:30 Yolanda Revilla: ASFV Tropism and Genes Interfering Host Cellular Pathways: Actors for ASF vaccine development

11:30-11:50 Gunther Keil: *Quo vadis* African Swine fever vaccine development?

11:50-12:05 Ferdinando Freitas: Studies on the role of ASFV E2 ubiquitin-conjugating enzyme in viral replication

12:05-12:20 Fernando Ferreira: Binding parameters of a functional histone-like protein encoded by African Swine Fever Virus

12:20-13:00 Discussion on ways to fulfil the gaps identified for future developments and Summary of discussion on developments for ASFV vaccines (Maria Montoya)

Workshop: Researchers link European industrial partners

Calendar and venue

This workshop will take place in Pulawy, Poland, on the 8th December 2016, 9.00-13.00 (<http://www.piwet.pulawy.pl/asf-stop/>)

Bakground and objectives

The workshop brings together non-academic actors from industry and researchers and is open to all participants.

The workshop aims at enabling fruitful collaboration between scientists and business, facilitating application of research into commercial development of tools to combat ASF. The scientists will interact directly with the industrial partners to explore opportunities to translate research findings into commercial products such as development of improved diagnostic kits, innovations and advances in ASF vaccines, safe and practical devices for transport of samples, protective clothes to work on pig farms enabling high biosecurity, and techniques for non-invasive sampling of wild boar, such as materials to collect saliva for virus and antibody detection.

Facilities

There will be 10 exhibition booths free of charge for the first 10 industrial partners who register to the workshop and would like to present their products.

Workshop Program

09:00-09:20 Opening and introduction. Francisco Ruiz-Fons (Vice-chair ASF-STOP) and Dolores Gavier-Widén (Chair ASF-STOP)

09:20-09:30 Tour de table

09:20-9.50 Advances in ASF diagnosis in domestic and wild suids. Sandra Blome, Friedrich-Loeffler-Institut, Germany

9.50-10:20 Mitigation of health conflicts at the wildlife-livestock interface: know-how transference. Mariana Boadella, SABIOTEC, Spain

10:20-10:50 Coffee break

10:50-11:20 Opportunities for industrial interactions on ASFV vaccines, Linda Dixon, The Pirbright Institute, UK

11:20-11:50 Implementation of control and biosecurity measures to avoid African Swine Fever spread in pig premises. Silvia Bellini, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia, Italy

11:50-12:10 SAFOSO role in projects on ASF and at the interface between science, policy makers and industries. Marco de Nardi, SAFOSO, Switzerland

12:15-13:00 Round table discussion: What can we do to improve transference between researchers and the industry in relation to ASF?

Invited Speakers (Linda Dixon, Sandra Blome, Mariana Boadella, Silvia Bellini), Moderators (Francisco Ruiz-Fons and Dolores Gavier-Widén), SME Representative (Marco de Nardi).

Contents

African Swine Fever: a call for action <i>Lina Mur</i>	15
Compartmentalisation: general principles and potential applications in African swine fever control <i>Silvia Bellini</i>	17
The role of wild boar in the maintenance of African swine fever virus in Poland, 2014–2016 <i>Krzysztof Smietanka</i>	19
Recent epidemic of African swine fever in pigs in Poland <i>Zygmunt Pejsak</i>	21
Experience from recent animal trials using recent genotype I and II ASFV strains– implications for epidemiology and control <i>Sandra Blome</i>	23
African swine fever in Sardinia: lessons learned that might be useful to the rest of Europe , <i>Alberto Laddomada</i>	25
ASF virus contagiousity and wild boar behavior <i>Klaus Depner</i>	27
Cross-protective immunity and African Swine Fever virus serotype-specific proteins , <i>Alexander Malogolovkin</i>	29
African swine fever virus entry and uncoating <i>Covadonga Alonso</i>	31
Interaction of porcine conventional dendritic cells with African swine fever virus <i>María Montoya</i>	32
Comparison of three isothermal amplification methods for detection of African swine febravirus in blood from infected wild boars and pigs. <i>Grzegorz Wozniakowski</i>	33
Behaviour of free ranging wild boar towards their dead fellows –potential implications for the transmission of African swine fever virus <i>Carolina Probst</i>	35
Potential implications of wild boar carcass and hunting remains consumption for African Swine Fever transmission in Mediterranean areas <i>Francisco Ruiz-Fons</i>	36
Breeding disease and biosecurity measures on pig commercial Farm in the Republic of Serbia <i>Jovan Bojkovski</i>	37
Risk factors for introduction of African swine fever to Slovenia <i>Marina Štukelj</i>	38

Measuring the interactions between wild boar and domestic pigs <i>Joaquín Vicente</i>	40
African swine fever in domestic pigs in Estonia in 2015 and 2016: epidemiological analysis of outbreak investigations <i>Imbi Nurmoja</i>	41
Modelling the spatial distribution of reported ASF outbreaks in the Russian Federation using national surveillance data, 2007-2014. <i>Timothee Vergne</i>	43
Alternative sampling strategies for African swine fever surveillance in wild boar – Assessment of African swine fever virus antibody detection from dry blood swabs <i>Jolene Carlson</i>	44
Diagnostic testing of dead animals is the optimal surveillance tool for control of epidemics of African Swine Fever <i>Tariq Halasa</i>	45
Hybridization levels in European <i>Sus scrofa</i> , comparison between genetic and survey data <i>Laura Iacolina</i>	47
African swine fever is a global threat requiring concerted efforts: lessons from an endemic country, Uganda <i>Charles Masembe</i>	49
Does wild boar behaviour predict slow spread of African Swine Fever? <i>Tomasz Podgorski</i>	51
Moving ASF risk assessments to the local level <i>Klaas Dietze</i>	52
The surveillance of African swine fever in wild boar population in Serbia <i>Tamas Petrovic</i>	53
Knowledge and Attitude Related to African Swine Fever of Backyard Farmers, Hunters and Governmental Veterinarians in Armenia <i>Marco De Nardi</i>	55
Non-invasive sampling of wild boar– Applicable for ASF virus detection? <i>Anja Globig</i>	57
Participatory epidemiology – a way to support the successful design of surveillance strategies and control measures <i>Katja Schulz</i>	58
African swine fever and its way through Asia and towards Europe (Epidemiology and risk assessment in Ukraine) <i>Borys Stegnyy</i>	59
Analysis of the threat of spread of African swine fever in Ukraine’s wild boar population: Current knowledge and future CBEP-Ukraine sponsored research <i>Olga Fedorenko</i>	60

Development and Implementation of Public Outreach, Biosurveillance, and Policy Development Activities for African Swine Fever within the Cooperative Biological Engagement Program in Ukraine <i>Natalija Mykhalovskaia</i>	62
Determination of sensitivity and timeliness of surveillance strategies using a simulation model <i>Katja Schulz</i>	64

Posters

Understanding the dynamics of ASF spread at the interface between wild boar and domestic swine <i>Joao Costa</i>	65
Comparison of different sample preparation methods intended for rapid identification of African swine fever under field condition <i>Lihong Liu</i>	66
Study of the dynamics of wild boar's population in Albania (<i>Sus scrofa</i>) <i>Kastriot Korro</i>	67
The wild boars population characteristics in the Republic of Serbia <i>Jasna Prodanov-Radulovic</i>	68
Preventive measures to minimize the risk of African swine fever spread during wild boar hunting <i>Silvia Bellini</i>	69
Estimation of wild boar movement based on rabies antibody detection in Finland. <i>Tiina Nokireki</i>	70
Presence verification of classical swine fever in Albania <i>Luigi Turmalaj</i>	71
Modelling the spread of African swine fever in Germany <i>Christiane Breidenstein</i>	72

Workshop Advances in vaccinology

Development of capripoxvirus vaccines and potential to transfer knowledge to the development of African swine fever vaccines <i>Shawn Babiuk</i>	73
Global Alliance for research on African swine fever virus (GARA): Criteria for ASFV vaccine development <i>Linda Dixon</i>	74

What do we have to explore in ASFV-cell interactions to develop a vaccine? <i>Covadonga Alonso</i>	75
ASFV Tropism and Genes Interfering Host Cellular Pathways: Actors for ASF vaccine development <i>Yolanda Revilla</i>	76
<i>Quo vadis</i> African Swine fever vaccine development? <i>Gunther Keil</i>	77
Studies on the role of ASFV E2 ubiquitin-conjugating enzyme in viral replication <i>Ferdinando Freitas</i>	78
Binding parameters of a functional histone-like protein encoded by African Swine Fever Virus <i>Gonçalo Frouco</i>	79

Workshop
Researchers link European industrial partners

Advances in ASF diagnosis in domestic and wild suids <i>Sandra Blome</i>	80
Mitigation of health conflicts at the wildlife-livestock interface: know-how transference. <i>Mariana Boadella</i>	81
Opportunities for industrial interactions on ASFV vaccines <i>Linda Dixon</i>	82
Implementation of control and biosecurity measures to avoid African Swine Fever spread in pig premises <i>Silvia Bellini</i>	83
SAFOSO role in projects on ASF and at the interface between science, policy makers and industries <i>Marco de Nardi</i>	84

African Swine Fever: a call for action

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Keywords: epidemiology, gap knowledge, re-emerging disease, risk.

African swine fever (ASF) is notifiable to the World Organisation of Animal Health (OIE). Its presence in affected territories invokes immediate trade restrictions and, consequently, important negative socio-economic impacts. ASF is caused by a complex, double stranded DNA virus that affects swine of all age and breeds, and for which no vaccine is available. All these features makes ASF one of the most important and devastating infectious disease of swine. The disease was first described in Africa, and is still present nowadays in the majority of sub-Saharan countries. The first reported outbreaks of ASFV outside the African continent occurred in 1957 and 1960 in Portugal, from where ASFV infections rapidly spread to Spain. During more than three decades (1960s-1990s) ASF was present in the Iberian Peninsula, which provided a source for sporadic outbreaks in Europe, the Caribbean and South America. It was during these years that the fundamentals of ASF research were established and the biggest advances were made, including the discovery of the virus infection mechanisms; the identification of the major proteins and epitopes; the description of many of the diagnostic tests (which are still in use today); and the isolation of some attenuated strains employed in vaccine research.

However, since ASF was eradicated from Europe in 1995 (with the exception of Sardinia which remains endemic for the disease), funding for ASF research gradually declined. For some years, ASF was neglected and considered an exotic disease. Research efforts were limited compared with other infectious animal diseases such as classical swine fever, foot and mouth disease and avian influenza. As a result, the number of ASF scientific publications reached a trough in 2005 (11 articles in that year). In April 2007, ASF was re-introduced into the European continent, reportedly through contaminated food waste. This time it affected the eastern region, starting in Georgia. From there, ASF spread slowly but continuously towards north and western territories, affecting domestic pigs and wild boar populations of all surrounding countries. This situation raised alarm in the scientific community and since 2010, several international research projects were conducted, leading to the publication of more than 300 articles (an average of 53 scientific articles per year have been published since then).

Nevertheless, the difficulties in stopping ASF spread through Europe have revealed that many challenges remain and there is still much work to be done. Basic issues need to be addressed including collection of comprehensive field transmission data; better understanding the role of wild boar, ticks, and carriers in the epidemiology; better describing the immune-pathology and protection processes; and assess the different vaccine platforms. Therefore, this is the time for the scientific community to take joint and collective action to fight ASF, to build on the knowledge acquired during previous decades, identify the existing gaps, develop realistic and applicable control options, and use innovative ideas and the latest technologies to address them. If we don't act now, ASF will continue to spread through Europe and to other regions, and will become an additional drain on our already damaged economies.

Afrykański pomór świński : wezwanie do działania

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Słowa kluczowe: epidemiologia, luki w wiedzy, powracające choroby, ryzyko.

Afrykański pomór świński (ASF) podlega obowiązkowi zgłaszania do światowej Organizacji Zdrowia Zwierząt (OIE). Obecnie tej choroby na danym terytorium wiążemy się z nałożeniem natychmiastowych restrykcji w handlu, co prowadzi do negatywnych konsekwencji społeczno-ekonomicznych. ASF wywołany jest przez duży, posiadający dwuniciowy DNA wirus, zdolny do zakażenia wiekszości ras i w każdym wieku i przeciwko któremu nie opracowano skutecznej szczepionki. Z tych względów ASF jest jedną z najwciążniejszych i najgroźniejszych chorób zakaźnych. ASF został opisany po raz pierwszy w Afryce, gdzie ciągle jest obecny w większości krajów części subsaharyjskiej kontynentu. Pierwsze ogniska poza Afryką wystąpiły po raz pierwszy w 1957 i 1960 r. w Portugalii, skąd zakażenie przeniosło się szybko do Hiszpanii. ASF był obecny na Półwyspie Iberyjskim przez ponad trzy dekady (od lat 60. do 90. XX wieku), skąd przedostawał się cyklicznie do innych krajów Europy, Ameryki Południowej i w rejon Karaibów. W tym czasie położono fundamenty pod badania naukowe i został w tym zakresie poczyniony ogromny postęp. Poznano mechanizmy infekcji, zidentyfikowano główne białka i epitopy wirusa, opracowano szereg testów diagnostycznych (ciągle stosowanych), izolowano szczepy atenuowane, które zastosowano w badaniach nad szczepionkami.

Jednak od czasu, gdy ASF został w Europie wyeliminowany w 1995 r. (z wyjątkiem Sardynii, gdzie choroba cały czas utrzymuje się endemicznie), finansowanie badań nad ASF zaczęło się stopniowo zmniejszać. Przez pewien czas ASF uznawany był za chorobę egzotyczną i nie poświęcano jej zbyt wiele uwagi. Liczba badań naukowych była ograniczona w porównaniu z innymi chorobami zakaźnymi, takimi jak klasyczny pomór świński, pryszczycza czy grypa ptaków. W konsekwencji liczba publikacji naukowych spadła w 2005 r. do 11. W kwietniu 2007 r. ASF został ponownie wprowadzony do Europy, najprawdopodobniej za pośrednictwem zlewek. Tym razem choroba zaatakowała rejon Europy wschodniej, zaczynając w Gruzji, skąd wirus powoli, ale konsekwentnie przemieszczał się w stronę obszarów leśnych na północ i na zachód, a zakażenie objęło populacje wiejskie i dzików we wszystkich krajach sąsiadujących. Sytuacja stała się alarmująca dla środowiska naukowego i od 2010 r. rozpoczęto realizację projektów naukowych, co zaowocowało publikacjami ponad 300 artykułów (średnio 53 artykuły rocznie).

Niemniej jednak trudno ci w zatrzymaniu szerzenia się ASF w Europie ujawniło jak wiele pracy i wyzwania ciągle stoi przed nami. Dotyczy to zagadnień podstawowych, takich jak szeroko zakrojone badania terenowe dotyczące mechanizmów transmisji zakaźnej, lepsze zrozumienie roli dzików, kleszczy, czy bezobjawowych nosicieli, jak również procesów immunopatologicznych i protekcyjnych, oszacowanie wartości różnych szczepionek i platform immunoprofilaktyki. Nadszedł właściwy czas dla środowiska naukowego by podjąć wspólne działania w walce z ASF, rozwijać wiedzę zdobytą w minionych dekadach, identyfikować luki w wiedzy, opracowywać realistyczne i możliwe do zastosowania programy zwalczania, a w tym celu wykorzystywać innowacyjne pomysły i najnowsze technologie. Jeśli nie zaczniemy działać teraz, ASF będzie szerzył się dalej w Europie i do innych rejonów świata i stanie się dodatkowym ciężarem finansowym dla naszych i tak już nadwierzonych gospodarek.

Compartmentalisation: General principles and potential applications in African Swine Fever control

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Keywords: ASF, control, compartmentalisation.

The OIE *Terrestrial Animal Health Code (Terrestrial Code)* sets out the standards for the improvement of terrestrial animal health and welfare and veterinary public health worldwide, and for safe international trade in terrestrial animals and their products. The health measures in the *Terrestrial Code* should be used by the Veterinary Authorities for early detection, reporting and control of agents pathogenic to terrestrial animals and for humans, and to prevent their transfer via international trade in terrestrial animals and their products, while avoiding unjustified sanitary barriers to trade.

Zoning and compartmentalisation are procedures implemented by a Member Country for defining subpopulations of distinct health status within its territory for the purpose of disease control and/or international trade. While zoning applies to an animal subpopulation defined primarily on a geographical basis, compartmentalisation applies to an animal subpopulation defined primarily by management and husbandry practices related to biosecurity. In practice, spatial considerations and good management including biosecurity plans play important roles in the application of both strategies. Compartmentalisation is not a new concept for Veterinary Services; in fact, it has been applied for a long time in many disease control programs that are based on the concept of disease free herds/flocks.

The *Terrestrial Code* chapter on OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization provides a process for trading partners to follow in achieving recognition of the health status of such subpopulations. These procedures are best implemented by trading partners through establishing parameters and gaining agreement on the necessary measures prior to disease outbreaks, rather than during outbreaks.

Zoning and compartmentalisation are not applicable to all diseases and in all situations but separate requirements are developed for each disease for which the application of zoning or compartmentalisation is considered appropriate. Indeed, the effective implementation of compartmentalisation depends, amongst others, on the epidemiology of the disease, country factors, environmental factors, the biosecurity measures, which may be applicable, the health status of animals in adjacent areas, surveillance and the relationship between the public and private sectors.

The proposed revised *Terrestrial Code* chapter on African swine fever (ASF) gives detailed technical recommendations for a country, zone or compartment to be considered as free from ASF, and addresses the disease in domestic and captive wild pigs, wild and feral pigs and African wild suid species.

Kompartmentalizacja: ogólne zasady oraz potencjalne zastosowania w zwalczaniu afrykańskiego pomoru świń

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OIE Scientific Commission for Animal Diseases (SCAD)*

Słowa kluczowe: ASF, zwalczanie, kompartmentalizacja.

Opracowany przez Wiatow Organizacji Zdrowia Zwierząt (OIE) Kodeks zdrowia zwierząt i ludzkiego zdrowia ustala standardy dla poprawy zdrowia zwierząt, ich dobrostanu oraz zdrowia publicznego na całym świecie oraz dla bezpieczeństwa w handlu międzynarodowym zwierzętami oraz produktami pochodzenia zwierzęcego. Rodziki opisane w Kodeksie powinny być stosowane przez władze weterynaryjne w przypadku wczesnej diagnostyki, raportowania oraz zwalczania czynników patogennych dla zwierząt i ludzkiego zdrowia oraz ludzi, a także w celu zapobiegania ich przeniesienia drogą handlu międzynarodowego, jak również uniknięcia stosowania nieuzasadnionych barier sanitarnych w obrocie.

Tworzenie stref oraz *kompartmentalizacja* są procedurami implementowanymi przez państwa członkowskie w celu zdefiniowania subpopulacji zwierząt o odrębnym stanie zdrowia na obszarze jego terytorium, dla celów zwalczania i/lub międzynarodowego obrotu. Podczas gdy określanie stref odnosi się do subpopulacji zwierząt zdefiniowanych przede wszystkim na podstawie kryterium geograficznego, to *kompartmentalizacja* odnosi się do subpopulacji zwierząt zdefiniowanej na podstawie praktyk zarządzania i bioasekuracji. W praktyce, względy geograficzne i dobre zarządzanie zawierające plany bioasekuracji odgrywają ważną rolę w aplikacji obu strategii. *Kompartmentalizacja* nie jest nowym pomysłem w działalności Inspekcji Weterynaryjnej; w rzeczy samej była stosowana przez długi czas w programach zwalczania wielu chorób, które były oparte na koncepcji wolnych od choroby stad zwierząt.

Rozdział Kodeksu dotyczący procedur OIE istotnych z punktu widzenia Porozumienia w sprawie Stosowania Rodków Sanitarnych i Fitosanitarnych Wiatowej Organizacji Handlu dostarcza modelu działania dla stron porozumienia, którym mogą się kierować w celu uznania statusu zdrowotnego w takich subpopulacjach. Najlepiej, gdy procedury te są implementowane przed wystąpieniem ognisk czy epidemii (a nie w trakcie ich trwania), poprzez ustalenie odpowiednich parametrów oraz uzgodnienie niezgodnych rodków Regionalizacja i *kompartmentalizacja* nie są stosowane przy wszystkich chorobach i we wszystkich sytuacjach, lecz dla każdej choroby i dla każdej sytuacji są tworzone oddzielne wytyczne, najwłaściwsze w konkretnej sytuacji. Dlatego też, efektywna implementacja *kompartmentalizacji* zależy, między innymi, od epidemiologii choroby, czynników charakterystycznych dla danego państwa, czynników środowiskowych, rodków bioasekuracyjnych, które mogą zostać wdrożone, statusu zdrowotnego zwierząt na siedzących terenach, systemu nadzoru oraz relacji między sektorem publicznym i prywatnym.

Zaproponowany przegląd rozdziału dotyczącego Afrykańskiego pomoru świń w Kodeksie Zdrowia Zwierząt i Ludzkiego Zdrowia OIE dostarcza szczegółowych zaleceń dla danego państwa, strefy lub kompartamentu, tak aby stał się wolny od ASF oraz odnosi się do świń domowych, dzików, wolno żyjących świń oraz wolno żyjących afrykańskich gatunków winiowatych.

The role of wild boar in the maintenance of African swine fever virus in Poland, 2014 - 2016

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Keywords: African swine fever, wild boar, epidemiology.

African swine fever virus (ASFV) of genotype II was first diagnosed in wild boar (WB) in Poland in February 2014 and up to now (October 2016) more than 180 WB in 114 locations were found positive for ASFV. Analysis of the results of active and passive surveillance (restricted to infected areas, i.e. Part II+III according to Commission Implementing Decision 2014/709/EU) showed that the annual detection rate in WB found dead varied between 40 – 50%. On the other hand, none of the WB killed in road accidents has ever been found ASFV-positive. Since examinations of wild boar killed by vehicles are also part of passive surveillance (in addition to WB found dead and WB shot while showing abnormal behavior), we suggest that the components of passive surveillance should not be combined but reported separately. On the contrary, the annual prevalence of ASFV estimated in the framework of active surveillance (testing hunted and apparently healthy wild boar) is very low and in areas under restrictions is less than 0.5% on average. Therefore, testing wild boar found dead seems to be the only effective tool for early detection and early warning. Seasonal pattern was also observed with the highest rate of ASFV-positive carcasses in July (80%, with the annual average of 45%). Spatial analysis showed that the size of infected area is small and is expanding slowly. The most recent cases (September/October 2016) in an area without previous history of ASF in WB but with the recent occurrence of outbreaks in pigs may suggest possible transmission of ASFV from pigs to WB.

The initial hypotheses forecasting the fast spread of the epidemic or, alternatively, rapid “self-implosion” due to the high virulence of the virus (Depner & Guberti, 2016), were apparently based on incorrect assumptions that contagiousness of the disease is high and the virus is easily transmitted between sick and susceptible hosts. Currently it seems plausible to conclude that the disease is characterized by unique epidemiological features: ASF is an intrinsically vector-borne disease that has spread into a geographical region where no susceptible vectors (ticks) exist but the virus entrance into the bloodstream seems to be a required pre-requisite of successful infection. Thus, contact with infected blood or blood-contaminated material (carcasses, environment) is suggested as the primary transmission mode. There is no decisive evidence that WB are cannibals but rare contacts with WB carcasses and nearby soil have been observed using video monitoring (K. Depner, personal communication).

To summarize, reorientation of the common epidemiological concepts towards the understanding of complex interdependencies between high virulence of the virus, carcasses as a major source of infection, rare contacts of healthy WB with carcasses of other WB, high tenacity of the virus in carcasses, specific behavior of WB (site fidelity), may be crucial for the comprehension why the virus has not spread rapidly beyond the primary focus and affected a relatively small percentage of WB population.

References

Depner K. Guberti V.: Wild boar as a ASF driver. Proceedings of the 3rd Annual GARA Scientific Workshop, Ploufragan, France, 6-8 September 2016, p. 29.

Rola dzików w utrzymywaniu się wirusa Afrykańskiego pomoru świń w Polsce w 2014-2016 r.

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Słowa kluczowe: Afrykański pomór świń, dziki, epidemiologia.

Wirus Afrykańskiego pomoru świń (ASF), należący do genotypu II, pojawił się w Polsce w lutym 2014 r. i do listopada 2016 r. stwierdzono 128 przypadków tej choroby (jeden przypadek może obejmować więcej niż jednego dzika). Analiza wyników badań przeprowadzonych w ramach monitoringu czynnego (badanie dzików odstrzelonych) i biernego (badanie dzików padłych i powypadkowych) wykazała, że 40-50% dzików znalezionych jako martwe na obszarze „zaka onym” (człony II i III załącznika Decyzji Komisji 2014/709/EU) było dodatnich w kierunku ASF. Z drugiej strony, jak dotychczas nie stwierdzono ani jednego wyniku dodatniego u dzików zabitych w wypadkach komunikacyjnych. Dlatego te obydwa komponenty monitoringu biernego powinny zostać rozdzielone i raportowane osobno. Z kolei roczna prevalencja zakażenia ASF u dzików odstrzelonych, nie wykazujących objawów chorobowych w momencie odstrzału, jest niższa niż 0,5%. Zatem jedynym skutecznym rodzajem wczesnego wykrywania obecności wirusa na danym terenie jest badanie dzików padłych. Zaobserwowano również trend sezonowy, tzn. najwyższy odsetek padłych dzików dodatnich w kierunku ASF występuje w lipcu (80%, przy rocznej średniej 45%). Analiza przestrzenna wykazała, że zasięg obszarów na których występuje ASF w populacji dzików powiększa się powoli. Pojawienie się w ostatnim czasie wirusa u dzików na obszarach dotychczas wolnych od ASF, ale gdzie między czerwcem i wrześniem b.r. choroba występowała u świń, sugeruje możliwość transmisji wirusa z populacji domowej do wolno żyjącej. Hipotezy dotyczące rozwoju sytuacji w zakresie ASF sformułowane po pojawieniu się pierwszych przypadków w 2014 r. (Depner i Guberti, 2016), okazały się nieprawdziwe. Według pierwszej z nich choroba miała szybko wygasnąć, gdy wirus, z uwagi na swój wysoki zjadliwość, szybko doprowadzi do depopulacji dzików. Druga hipoteza zakładała szybkie rozprzestrzenienie się choroby w kierunku zachodnim. Obydwie hipotezy błędnie zakładały wysoki zaraźliwość wirusa, który łatwo będzie ulegał transmisji między osobnikami chorymi i wrażliwymi. Aktualnie wydaje się uzasadnione sformułowanie konkluzji, że choroba w naszym regionie charakteryzuje się do unikalnymi cechami epidemiologicznymi. ASF jest w Afryce typową chorobą przenoszoną przez wektory (kleszcze), natomiast po pojawieniu się jej na obszarach gdzie kleszcze nie występują, choroba nie wygasła, jednak wnikiwie wirusa do krwiobiegu w dalszym ciągu jest warunkiem koniecznym do wywołania zakażenia. Dlatego te do infekcji dochodzi przez kontakt z krwią lub materiałem zanieczyszczonym krwią zawierającym wirus (zwłoki, rodowisko). Jak dotychczas nie ma dowodu na to, aby dziki czy sto wchodziły w kontakt z padliną innych dzików, chociaż jednak penetrują glebę w okolicach zwłok, co wykazano przy użyciu monitoringu wideo (K. Depner, informacja ustna). Może to tłumaczyć, dlaczego ASF objął stosunkowo niewielki odsetek populacji (ok. 5%).

Podsumowując, konieczne jest przeorientowanie poglądów na temat epidemiologii ASF u dzików w Europie wschodniej, celem zrozumienia słabo poznanych współzależności między wysoką zjadliwością wirusa, rolą zwłok jako źródła zakażenia, odpornością patogenu w rodowisku, czy też specyficznego zespołu zachowań socjalnych dzików (mała mobilność watah).

Piśmiennictwo

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Recent epidemic of African swine fever in pigs in Poland

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Keywords: African swine fever, pigs, epidemic.

African swine fever (ASF) in Poland was first reported in wild boar in February 2014, until recently the occurrence of the disease in domestic pigs has been sporadic: in total 2 outbreaks in 2014 and 1 outbreak at the beginning of 2015 had been identified. Since the end of June 2016, a new wave of ASF outbreaks has occurred in North-Eastern Poland, and by 30th of September, twenty new outbreaks were detected. The affected pig herds varied in size from a few to 540 pigs. Clinical signs observed in infected pigs included: fever (41.5-42.0°C), cyanosis of the ears and skin, diarrhea as well as abortions in pregnant sows. The first clinical signs of infection were observed in sows. Morbidity and mortality rates were slowly increasing in the herd, indicating a slow transmission of the virus by direct contact with infected excretions (low contagiousity). At necropsy, enlargement of spleen was the most frequently observed changes, while haemorrhagic lesions in organs were less pronounced. Wild boar seem to be the most likely primary source of the virus for domestic pigs in case of a few outbreaks, but illegal trade of infected pigs contributed greatly to the unprecedented spread of ASF in the area. Swill feeding seems to be one of the major transmission modes of the virus to susceptible pigs while fomites played a minor role in the virus entrance into the holdings. In case of a few outbreaks, the source of infection remains inconclusive. The immediate control measures were applied in infected holdings accordingly to the Council Directive 2002/60/EC, and included stamping out, carcass disposal, cleansing and disinfection, establishment of surveillance and protection zones followed by the intensive surveillance. Moreover, extension of the Part I – III areas based on amendments to Commission Implementing Decision 2014/709/EU, that imposes specific restrictions with respect to local and international movement and trade, was also implemented. Since the detection of the 1st outbreak this year (end of June) to the beginning of October, approximately 35 000 pigs have been tested, mostly from the areas under various restrictions. Additionally, as a response to the recent situation, an interdepartmental team was urgently created under the supervision of the prime minister of Poland and extraordinary legislation is currently being prepared and adopted to contain the epidemic and prevent similar events occurring in the future. The set of new measures and recommendations will include: maximum possible reduction of the wild boar population in the whole area of the country, involvement of military troops for active search of wild boar carcasses, strict enforcement of ban on pig production in holdings that do not fulfill biosecurity regulations, intensive information campaign in local and national media, in-depth investigations (including next generation sequencing) to determine the source and transmission pathways of the virus, intensification of efforts aiming at early detection of food products at border posts in Eastern Poland. To summarize, the recent situation underlines the primary role of illegal human activity in the spread of ASF in domestic pigs.

Afrykański pomór świń w populacji świń w Polsce w 2016 r.

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Słowa kluczowe: Afrykański pomór świń, winie, epidemia.

Afrykański pomór świń (ASF) wykryto w Polsce po raz pierwszy w lutym 2014 r. u dzików, ale do niedawna występowanie tej choroby u świń było sporadyczne: ogółem 2 ogniska w 2014 r. i 1 ognisko na początku 2015 r. (wszystkie w chowie przyzgodowym). Pod koniec czerwca 2016 r., w północno-wschodniej Polsce wystąpiła nowa fala zachorowań na ASF i do 30 września b.r. wykryto obecnie wirusa w 20 ogniskach. Liczebność świń w zakładach hodowlanych wahała się od kilku do 540 zwierząt. Zaobserwowane objawy kliniczne obejmowały: gorączkę (41.5-42.0°C), zasinienia uszu i skóry, biegunkę, natomiast u niektórych świń wystąpiły powąły poronienia. Lochy były często pierwszymi zwierzętami w stadzie, u których wystąpiły objawy kliniczne. Współczynniki zachorowalności i śmiertelności w zainfekowanych stadach wzrastały stopniowo, co wskazuje na powolną transmisję wirusa przez kontakt bezpośredni (niska zarazyliwość). W badaniu sekcyjnym stwierdzano najczęściej uszkodzenia łądźni, natomiast zmiany o charakterze krwotocznym były słabiej wyrażone. W przypadku kilku ognisk dziki były najbardziej prawdopodobnym pierwotnym źródłem zakażenia dla świń, natomiast do znacznego rozprzestrzenienia się wirusa przyczynił się w największym stopniu nielegalny handel winiami. Jednym z głównych sposobów transmisji wirusa do wrażliwych zwierząt było również skarmianie zlewkami, natomiast przenoszenie patogenu na odzieży, butach czy sprzęcie wydaje się odgrywać drugorzędny rolę. W kilku przypadkach nie udało się precyzyjnie ustalić źródła zakażenia. Wprowadzone w trybie natychmiastowym środki zwalczania (zgodnie z Dyrektywą 2002/60/KE) obejmowały wybijanie zwierząt, utylizację padliny, mycie i dezynfekcję, ustanowienie okręgów zapowietrzonych i zagrożonych oraz intensywny monitoring. Ponadto, zgodnie z Decyzją Komisji 2014/709/EC, powieszono zasięgi stref I-III. Od wykrycia pierwszego ogniska w 2016 r. (koniec czerwca) do początku października przebadano 35 000 świń, głównie z obszarów objętych różnymi ograniczeniami. Utworzono pod nadzorem premiera ministerstwa resortowy zespół reagowania, opracowano i przygotowano specyfikację zestawu środków zwalczania i prewencyjnych, takich jak: maksymalne możliwości redukcji populacji dzikiej (do poziomu nie większego niż 0,5 dzika/km²), zaangażowanie wojska w poszukiwanie zwłok padłych dzików, ścisły zakaz produkcji świń w gospodarstwach niespełniających zasad bioasekuracji, kampanie informacyjne w mediach, dochodzenie epidemiologiczne (m.in. przy użyciu metod sekwencjonowania następnej generacji), celem ustalenia źródeł pochodzenia i dróg szerzenia się zakażenia, jak również intensyfikacja poszukiwań i konfiskat produktów pochodzenia zwierzęcego na punktach granicznych. Podsumowując, aktualna sytuacja podkreśla rolę nielegalnej działalności człowieka w rozprzestrzenianiu się wirusa ASF u świń.

Experience from recent animal trials using recent genotype I and II ASFV strains – implications for epidemiology and control

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Keywords: Experimental infections, attenuated phenotype, carrier state.

Recently, genotype II African swine fever virus (ASFV) strains have been characterized in different experimental infections using domestic pigs, European wild boar and mini pigs. In a first experiment, ten sub-adult wild boar were inoculated using a recent ASFV strain from Estonia. Under our experimental conditions, the virus showed high virulence, and nine out of ten animals succumbed to infection showing typical lesions. Subsequently, a virus re-isolated from the recovered animal was utilized in two trials with different pig breeds. Trial A comprised 12 mini pigs, trial B five domestic fattening pigs. In both trials, oronasal inoculation was carried out using a blood suspension from the survivor in its acute clinical phase. In trial A, all animals developed fever and unspecific clinical signs within the first week post inoculation. However, nine out of twelve mini pigs survived the acute phase and were slaughtered in good health status at 36 days post inoculation (DPI). Necropsy revealed that two of the inoculated mini pig sows were pregnant. Organ pools of the fetuses were tested by PCR and were found negative. Antibodies were detected in all convalescent animals by the end of the trial. Animals of trial B displayed a similar disease course with all animals surviving till the end of the trial at 36 DPI. Apart from a severe fibrinous pericarditis in one of the animals, necropsy did not reveal any signs indicative for ASF or any other disease. While clinical signs were completely absent from 19 DPI, high ASFV genome loads were still detectable by PCR till the end of the trial. Summarizing, an apparently attenuated virus strain was re-isolated from the initial trial. Animals of different pig breeds, i.e. mini pigs and domestic fattening hybrids, showed similar disease courses that were, in terms of virus detection, still comparable to the highly virulent ancestors. However, clinical signs and mortality were drastically reduced. Under field conditions, these unspecific clinical signs could easily go unnoticed and thus complicate disease control tremendously. For the wild boar situation in Estonia, circulation of attenuated strains is likely and should be further investigated.

In addition, an animal trial was carried out targeting the assessment of a potential carrier state of 30 pigs in total which were allowed to recover from infection with ASFV “Netherlands’86” for 99 days prior exposure to six healthy pigs for more than two month. Upon initial infection, a wide range of partly atypical clinical and pathomorphological signs were observed. After an initial acute phase in all experimentally inoculated pigs, 66.6 % recovered fully from infection including seroconversion. No ASFV transmission occurred over the whole in-contact phase. Similarly, ASFV was not detected in any of the numerous tissue samples from ASFV convalescent and in-contact pigs. These findings suggest that a carrier state is not imperative for all surviving animals.

Do wiadzenia z przeprowadzonych ostatnio bada na zwierz tach z u yciem aktualnych wirusów ASF genotypu I i II – konsekwencje dl epidemiologii i zwalczania

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Słowa kluczowe: zaka enia eksperymentalne, atenuowany fenotyp, nosicielstwo.

W ostatnim czasie szczepy genotypu II wirusa Afryka skiego pomoru wi (ASFV) zostały scharakteryzowane w ró nych układach eksperymentalnych na winiach, dzikach i winiach miniaturowych. W pierwszym eksperymencie, dziki były zaka ane szczepem wirusa aktualnie kr cym w Estonii. Wirus wykazał wysok zjadliwo , a 9 na 10 zaka onych zwierz t padło i stwierdzono w nich obecno typowych zmian sekcyjnych. Nast pnie wirus wyizolowany od dzika który prze ył zaka enie został wykorzystany w dwóch kolejnych do wiadzeniach, w których wykorzystano ró ne rasy wi . Eksperyment „A” obejmował 12 wi miniaturowych, a eksperyment „B” 5 tuczników. W obydwu do wiadzeniach przeprowadzono zaka enie *per os* i donosowe zawiesin krwi pobran od dzika-ozdrowie ca w ostrej fazie klinicznej. U wszystkich zwierz t w grupie „A” rozwin ła si gor czka i niespecyficzne objawy kliniczne w pierwszym tygodniu po zaka eniu, jednak 9 z 12 wi miniaturowych prze yło ostr faz i zostało poddanych eutanazji w 36 dniu po zaka eniu (pz). Badaniem sekcyjnym stwierdzono, e dwie samice były ci arne. Pulowane próbki narz dów pobranych od płodów badano metod PCR z wynikiem ujemnym. Wykryto obecno przeciwciał w surowicach krwi pobranej od ozdrowie ców pod koniec zaka enia. Zwierz ta w do wiadzenia „B” wykazały podobny przebieg choroby i wszystkie prze yły do dnia zako czenia bada w 36 dniu pz. Oprócz ostrego włóknikowego zapalenia osierdzia stwierdzonego u jednej wini, badanie sekcyjne nie wykazało zmian typowych dla ASF ani innej choroby. Podczas gdy nie obserwowano adnych objawów klinicznych pocz wszy od 19 dnia pz, wykryto du ilo materiału genetycznego wirusa w próbkach pobranych pod koniec do wiadzenia. Podsumowuj c, wszystko wskazuje na to, e wirus izolowany z pilota owego do wiadzenia na dzikach posiada cechy atenuacji. U wi miniaturowych oraz tuczników wykazano podobny przebieg kliniczny, a w kontek cie wykrywania wirusa - przebieg typowy dla zjadliwego wirusa-przodka. Jednak zmiany kliniczne i sekcyjne były znacznie słabiej wyra one. W warunkach terenowych tego rodzaju niespecyficzne objawy kliniczne mog zosta niezauwane i diametralnie utrudnia zwalczanie choroby. Bior c pod uwag aktualn sytuacj w Estonii, kr enie szczepów atenuowanych jest prawdopodobne i wymaga dalszych bada .

Dodatkowo przeprowadzono eksperyment, którego celem była ocena potencjalnego stanu nosicielstwa 30 wi które prze yły zaka enie szczepem wirusa “Netherlands’86” przez 99 dni przed ekspozycj na 6 zdrowych wi . Po ekspozycji zwierz ta obserwowano przez okres 2 miesi cy. Po zaka eniu obserwowano szerokie spektrum cz ciowo atypowych zmian klinicznych i patomorfologicznych. Po pocz tkowej ostrej fazie odnotowanej u wszystkich zaka onych wi , 66.6 % z nich całkowicie wyzdrowiało i wytworzyło przeciwciała. Nie zaobserwowano transmisji wirusa do wi kontaktowych w trakcie trwania eksperymentu. ASFV nie był równie wykrywany w adnej z licznych tkanek pobranych od wi zaka onych i kontaktowych. Wyniki te dowodz , e nosicielstwo nie jest imperatywem w odniesieniu do wszystkich zwierz t, które prze yj zaka enie.

African swine fever in Sardinia: lessons learned that might be useful to the rest of Europe

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Keywords: African swine fever, virus evolution, epidemiology, risk factors, seasonal trend analysis.

African swine fever (ASF) has been endemic in the island of Sardinia since 1978. The Sardinian ASF virus owns to p72-genotype I, like those that circulated in the Iberian Peninsula from 1957 until the 1990s. Molecular epidemiology studies on the Central Variable Region and on the EP402R genes show that the Sardinian isolates can be divided into two different temporally related sub-groups: the first group including isolates from 1978-1990 and the second group including all isolates, except one, as from 1990. The most recent ASF viruses seem to have rapidly and completely replaced the oldest viruses, perhaps due to some selective advantage of this variant, even though field data do not suggest any change in virus virulence. Studies of the full sequence of a 2010 ASFV isolate confirmed that in Sardinia the virus has undergone genetic variations in the two genome regions above and that there are also genetic differences with the 16 isolates from the rest of the world.

Several epidemiology studies have clearly demonstrated that the most important risk factor for disease persistence in Sardinia is the illegal practice of keeping domestic pigs at free range, that is still common in some inner areas of the island. Other irregular practices such as moving pigs, pigmeat and pigmeat products without the required veterinary checks are also important risk factors. The wild boar seems to play a less important role in virus spread and persistence, although a statistically significant association was observed between ASF occurrence and wild boar density. However, field observations suggest that in those areas of Sardinia where the disease was successfully eradicated from domestic pigs, ASF virus showed then the tendency to spontaneously fade out from the wild boar.

Preliminary results of an analysis of the seasonality of ASF and bluetongue (BT) in Sardinia and ASF in Russia show statistically significant differences. However, the seasonal curve of ASF in Russia looks more similar to the one of BT in Sardinia; if confirmed, these data would suggest that ASF in Russia is associated with a strong seasonal risk factor.

In Sardinia, socio-cultural factors behind the practice of keeping pigs at free range have so far made disease eradication attempts unsuccessful. An association between Material Deprivation Index and disease persistence has also been shown. Since 2015, an ASF extraordinary eradication programme is in place that includes not only better targeted veterinary measures but also measures to eliminate free ranging pigs and to incentivize on-farm biosecurity and good practices. The first results of this ongoing programme seems to be encouraging.

Afrykański pomór wi na Sardynii – lekcja, z której Europa może wyciągnąć wnioski

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Słowa kluczowe: Afrykański pomór wi, ewolucja wirusa, epidemiologia, czynniki ryzyka, analiza trendów sezonowych.

Afrykański pomór wi (ASF) występuje na Sardynii (Włochy) endemicznie od 1978 r. Wirus należy do genotypu I (w oparciu o analizę genu p72) - tego samego, który krążył na Półwyspie Iberyjskim od 1957 roku do lat 90. XX wieku. Badania molekularne przeprowadzone na podstawie analizy regionu zmiennego oraz genów EP402R wykazały, że izolaty ASFV z Sardynii można podzielić na dwie podgrupy. Pierwsza z nich obejmuje wirusy z lat 1978-1990, a druga wszystkie (z wyjątkiem jednego) wykrywane od 1990 roku. Wirusy z drugiej podgrupy szybko i całkowicie wyparły szczepy “wczesne”, prawdopodobnie na skutek przewagi selekcyjnej nowych wariantów, aczkolwiek dane terenowe nie wykazują, aby zaszły zmiany jeżeli chodzi o zjadliwość wirusa. Analiza całej sekwencji genomu izolatu ASFV z 2010 roku potwierdziła, że wirus na Sardynii uległ znaczącym zmianom genetycznym w obydwu wyżej wspomnianych regionach genomu, stwierdzono również różnice w porównaniu z 16 szczepami uwzględnionymi w badaniach, a wykrywanymi w różnych rejonach świata.

Liczne badania epidemiologiczne jasno wykazały, że głównym czynnikiem ryzyka długotrwałego utrzymywania się tej choroby na Sardynii są nielegalne praktyki utrzymywania wi systemem wolno wybiegowym, co jest zjawiskiem ściśle powiązanym z powszechnym w niektórych centralnie położonych obszarach wyspy. Inne, występujące z mniejszą regularnością praktyki, takie jak przemieszczanie wi i wieprzowiny bez wymaganych certyfikatów weterynaryjnych, stanowi również ważny czynnik ryzyka. Dzikie odgrywają mniejszą rolę w utrzymywaniu się wirusa i jego rozprzestrzenianiu, chociaż stwierdzono statystycznie istotną korelację między występowaniem ASF i gęstością populacji. Obserwacje terenowe wskazują jednak, że na obszarach, gdzie wirus został skutecznie wyeliminowany z populacji wi, ASF ma tendencję do zanikania również w populacji dzików.

Wstępne analizy sezonowości ASF i choroby niebieskiego języka (BT) na Sardynii oraz ASF w Rosji wykazują statystycznie istotne różnice, jakkolwiek trend sezonowy ASF obserwowany w Rosji jest zbliżony do obserwowanego w przypadku BT na Sardynii; jeżeli zostanie to potwierdzone, wskazywałoby to, że w Rosji sezonowo ASF jest ważnym czynnikiem ryzyka. Czynniki społeczno-kulturowe polegające na utrzymywaniu wi na Sardynii w wolnym systemie chowu uniemożliwiają jak na razie skuteczną eradykację ASF. Udowodniono również zależność między wskaźnikiem zagrożenia ubóstwem a utrzymywaniem się ASF. Od 2015 r. realizowany jest nadzwyczajny program eradykacji, obejmujący nie tylko wdrażanie lepiej ukierunkowanych weterynaryjnych środków zwalczania, ale również środków zmierzających do wyeliminowania praktyk wolnego chowu wi oraz tworzenie systemu zachęt dla hodowców przestrzegających zasad bioasekuracji i dobrej praktyki w gospodarstwach. Pierwsze wyniki tego programu wyglądają obiecująco.

ASF virus contagiousity and wild boar behavior

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Keywords: contagiousity, virus tenacity, wild boar ecology.

Contagiosity describes the property of a pathogen (e.g. African swine fever virus) to be transmitted directly without transmission factors (e.g. mechanical vectors, soft ticks) from an infected animal to a non-infected animal resulting in a new infection. The degree of contagiousness (contagiosity index) depends on several factors including characteristics of the host (susceptibility to disease, receptor availability), dose and characteristics of the pathogen (virulence, tenacity) and the likelihood of effective contact (infection route, behavior of the host, etc.). The contagiousity index describes the percentage of non-immune animals that get infected after contact with an infectious agent. It also indicates the probability that an animal picks up an infection. Highly contagious diseases like foot and mouth disease or Influenza have a high contagiousity index (>0.9) since nearly all susceptible animals of an epidemiological unit get infected shortly after the pathogen is introduced. Highly contagious diseases are mostly airborne and can spread very fast and die out when the number of susceptible animals decreases.

Recent field studies in the Baltic States as well as experimental animal trials provided evidence that ASF has a relatively low contagiousity index (<0.2) (Pietschmann et al., 2015; O ševskis et al., 2015). Furthermore, it can be observed that the disease persists in wild boar populations for years in the same areas in the Baltics and Poland. In this context the role of infectious wild boar carcasses may play a crucial role. How healthy wild boar behave towards their dead fellows has been analysed in a field study conducted over a 12-month period in Germany (Probst et al. 2016).

In this presentation, the findings from the carcass study are linked with the contagiousity data of ASF and to the tenacity characteristics of ASF virus for a better understanding of the current picture of ASF spreading among European wild boar populations. Particularly the quality and intensity of contacts between healthy wild boar and wild boar carcasses as well as between healthy wild boar and potentially contaminated soil during different seasons of the year are evaluated. Finally, knowledge gaps for a better understanding of ASF epidemiology in wild boar populations are addressed.

Zara liwo wirusa ASF a behawior dzika

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Słowa kluczowe: zara liwo , oporno wirusa, ekologia dzika.

Termin „zara liwo ” definiowany jest jako zdolno patogenu (np. wirusa Afryka skiego pomoru wi , ASF) do bycia przekazywanym bezpo rednio, z wył czeniem po rednich czynników transmisji (np. wektorów mechanicznych, kleszczy), mi dzy zwierz ciem zaka onym i niezaka onym. Stopie zara liwo ci (wyrany indeksem zara liwo ci) zale y od wielu czynników, w tym wła ciwo ci gospodarza (wra liwo na zaka enie, dost pno receptorów), dawki i charakterystyki patogenu (zjadliwo , oporno na warunki rodowiska) jak równie prawdopodobie stwa skutecznego kontaktu (droga zaka enia, behawior gospodarza, itp.).

Indeks zara liwo ci opisuje odsetek wra liwych zwierz t ulegaj cych zaka eniu po kontakcie z czynnikiem zaka nym. Wskazuje równie na prawdopodobie stwo, e zwierz ulegnie infekcji. Wysoce zara liwe choroby, takie jak pryszczycyca czy grypa, cechuj si wysokimi warto ciami wska nika zara liwo ci ($>0,9$), poniewa niemal wszystkie wra liwe zwierz ta w danej jednostce epidemiologicznej ulegaj zaka eniu w krótkim czasie po wprowadzeniu wirusa. Choroby wysoce zara liwe najcz ciej przenosz si drog powietrzn i szerz si szybko, ale równie szybko wygasaj , kiedy liczba osobników wra liwych spada.

Ostatnio przeprowadzone w Krajach bałtyckich badania terenowe, jak równie badania eksperymentalne na zwierz tach, dostarczaj dowodów, e w przypadku ASF indeks zara liwo ci jest niski ($<0,2$) (Pietschmann i wsp., 2015; O ševskis i wsp., 2015). Co wi cej, mo na zaobserwowa , e choroba utrzymuje si w populacji dzików w tych krajach, jak równie w Polsce, od kilku lat. W tym kontek cie rola zwłok dzików padłych na ASF wydaje si kluczowa. Zachowanie dzików zdrowych w stosunku do zwłok dzików padłych zostało przeanalizowane podczas 12-miesi cznej obserwacji terenowej w Niemczech (Probst et al. 2016).

Podczas prezentacji wyniki bada nad rol padliny zostan powi zane z danymi dotycz cymi zara liwo ci i oporno ci ASFV na warunki rodowiska, celem lepszego zrozumienia cech szerzenia si ASF w europejskiej populacji dzików. W sposób szczególny zostanie przeanalizowany aspekt cz sto ci i charakteru kontaktów mi dzy dzikami zdrowymi i padłymi, jak równie mi dzy dzikami zdrowymi i zanieczyszczon wirusem gleb w ró nych okresach roku. Zostan równie zasygnalizowane luki w wiedzy na temat epidemiologii ASF u dzików.

Cross-protective immunity and African Swine Fever virus serotype-specific proteins

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Keywords: African swine fever virus, serotype-specific antigens, cross-protective immunity.

African swine fever (ASF) is arguably the most significant emerging disease threat for the swine industry worldwide. Devastating ASF outbreaks and the continuing epidemic in Russia (2007 – to date) and Eastern Europe (2014 - to date) underscore the significance of this disease. There is no ASF vaccine available; however, it is clear that vaccination is possible since protection against homologous reinfection has been definitively demonstrated. Vaccine development has been hindered by large gaps in knowledge concerning ASFV infection and immunity, the extent of ASFV strain variation in nature and the identification of viral proteins (protective antigens) responsible for inducing protective immune responses in the pig. Available data from vaccination/challenge experiments in pigs suggest that ASF protective immunity is hemadsorption inhibition (HAI) serotype-specific. A safe and efficacious DIVA (Differentiate Infected from Vaccinated Animal) compatible ASFV vaccine would provide a critical tool for emergency disease response and control and reduce the risk for pork producers. Better understandings of ASFV HAI serologic group diversity in nature as well as identification of serotype-specific antigens (SSAs) involved in protective immunity are needed for eventual vaccine design and development. Recently, we have shown that two ASFV proteins, CD2v (EP402R) and C-type lectin (EP153R), are necessary and sufficient for mediating HAI serologic specificity (J. Gen. Virol. 96:866-873).

Here, using ASFV inter-serotypic chimeric and/or gene-deleted ASF viruses and vaccination/challenge experiments in pigs we demonstrate that serotype-specific CD2v and C-type lectin proteins are necessary for generation of homologous protective immunity in pigs. Thus, CD2v and C-type lectin proteins represent significant ASFV SSAs that should be targeted in vaccine design and development. Additionally, data are consistent with and support the emerging concept that ASF protective immunity is HAI serotype-specific.

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Krzy owa immunogenno białek swoistych dla serotypu wirusa afryka skiego pomoru wi

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Słowa kluczowe: afryka ski pomór wi , antygeny serotypowo-swoiste, immunogenno krzy owa.

Afryka ski pomór wi (ASF) jest prawdopodobnie najpowa niejsz chorob zaka n wi o mi dzynarodowym znaczeniu ekonomicznym dla hodowli trzody chlewnej. Fatalne dla dobrostanu hodowli ogniska i epidemia ASF w Rosji (od roku 2007 – do chwili obecnej) oraz w Europie Wschodniej (od roku 2014 – do chwili obecnej) podkre laj bardzo du e znaczenie tej choroby. Dotychczas brak jest dost pnej szczepionki przeciwko ASF, jednak e wiadomo, e szczepienie jest mo liwe, poniewa wykazano protekcj poszczepienn przeciwko homologicznym szczepom ASFV. Opracowanie szczepionki jest utrudnione w zwi zku z brakami w zrozumieniu mechanizmu zaka enia i immunogenno ci wirusa, poziomu zmienno ci szczepów ASFV w rodowisku i identyfikacji białek wirusowych (antygenów protekcyjnych) odpowiedzialnych za indukcy odpowiedzi immunologicznej organizmu wini. Dost pne informacje pozyskane z eksperymentalnego szczepienia/zaka enia wi wskazuj , e immunogenno jest swoista dla serotypu okre lonego testem zahamowania hemadsorpcji (HAI). Bezpieczna i skuteczna szczepionka, opracowana z wykorzystaniem strategii DIVA (Differentiate Infected from Vaccinated Animal) powinna by kluczowym narz dziem w zapobieganiu i kontroli tej choroby oraz redukcji poziomu ryzyka dla producentów wieprzowiny.

Lepsze zrozumienie ró norodno ci w rodowisku serotypowo HAI-swoistej grupy szczepów ASFV, jak równie identyfikacja swoistych serotypowo antygenów (SSAs) bior cych udział w zapewnieniu protekcji poszczepiennej s konieczne w celu zaprojektowania i opracowania szczepionki. W ostatnim czasie wykazano, i dwa białka ASFV, tj. CD2v (EP402R) i lektyna typu C (EP153R) s niezb dne i warunkuj swoisto HAI-serotypowo specyficzn (J. Gen. Virol. 96:866-873). W niniejszej pracy, zastosowano mi dzy-serotypowe, chimeryczne i/lub delecyjne szczepy ASFV do eksperymentalnego szczepienia/zaka enia wi . Uzyskane wyniki wykazały, e serotypowo swoiste białko CD2v i lektyna typu C s wymagane do uzyskania homologicznej protekcji poszczepiennej u wi . W zwi zku z powy szym białko CD2v i lektyna typu C ASFV wykazuj własno ci SSAs, które powinny by brane pod uwag przy projektowaniu i opracowaniu szczepionki. Dodatkowo, uzyskane dane potwierdzaj ide immunogenno ci ASFV, która jest swoista dla okre lonego serotypu HAI.

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African swine fever virus entry and uncoating

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Keywords: ASFV endosomal entry, Uncoating, Proteasome degradation.

African swine fever virus (ASFV) infectious entry traffics the endocytic pathway. Soon after entering the cell, viral decapsidation occurs in the acidic lumen of multivesicular late endosomes. From these vesicles, virions gain access to the cytosol to start replication. In contrast to the case for other DNA viruses, such as vaccinia or adenovirus 5, controlled cholesterol efflux at this level is required for successful viral fusion with the membrane and ASFV release to the cytosol. ASFV also remodels intracellular cholesterol by increasing its cellular uptake and redistributes free cholesterol to viral replication sites. Some innate immunity proteins target this lipid flux conducting virions to lysosomal degradation. ASFV also manipulates the ubiquitin-proteasome system. Ubiquitination has a central role in a variety of cellular processes, including the recognition and clearance of pathogens by the innate immune response. However, viruses have evolved several ways to exploit the ubiquitination system, for example by targeting key immune proteins for degradation or decreasing the presentation of antigens. The virus encodes for a gene homologous to the E2 ubiquitin conjugating enzyme (UBC) which could be able itself to catalize ubiquitination below E1, given that the inhibition of UB-activating enzyme E1 does not affect viral production. In contrast, inhibition of the final stage of the pathway by proteasome inhibitors inhibited viral replication and virus production. The viral UBC_v is present in the viral particle suggesting it might play a role early at infection. Also, core-associated and/or viral proteins involved in DNA replication may be targets for the ubiquitin-proteasome pathway that could possibly assist the virus in either core uncoating or DNA replication. Our analysis shows that ASFV manipulates both lipid flux and the ubiquitin-proteasome system at early infection, probably to surpass innate immunity and ensure a productive infection.

Interaction of porcine conventional dendritic cells with African swine fever virus

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African swine fever virus (ASFV) infection can cause an acute haemorrhagic fever in pigs with high mortality. The recent spread of disease in Eastern Europe has had a high socio-economic impact and threatens the global pig industry. No vaccine is available which limits the options for disease control.

An efficient immune response against a particular pathogen depends on the efficient recognition of the pathogen/antigen by the immune system and dendritic cells (DC) play an essential role in priming this response. Dendritic cells are competent antigen-presenting cells (APCs) responsible for the activation of naïve T cells and the generation of primary T-cell responses and they constitute the bridge between the innate and adaptive immune responses. According to their functionality and phenotype, DCs can be classified as conventional DCs (cDCs) known as professional APCs, or plasmacytoid DCs (pDCs), which naturally produce high levels of type-I interferon. Previous work has analysed the ability of negatively-selected pDC to produced large quantities of biologically active IFN when exposed to both virulent and low virulent strains of ASFV. However, ASFV interaction with cDC has not been previously analysed. Porcine DC (cDC) were purified from peripheral blood of healthy pigs by negative and positive selection. They were subsequently exposed to low or high virulent ASFV strains *in vitro* at a MOI of 1 and parameteres such as activation, viral replication and cytokine secretion were measured at 24 and 48 h post infection. ASFV viral factories in the cytoplasm of infected cDC were detected. Cytokines as well as changes in activation by lymphoproliferation acitivity were also detected, which dependent on the strain of virus. These new findings pave the way to elucidate the role of conventional DCs during the course of ASFV infection in pigs.

Comparison of three isothermal amplification methods for detection of African swine fever virus in blood from infected wild boars and pigs

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Keywords: ASFV, isothermal assays, comparison, cross-priming amplification, loop-mediated isothermal amplification, polymerase cross-linking spiral reaction.

The spread of the African swine fever virus (ASFV) among infected pigs and wild boars, is currently one of the most important facets of virus transmission in eastern Europe. Because of lack of vaccine against ASF the only available measures of ASF control include, stamping out of pigs from outbreaks, wild boar hunting, collection of wild boar carcasses administrative regulations as well reliable and fast diagnosis. The officially approved, diagnostic methods include, virus isolation, serological assays, including ELISA and immunoperoxidase assay (IPT) and different modifications of the polymerase chain reaction (PCR). So far, isothermal amplification methods including cross-priming amplification (CPA), loop-mediated isothermal amplification (LAMP) have been used as an alternative among ASFV detection methods. The third isothermal method has been designed by the National Reference Laboratory for diagnosis of ASF at the NVRI in Pulawy. The method is called polymerase cross-linking spiral reaction (PCLSR) and relies on application of 2 spiral primers and 1 cross-linking primer along with DNA polymerase with strand-displacement activity. Briefly, three methods have been used for detection of DNA extracted from blood of pigs originating from 3 outbreaks and wild boars from 17 selected cases. The results obtained using LAMP and CPA showed from 5 to 10% of false positive and negative results. The sensitivity of PCLSR was slightly below the sensitivity of LAMP and CPA but offered improved repeatability and reliability. The PCLSR was capable of detecting ASFV DNA in all examined blood samples, originating from pigs (3 outbreaks) and wild boars (17 cases). In case of LAMP and CPA the DNA of ASFV was not detected in 2 samples. No cross-reactivity with cDNA of classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRS) or porcine epidemic diarrhea virus (PEDV) have been observed in case of any compared method. The developed PCLSR presents a specific and sensitive method for on-site detection of ASFV in blood of wild boars in pigs. It might be further used by local and county veterinary officers, hunters or pig farmers, for preliminary ASF diagnosis.

Porównanie trzech metod amplifikacji w warunkach stałej temperatury do wykrywania wirusa afrykańskiego pomoru świń w krwi zakażonych dzików i świń

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Słowa kluczowe: ASFV, metody amplifikacji kwasów nukleinowych w warunkach stałej temperatury, porównanie, amplifikacja krzyżowa, amplifikacja w warunkach stałej temperatury, polimerazowa reakcja krzyżowa spiralna.

Szerzenie się wirusa afrykańskiego pomoru świń (ASFV) pomiędzy winiami domowymi oraz dzikami jest ostatnio jednym z najważniejszych aspektów transmisji wirusa we wschodniej Europie. Ponieważ nie istnieje szczepionka przeciwko ASF jedynym dostępnym narzędziem kontroli ASF jest wybicie świń w ognisku choroby, polowanie na dziki, regulowane przepisami administracyjnymi zbieranie zwłok padłych dzików, jak również wiarygodna i szybka diagnostyka. Oficjalnie uznane metody diagnostyczne zawierają izolację wirusa, testy serologiczne, w tym ELISA i test immunoperoksydazowy (IPT) i różne modyfikacje łańcuchowej reakcji polimerazowej (PCR). Jak dotychczas metody amplifikacji w warunkach stałej temperatury włączają amplifikację krzyżową (cross-priming amplification CPA), amplifikację w warunkach stałej temperatury (loop-mediated isothermal amplification LAMP) oraz jako metody alternatywne do detekcji ASFV. Trzeci metod amplifikacji w warunkach stałej temperatury zaprojektowano w Krajowym Laboratorium Referencyjnym ds. diagnostyki ASF w PIWet-PIB w Puławach. Metoda została nazwana polimerazową reakcją spiralno-krzyżową (polymerase cross-linking spiral reaction PCLSR) i polega na zastosowaniu 2 spiralnych starterów i 1 startera krzyżowego oraz polimerazy DNA posiadającej zdolność rozlatania podwójnej nici DNA. W skrócie, użyto trzy metody detekcji DNA wyizolowanego z krwi od świń pochodzących z 3 ognisk oraz dzików z 17 wybranych przypadków ASF. Wyniki otrzymane przy użyciu LAMP i CPA wykazały od 5 do 10% wyników badań fałszywie pozytywnych i negatywnych. Człono PCLSR była nieznacznie poniżej czułości metody LAMP i CPA, lecz dostarczała wiarygodnych wyników wskazujących na jej powtarzalność i niezawodność. Metoda PCLSR pozwalała na wykrywanie DNA ASFV we wszystkich poddanych badaniom próbkach krwi, pochodzących od świń (z 3 ognisk) oraz dzików (z 17 przypadków). W przypadku metod LAMP i CPA obecność DNA ASFV nie została wykryta w 2 próbkach. Nie zaobserwowano reakcji krzyżowych z cDNA wirusa klasycznego pomoru świń (CSFV), wirusem zespołu rozrodczo-oddechowego (PRRSV) oraz wirusem epidemicznej biegunki (PEDV) z użyciem badanych porównywanych metod. Opracowana metoda PCLSR jest specyficzna i czuła metod do wykrywania DNA ASFV we krwi dzików i świń. W przyszłości może być wykorzystywana przez inspektorów inspekcji weterynaryjnej, myśliwych lub właścicieli trzody chlewnej do wstępnego diagnostyki ASF.

Behaviour of free ranging wild boar towards their dead fellows – potential implications for the transmission of African swine fever virus

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Keywords: Wildlife, Wild boar, cannibalism, scavenging, African swine fever.

African Swine Fever virus (ASFV) is very stable in the environment and efficiently transmitted via blood and meat of infected animals. Therefore, the behavior of wild boar towards potentially infectious carcasses can significantly influence the course of an ASF epidemic. Although several studies have examined the phenomenon of carrion use, none of them has explicitly focused on wild boar. The aim of this study was to obtain a better understanding of the behavioral response of wild boar towards their dead fellows, in particular the frequency and intensity of contacts and potential cannibalism.

We monitored thirty wild boar carcasses under field conditions by digital cameras from October 2015 to September 2016 in the federal state of Mecklenburg-Western Pomerania, north eastern Germany.

Altogether, sixteen species were identified at the study sites, including eleven species of mammals and five species of birds. All species except for wild boar, deer, raccoon and squirrel, were observed scavenging on the carcasses.

In most occasions, wild boar did not take direct contact with the carcasses, but maintained a distance of at least 1 meter. They were also observed to sniff and poke on the carcasses, but without leaving any sign of cannibalism. However, several ruminant carcasses used as “controls” were consumed by wild boar in a matter of a few days, including large bones. Most often, wild boar spent some time rooting belowground in close proximity to or directly at the carcass site after its complete decomposition. In this context the infectiosity of the soil underneath an infected carcass remains to be investigated.

The results indicate that direct contacts of wild boar with their fellows’ carcasses do occur but are rather sporadic events. These findings are of particular interest for understanding the epidemiology of ASF and may help to understand and interpret the dynamics of its perpetuation in a wild boar population.

**Potential implications of wild boar carcass
and hunting remains consumption for African Swine Fever
transmission in Mediterranean areas**

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Keywords: Canibalism, shared diseases, transmission, wildlife-livestock interface.

While obligate scavengers effectively remove infectious tissues from carrion, the consumption of infected ungulate carrion and hunting remains by facultative scavengers may contribute to the spread and persistence of mammalian disease, such as African Swine Fever (ASF) in wild boar. Here we describe the guild of vertebrate scavengers and evaluate the potential for disease spread in two wildlife areas in South Central Spain, in one of which obligate scavengers (vulture) were absent during the study period, but wild boar is always present. We evidenced an active scavenging vertebrate community in which either obligatory scavengers (griffon *Gyps fulvus* and monk *Aegypius monachus* vultures) or medium-large sized mammals (wild boar *Sus scrofa* and red fox *Vulpes vulpes*) predominated according to local conditions. This occurred to the extent that wild boar, in absence of vultures, became locally predominant among the vertebrate scavenging guild. The boar showed greater importance in the consumption of residues (cannibalism) than of complete carcasses. Limiting the access of facultative mammal scavengers to abandoned carcasses and gut piles, while guaranteeing food supply for obligate scavengers (of conservational interest), should reduce sanitary risks for disease transmission and persistence among mammals, and specifically those infectious diseases for which the wild boar plays a relevant epidemiological role. Altogether, our observations evidenced that the necessary conditions for food borne pathogen transmission, such as ASF, towards scavenging cannibalism by wild boar occurred in Mediterranean West Europe.

Breeding disease and biosecurity measures on pig commercial farm in the Republic of Serbia

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Keywords: breeding disease, biosecurity measures, pig.

Production of pigs on commercial farms requires a great concentration of animals in a relatively small area. This situation requires the introduction of specific measures in order to preserve herd health. The aim of study was to monitor the health status of the herd on two commercial domestic swine farms that had a complex pathology and implementation of the required biosecurity measures at the satisfactory level. (that are applied for the satisfactory level of biosecurity). On two commercial pig farms we analyzed current breeding disease (health status and diseases in the category of breeding animals) and applied biosecurity measures. Balance on one of the animal production flow is not including the all-in/all-out system i.e) tested farms is such that suckling, and space for weaning piglets and space for finisher are in the same building. Separate compartments are just the latest. There is a possibility of direct contact directly between the newly purchased animals with animals that have for long been present on the farm. On both farms, there is a test area for quarantine. In the category of the weaning piglets, on the both swine farms, the significant health problems that include diseases of digestive and respiratory organs exist. On both farms there are diseases of the digestive and breathing in the category of weaning. Also, on commercial pig farms was diagnostic complex respiratory diseases was diagnosed. Studies were conducted to determine the prevalence of certain biological agents that participate in the development of complex respiratory diseases. Of great importance are the variations of pathogenic microorganisms not only in the expression of resistance to antimicrobials drugs already genetic recombination phenomena that certainly affect the clinical course of the disease and making diagnosis difficult. Our recommendation given to commercial pig farms is related to reducing the risk of effects of various environmental contaminants, the implementation of multi monitoring quality of raw materials and finished products as well as application of adequate protectors of the toxic effects of agents and improvement of external and internal biosecurity.

Risk factors for introduction of African swine fever into Slovenia

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Keywords: ASF, domestic pig, wild boar, epidemiology.

Prior to Slovenia has joined the European Union (EU) in 2004, a pig production in our country was for years under the systematic monitoring and control for a variety of important diseases. Significant number of pig samples were tested each year for the following diseases: Aujeszky's disease AD, transmissible gastroenteritis, porcine reproductive and respiratory syndrome (PRRS) and pig brucellosis. Besides monitoring, the obligatory quarantine for all imported animals are helping to maintain a very good health situation in Slovenia. In the EU, a quarantine for animals coming from the other EU members is not mandatory. Thus a free import of pigs unfortunately worsened a health status of pigs in our country. Therefore epidemiological situation in our country is now very different. Systematic monitoring is currently implemented only for the AD (we are country officially free of AD) and for 1% of died pigs for Classical and African swine fever (we are free of both). The health status for other diseases is mostly unknown. After joining the EU, Slovenia imported some economically very important diseases, mainly because the new breeding animals and fatteners were introduced to the farms without quarantine or serological testing. Usually the farmers have not been asking the seller about the health status of the pigs that they wanted to buy. Thus, after joining the EU, Slovenia imported: PRRS, porcine dysentery, new serotypes of *Actinobacillus pleuropneumoniae*, porcine epidemic diarrhoea, porcine intestinal adenomatosis.(1, 2).

Since 2004, a significant decrease in pig meat production in Slovenia was noticed. Additionally the number of animals and the number of large farms has been decreasing for several years. In the past, there were 8 large farms, wherefrom only two are operative now. Most pig farms in Slovenia are small-sized, as well as one site production farms. All together there are 276.747 pigs, from which 27.845 are the breeding pigs. Currently there are 3.909 farms with 1 to 20 breeding pigs, 206 farms with 21 to 50 breeding pigs, 31 farms with 51 to 100 breeding pigs, 12 farms with 101 to 200 breeding pigs, 2 farms with 501 to 1.000 breeding pigs and only 2 farms with more than 1.000 breeding pigs (3). The highest density of domestic pig population is located in the eastern part of the country. Moreover, the majority of the Slovenian pig industry involves traditionally small pig farms with less than 50 breeding sows. Since they are usually located closely to each other this presents an easy way to transmit the infection. These small farms have mostly no close contacts with wild boars population. Nevertheless, such "backyard" pig operations might be a potential risk for the introduction and spread of diseases from the wild boars to the domestic pigs, even more so, since wild boars are present all over Slovenia. However, the highest density of the wild boars can be found in the south-west part of the country (4). In the recent years there has been a growing interest in the role of the wild boar populations for the epidemiology of the important infectious diseases in swine. This interest occurs due to an increase in the wild boar population density worldwide (also in Slovenia), leading to a higher probability for the disease transmission. We made the study in which we try to estimate the prevalence of selected pathogens in wild boar population in Slovenia and estimate the risk of infection for the domestic swine. A conclusion for this study was, that there

is no transmission of the diseases from wild boars to the domestic swine or vice versa in pig production system used in Slovenia (5).

The main risk factors for introduction of the ASF may represent the transport vehicles that cross Slovenia from East part of the Europe, as well as all meat products that may be illegally introduced from the ASF positive country. An increasing number of the wild boar population and an intensive trade of pigs with the rare use of the quarantine by our farmers, may additionally represent some risk for the introduction of the ASF into the territory of Slovenia.

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Measuring the interactions between wild boar and domestic pigs

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Keywords: Interaction, wild boar, wildlife-livestock interface.

This presentation deals with how to get contact data or parameters that approach to contact at the wild boar/pig interface. Particularly, we focus on stakeholder interviews, direct observations, camera trapping (CT), GPS collaring, proximity data logger and pathogen genetic markers. We must have present that wildlife and livestock share the same needs; although wildlife usually avoid contact with livestock and people. The interface between livestock and wildlife is usually not a direct interaction since they asynchronously share space: Soil, food, water, etc, but it can occur directly, for exempling, during mating. Before setting up any study design, we need a previous understanding of the system. Often we lack information on where interspecific contacts might take place or whether the environment is relevant, where and when (seasonal). This previous understanding informs where to place, for instance, the camera traps, loggers and traps, which host species we must study, quality of data we will get, analytical approaches, etc. Which method(s) to choose? It will depend on the objectives of the study area and different aspects: (i) pathogen: life cycle, host range, routes of infection, are contacts relevant? (ii) host behavior; (iii) environment; (iv) logistics (e. g. downloading data, access to animals); (v) budget; (vi) data processing and analysis capacity; (vii) is intra-group/spp needed and/or relevant?; (viii) is individual relevant? (super-shedders, super-susceptible? (ix) are we performing social Network Analysis and Network modeling? (x) it is possible and desirable the combination of methods (Any & CTs, Proximity loggers and GPS). The method we select will also determine the definition of contact. Nonetheless this definition should have a biological basis, that us, how a pathogen is transmitted between wild boar and pigs. The extent of pathogen spread on a contact network (further modeling) is dependent on how a contact is defined. The main constraints of biologging are logistical challenges, undesirable effects on the animals during the capture handling and along the period on which the individuals are tagged, and the limitation in the number of animals that can be studied, restricted by the number of tags deployed, which are often expensive. Proximity loggers are transceiver tags that both transmit and receive (scan) radio signals (UHF). Each tag emits uniquely ID-coded radio pulses at regular, user-defined time intervals. They continually 'listen' for the signals of other tags, and therefore operate nearly continuously and are better suited to quantify contacts because, unlike GPS collars, there is no programmed interval when data cannot be gathered. Location error in radiotelemetry and GPS telemetry prevent these technologies from producing the fine-scale spatial and temporal resolution needed in some disease studies. Therefore, contacts often are inferred from close associations in space and time. Interestingly, the integration of GPS and proximity loggers avoids uncertainty about which individuals had the opportunity to interact (home range does not overlap, a problem encountered by studies based solely on proximity loggers. With molecular biomarkers. Instead of focusing on the host, we focus on the pathogen to infer transmission networks. Ideally, genetic studies should be conducted in parallel with detailed contact studies in order to define which interactions are important for transmission. In summary, there are a range of methods to collect information on wild boar-domestic pig overlap, all valid, depending on the local circumstances and objectives. The collection of contact data is getting easier due to new technologies, improving our ability to collect extensive contact data on (entire) study populations.

African swine fever in domestic pigs in Estonia in 2015 and 2016: epidemiological analysis of outbreak investigations

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Keywords: African swine fever, epidemiology, domestic pigs.

The first case of ASFV in Estonia was detected in September 2014, when an infected dead wild boar was found in southern part of the country, close to the Latvian border. In total, 73 ASF positive wild boar were found in 2014. From January to June 2015, the area affected by ASFV in wild boar was slowly increasing. Starting from July 2015, the ASFV situation in wild boar changed drastically, when the number of reported ASF positive wild boar was two to three times higher compare to previous months and the virus spread fast to new previously uninfected areas. By the end of the year in total, 1095 ASF positive wild boar were detected in eleven counties. In 2016, the spread of the virus in wild boar has been constant towards to the disease free areas in western Estonia. By the end of November only one county out of 15 (the island of Hiiumaa) was free of ASF.

The first case of ASF in domestic pigs in Estonia was discovered in the end of July 2015 in a backyard farm 5 km from the Latvian border. Subsequently, during next 9 weeks 17 other outbreaks were confirmed in seven counties. In 2016, the first two domestic pig outbreak were detected in central part of Estonia in the end of June. Next 3 outbreaks were reported in July and last outbreak (until 24th of November) was discovered in the middle of August in western Estonia, on the biggest island, previously free of ASF. As a result of these 24 outbreaks, a total of 29 076 domestic pigs were culled.

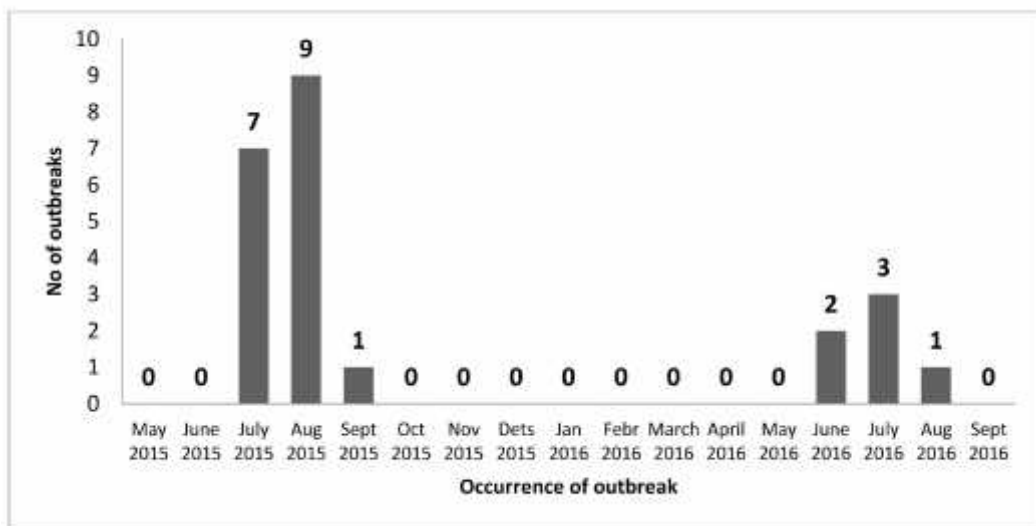


Figure 1. Time distribution of ASF outbreaks in domestic pig herds in Estonia in 2015 and 2016, based on the date of appearance of clinical symptoms

Epidemiological investigation was conducted for every outbreak farm by the team of experts from the Estonian University of Life Sciences.

The outbreaks occurred in farms of all size categories including small backyard farms (1-15 pigs), small-producers (up to 500 pigs) as well as large industrial farms (>1000 pigs). Farm to farm transmission was observed in two occasions only in 2015, when two affected farms belonged to the same owner. The outbreak farms were of different type, including farrow to finish, multiplier and fattening farms.

20 outbreaks were detected in counties, where ASF was previously diagnosed in wild boar population. The virus was most probably introduced to the farms via indirect transmission routes. Only in two outbreaks the direct contact with wild boar could not be completely excluded.

Most frequently inadequate biosecurity measures were considered as the most probable cause of ASFV introduction. In five cases contamination of cereal feeding stuffs, in two case grass feeding and in one case contaminated bedding, were suspected to be the cause of virus introduction.

Modelling the spatial distribution of reported ASF outbreaks in the Russian Federation using national surveillance data, 2007-2014

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Keywords: spatial modelling, ASF, zero-inflation, risk factors, surveillance.

African swine fever (ASF) is a viral disease of swine that has been present in the Russian Federation since 2007. Counts of ASF outbreaks reported in the Southern regions of the country (2007–2014) were aggregated to a grid of hexagons, and a zero-inflated Poisson model accounting for spatial dependence between hexagons was used to identify concomitantly 1) factors associated with the presence of ASF outbreaks and 2) factors associated with the number of ASF reports in affected hexagons. The putative explanatory variables used in this analysis focused on anthropogenic, swine-related and environmental variables, including human population density, distance to the nearest regional capital, distance to the nearest diagnostic laboratory, road density, density of pigs raised in high and low biosecurity farms and forest coverage. All analyses were performed in a Bayesian framework. The ability of the model to correctly predict presence or absence of at least one reported outbreak at the hexagon level (discriminatory power) was assessed by using the posterior distribution of the probability that at least one outbreak was reported in each hexagon. Increasing density of pigs raised on low biosecurity farms was found to be positively associated with the probability of occurrence of at least one ASF outbreak in a hexagon and with the average number of reported ASF outbreaks amongst affected hexagons. Increasing human population density and increasing distance from the closest diagnostic laboratory were additional variables associated with number of reported ASF outbreaks amongst affected hexagons. The model was shown to have good discriminatory power. Using the spatial distribution of the variables retained in the model, the median predicted probability of occurrence of ASF in domestic pigs, the median predicted sensitivity of ASF detection at hexagon level and the median predicted probability that at least one outbreak was reported were mapped. At the time this research was conducted, i.e. eight years after ASF virus had started circulating in the Russian Federation causing significant economic losses to the national pig industry, very few studies have tried to identify factors associated with the spatial pattern of the epidemic. The present study contributed to filling this gap. The results of this study are consistent with previous findings about the epidemiology of ASF in the Russian Federation. It also provides useful complimentary information as the structure of the zero-inflated model allows more detailed inference. For example, it indicated that human population density was associated with an increase in the average number of ASF reports if ASFv was circulating in the area (i.e. with an increase in the probability that at least one outbreak would be reported in affected areas), but not with an increase in the risk that ASFv circulates in the area (as was suggested by previous studies). Importantly, these results could assist the Russian veterinary services in defining high risk areas to be targeted for improved early detection and early reporting in order to minimise the impact of ASF on pig farmers' livelihoods.

Alternative sampling strategies for African swine fever surveillance in wild boar – Assessment of African swine fever virus antibody detection from dry blood swabs

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Keywords: surveillance, wild boar, alternative sampling, antibody detection.

Since early 2014, African swine fever continues to occur in the wild boar populations of the Baltic EU Member States and Poland. There is a high risk of introducing ASF into free areas as was confirmed by the occurrence of ASF in Moldova a couple of days ago (OIE WAHID, visited October 6th, 2016). Thus, targeted surveillance is of utmost importance for early detection. The introduction of ASF is usually accompanied by an increased occurrence of animals found dead and these fallen wild boar are the main target for passive surveillance. However, encouraging reporting by hunters and sampling of these animals is difficult. Partly, these problems could be solved by providing a pragmatic sampling approach.

We recently reported on the use of dry blood swab samples for passive classical and African swine fever (CSF and ASF) surveillance in wild boar (Petrov et al., 2014). In this context, we used real-time polymerase chain reactions for detection. Upon availability of the article online, we were asked by national and international colleagues whether this approach would be suitable also for antibody detection. In a very rough and brief evaluation, we could show that ASFV antibody detection is possible (Blome et al., 2014).

Here, we present the further evaluation and validation of antibody detection from dry blood swabs using the filter paper protocol of a commercially available antibody ELISA (ID Screen® African Swine Fever Indirect, IDVet). We were able to use serial blood samples from different animal trials with both domestic pigs and European wild boar and included different ASFV genotypes.

In general, fitness for the purpose could be confirmed, and swab samples could, therefore, be a valuable alternative for the sampling of wild boar in affected regions.

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Diagnostic testing of dead animals is the optimal surveillance tool for control of epidemics of African swine fever

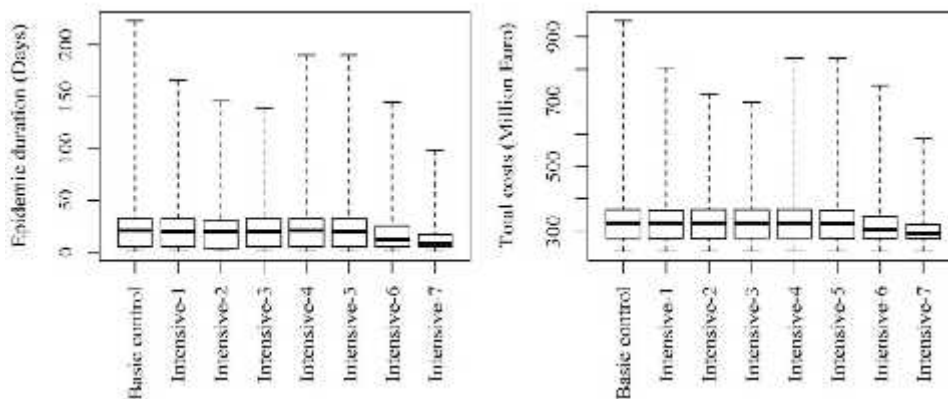
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Keywords: African swine fever; simulation model; spread; control; surveillance.

African swine fever (ASF) is a notifiable infectious disease with a high impact on animal health. At present, there is a significant risk of ASF spread towards Western Europe. Therefore, as part of contingency planning, it is important to explore strategies that can effectively control an epidemic of ASF. The epidemiological and economic effects of several strategies to control the spread of ASF between swine herds in Denmark were examined using a previously published stochastic and dynamic spatial spread model. The strategies were: A) the basic EU and national strategies (Basic control), which includes depopulation, cleaning and disinfection of detected herds, implementing a 3 km protection zone, in which swine herds are surveyed clinically at start and tested with serology before lifting the zone, and a 10 km surveillance zone, in which herds are clinically surveyed before lifting the zone, movement restrictions in the zones, tracing of movements and contacts, and implementing a 3 days national standstill on movements; and B) The basic control plus scenarios of intensive surveillance (Intensive) of live animals or dead animals in herds in the protection and/or the surveillance zones as follows: Intensive-1: PCR-testing of live animals within herds in the protection zone at start; Intensive-2: Serological testing of live animals within herds in the surveillance zone before lifting the zone; Intensive-3: Intensive-2 plus clinical surveillance of live animals within herds in the surveillance zone at start; Intensive-4: Intensive-1 plus Intensive-2; Intensive-5: In both zones for live animals, PCR testing at start and serological testing before lifting the zone; Intensive-6: PCR and serological testing of 1-5 dead animals per week from herds in protection zones; Intensive-7: PCR and serological testing of 1-5 dead animals per week from herds in both zones.



The figure shows boxplots of epidemic duration and the total costs using different control scenarios for an ASF epidemic. Using the basic control, the median epidemic duration was predicted to be 21 days (5th and 95th percentiles; 1-55 days), the median number of infected herds was predicted to be 3 (1-8), and the total costs were predicted to be €326 million (€256-442 million). Adding diagnostic testing of dead animals in the protection and surveillance zones (Intensive-7) was predicted to be the optimal control scenario for an ASF epidemic in industrialized swine populations. This scenario reduced the predicted epidemic duration to 9 days (1-38) and the total costs to €294 million (257-392 million). Adding intensive surveillance by testing live animals (Intensive-1 to Intensive-5) resulted in limited improvements in the control of the epidemics (Figure).

Hybridization levels in European *Sus scrofa*, comparison between genetic and survey data

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Keywords: wild boar, domestic pig, hybridization, SNP, contact areas.

Outdoor farming has been traditionally implemented in some European rural areas, but was mostly limited to backyard or small farming systems, however we are now observing an increment in this practice, as a result of both increased attention to animal welfare and consumers interest in organic food. At the same time, Europe is showing interest in rewilding projects. This combination might expand the wildlife-livestock interface, with enhanced sanitary risk and hybridization.

Hybridization events between the domestic pig (*Sus scrofa domestica*; hereafter DP) and the wild boar (*Sus scrofa*; hereafter WB) have been reported in several European countries and might provide insight on human practices that can lead to increased, although spatially delimited, risk of contagion. The markers most commonly used to investigate this aspect are mitochondrial DNA (mtDNA), MC1R and microsatellite (STR). All of them present some advantages and some disadvantages. MtDNA and MC1R can be easily compared among laboratories, while STR require calibration. Furthermore, STR can only detect recent hybridization events, while MC1R and mtDNA can also identify more ancient episodes. On the other hand STR and MC1R are biparentally inherited, thus representing the whole population, while mtDNA only shows the maternal lineage. In recent years the development and decrease in price of genomics techniques have allowed the implementation of genomic markers (like whole genome sequencing or Single Nucleotide Polymorphisms - SNP) for the study of genomic ancestry and hybridization. SNP markers are easily comparable between laboratories, biparentally inherited and allow the simultaneous study of loci neutral and under selection.

Here we present a comparison based on data from several European countries of genetic hybridization levels, detected with SNP markers, with data on hybridization perception, collected through an online survey. The survey covers 29 countries, with a major contribution from academic researchers (70%) but not negligible answer rate from the non-academic component (28%). Genetic data are based on 235 WB from 22 areas 149 DP (49 from 5 commercial lines and 100 from 9 local breeds) analysed with the Porcine SNP60 Beadchip (64,232 SNP).

Although data are not perfectly comparable, due to incomplete overlap of sampled areas and differences of methods, results show some interesting aspects. Genetic data show a clear separation between WB and DP, with a limited number of hybrids in both populations. The introgression level varies considerably among populations, from non-detectable to very high. Perceived presence of hybrids, based on phenotypic characteristics or historic data, is usually higher and widespread than that observed with SNP data, especially in the WB population.

Participants that stated hybridization was a past issue, often still report the presence of introgressed phenotypes in WB (50%) or both WB and DP (29%).

These results show the importance of considering both available information and hard data as they might help interpretation of outcomes or complement each other. At the same time, they point out the need to investigate further the genomes of hybrids individuals, as they might show the presence of regions under selection introgressed from one of the two forms into the other that might help better understand selection, introgression and genetic basis of WB invasiveness and that would be useful in the development of contingency or management plans.

African swine fever is a global threat requiring concerted efforts: lessons from an endemic country, Uganda

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Keywords: African swine fever, bushpig, domestic pigs, wildpigs, wildlife-livestock interface.

With the exception of areas with cultural and religious norms, pig production is practiced at a global scale and supports the economies and livelihoods of millions of people. The pig industry in many sub-Saharan African (SSA) countries is on the rise and is expected to continue growing in the coming years. This growth is mostly happening in developing countries and 75% of this is practiced by smallholder farmers. The population of pigs in SSA has increased fourfold in the last 50 years probably because of the rapid rate of multiplication. Pigs have been promoted as a way of improving household incomes and food security among resource constrained small scale farmers. The pig industry is however faced by the lethal and devastating African swine fever (ASF) disease, which kills close to 100% of infected pigs. Unlike several animal diseases, this ASF virus disease has neither treatment, nor vaccine. This is further complicated by the presence of numerous wildlife reserves in SSA with large populations of wild pigs that are known to be reservoirs of ASF. Indeed ASF exists both in the wild and the domestic pigs. There is a high chance of ASF transmission between wild and domestic pigs due to the porous interface. The main wild species of concern are the warthog (*Phacochoerus africanus*), the bushpig (*Potamochoerus larvatus*), and the soft tick (*Ornithodoros genus*). In order to understand how the disease is maintained and transmitted in the pig population, concerted efforts are needed to investigate the existence, prevalence, and characteristics of the ASF virus from each of the above species. This keynote presentation will highlight the potential role played by the key species, the challenges faced while conducting ASF research in Africa, and emphasize the need for joint and global efforts for ASF control.

Afrykański pomór wi jako globalne zagrożenie wymagające połączonej siły: lekcje z kraju endemicznego, Ugandy

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Słowa kluczowe: afrykański pomór wi, dzikany zarołowe, winie domowe, dzikie winie, kontakt pomiędzy winiami wolnymi i domowymi.

Za wyjątkiem terenów, gdzie normy kulturowe i religijne to wykluczają, produkcja wi jest praktykowana na globalną skalę, jest bardzo ważnym wsparciem dla gospodarki i stanowi źródło utrzymania dla milionów ludzi. Chów przemysłowy wi w wielu krajach Afryki Subsaharyjskiej rośnie i oczekuje się, że w następnych latach będzie nadal wzrastał. Ten wzrost jest głównie obserwowany w krajach rozwijających się i w 75% dotyczy gospodarstw niskotowarowych. W ciągu ostatnich 50 lat populacja wi w Afryce Subsaharyjskiej wzrosła czterokrotnie, prawdopodobnie w wyniku tempa reprodukcji. Chów wi był promowany jako droga do poprawienia dochodów rodzinnych i zapewnienia bezpieczeństwa żywnościowego drobnym rolnikom dysponującym niewielkimi zasobami. Przemysł wiński jednak boryka się ze śmiertelnymi wyniszczającymi chorobami jak jest afrykański pomór wi (ASF), która zabija blisko 100% zakażonych wi. W przeciwieństwie do kilku innych chorób zwierząt, przeciwko ASF nie opracowano odpowiedniego leczenia ani szczepionki. Ponadto sytuacja jest komplikowana przez obecność licznych rezerwuarów tej choroby w Afryce, które składają się z dużych populacji dzikich wi, u których wirus krąży przez cały czas. Istnieje duża szansa na wystąpienie transmisji ASF pomiędzy dzikimi a domowymi winiami. Głównym rezerwuarem brzośnym pod uwagę w ród dzikich gatunków zwierząt są guce zwyczajne (*Phacochoerus africanus*), dzikany zarołowe (*Potamochoerus larvatus*) oraz kleszcze (rodzaj *Ornithodoros*). W celu zrozumienia w jaki sposób choroba utrzymuje się i przenosi się w populacji wi potrzeba zjednoczenia sił, podjęcia badań nad, prewalencją oraz charakterystyką wirusów ASF pochodzących od obydwu wspomnianych gatunków dzikich wi. Myśl przewodnią prezentacji będzie przybliżenie potencjalnej roli, jak odgrywają gatunki kluczowe, rodziców i wyzwania podczas prowadzenia badań nad ASF w Afryce oraz podkreślenie potrzeby połączonej i globalnych wysiłków w kierunku zwalczania ASF.

Does wild boar behaviour predict slow spread of African Swine Fever?

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Keywords: ASF, contact rates, movements, social behaviour, space use.

African Swine Fever (ASF) has been expanding rapidly in eastern Europe since its introduction in Georgia in 2007, affecting both wild boar and domestic pigs. The emergence of the ASF in eastern Poland in 2014 raised serious concerns about its further spread westwards. Given the previous spatio-temporal pattern of epidemic in eastern Europe and abundant wild boar populations, the ASF was anticipated to continue spreading at a fast rate.

Contrary to this prediction, the disease expanded slowly in time and gradually in space during two years following its first detection in the wild boar population in Poland. In 2014-2015, 139 wild boar were diagnosed positive for ASF, with the mean prevalence of 5%. Most infected animals (81%) were located <15km from Poland-Belarus border, indicating potential source of infections. Epidemic frontline has shifted away from the border steadily at the rate of 1.5 km/month, with the maximum distance of 33 km from the border. The size of infected area reached 2060 km² (minimum convex polygon of all cases) by the end of 2015.

This slow spread can be attributed to several factors related to the behaviour of wild boar. Firstly, inter-individual contacts in a wild boar population are strongly structured socially and spatially and this contact heterogeneity may represent constraint to spread of infectious diseases. Contact rates among wild boar from different social groups are lower by an order of magnitude than among animals from the same social group. Frequency of direct contacts declines sharply with the distance between animal's home ranges: contacts occur rarely between animals living more than 2 km away from each other and just very occasionally if home ranges are separated by 4 km or more. Secondly, spatial behaviour of wild boar plays a minor role in shaping patterns of ASF spread. Analysis of ASF epidemiological data and telemetry-derived data on wild boar movements revealed no relationship between monthly parameters of disease spread (prevalence, incidence rate, geographic expansion of infected area) and spatial behaviour of wild boar (range size and dispersal distance). Thirdly, existing evidence suggests that wild boar tend to avoid visiting wild boar carcass, thus limiting the chance of direct contact with potentially infectious material. However, the role of infectious wild boar carcass in the ASF dynamics is still poorly understood.

Overall, it appears that spatio-temporal patterns of the ASF spread observed in Poland in 2014-2015 represented natural course of the disease in the wild boar population. Current epidemiological situation indicates that wild boar behaviour alone does not contribute to rapid expansion of the ASF in the susceptible population.

Moving ASF risk assessments to the local level

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Keywords: disease management, local implementation, local risk, customized risk assessment.

Risk assessments are a tool of major importance for risk managers to plan and implement measures to mitigate the respective risks. In the veterinary field, qualitative risk assessments for disease introduction and spread are fairly well established, assisting the veterinary authorities in setting their priorities for prevention and control.

With African swine fever (ASF) becoming endemic in Eastern Europe, the European Food Safety Authority (EFSA), independent research teams and the national veterinary authorities of several European Union (EU) member countries have conducted risk assessments looking into possible paths of ASF introduction and spread within the EU or the respective country. As these assessments cover rather large areas, diverse landscapes and heterogeneous host populations, the strengths of these assessments is more to be found on the determination of the sense of urgency. The mitigation options that can be deducted from these approaches are usually of an unspecific nature and lack a practical bridge for the implementing arm of the risk managers – the local veterinary authority. At this local level, the veterinary authorities find themselves often stretched between limited resources for additional risk mitigation measures and their overall diverse work portfolio leaving little time to work out sophisticated risk assessments.

The ongoing effort presented here, is the development of a framework for an ASF risk assessment for veterinary authorities targeting the district level. It will be designed to guide the local risk managers to base their risk mitigation measures on conclusions of national and regional risk assessments combined with their local realities. The tool is expected to help the veterinary authorities at the level where mitigation options need to be translated into targeted activities taking the local circumstances and constraints into account. It will provide the guiding questions related to pig production practices, wild boar densities and hunting activities, the role of migrating work force from ASF infected countries as well as major transport routes and other aspects to identify the high risk areas for introduction and spread of ASF within a district. The important role of potential seasonal patterns of the different factors will also be captured. By identifying high risk periods a temporal dimension is added to the assessed spatial risk pattern.

With the expected output, a hands-on tool is made available for risk managers at the local level to work them through the steps of assessing the risk of ASF introduction and spread in their area of responsibility.

The surveillance of African swine fever in wild boar population in Serbia

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Keywords: ASF surveillance, wild boars, Serbia.

African swine fever (ASF) is a highly contagious haemorrhagic disease of pigs, and one of the most serious transboundary animal diseases with the potential for rapid international spread, and high socio-economic impact. In 2007, ASFV was introduced into Georgia and subsequently into other Transcaucasian Countries, the Russian Federation, Ukraine and Belarus, where it has caused outbreaks in wild boar and domestic pigs. In 2014 ASF entered the EU in Poland, Lithuania, Latvia and Estonia, where outbreaks in both wild boar and domestic pigs have been notified. There is a non-negligible risk of ASFV introduction to other parts of Europe via transport vehicles, movement of people and other human-induced activities. Prevention and control of ASF is based on two main principles: early detection and strict sanitary measures. Wild boar (WB) appears to be a key factor in maintaining the disease in endemic areas and local spread across EU borders. Therefore it is crucial to establish the monitoring the WB population for the presence of ASF. The population of WB in Serbia was approximately estimated on >25,000 heads and the population density ranges from 0.2/km² to over 20/km². In the country there are about 300 hunting grounds with WB and their surface ranges from 20 to 1000 km².

Surveillance of classical swine fever (CSF) in WB was established in Serbia since 2009 and maintained the last 6 years as part of the Program of control and eradication of CSF and rabies in Serbia. The surveillance includes serological testing of WB blood samples on presence of antibodies, and tissue samples on CSF virus presence. For each of 25 district in Serbia, based on estimated population size and projected annual hunting bag, the number of samples to be examined is determined to confirm the presence or absence of infection with CSF (5-10%, 95% CL). Due to the epidemiological situation of ASF in Europe, from 2013/2014 hunting season, 20% of WB samples collected for CSF testing were also tested on the presence of anti-ASF antibodies by commercial ELISA test. In the last three years (hunting seasons) about 900 WB blood samples collected throughout the whole country were tested per each season. All the blood samples tested in the past 3 years give negative result on the presence of anti-ASF antibodies. The short overview of methodology and results of testing of WB sera samples collected on the northern and on the southern part of Serbia is presented.

Out of 2383 WB samples from 13 districts (8 from northern and 5 from southern part) of Serbia, planned for CSF testing in 2013/2014 hunting season, in total 485 samples were tested for ASF antibodies presence. Tested samples belonged to the young, up to 6 months old animals (7.84%), to 6 - 18 months old WB (36.49%), 1.5 - 2.5 year old WB (21.03%) and more than 2 year old WB (34.64%). During hunting season 2014/2015, out of 2383 samples planned for CSF testing from the same afore mentioned territory, 475 samples were tested for ASF antibodies presence. Tested samples belonged to the young, up to 6 months old WB (17.26%), to 6 - 18 months old WB (47.58%), 1.5 - 2.5 year old WB (15.58%) and more than 2 year old animals (19.58%). Out of 2027 WB samples from 6 districts, all from northern part of Serbia, planned for CSF testing in 2015/2016 hunting season, in total 406 samples were tested for ASF antibodies presence. The samples were tested from young, up to 6 months old WB

(20.44%), from 6 - 18 months old WB (50.99%), 1.5 – 2.5 year old WB (21.67%) and more than 2 year old animals (6.99%). All samples tested during the whole surveillance period were negative on anti-ASF antibodies.

In the absence of efficient vaccine, ASF control relying in efficient early diagnosis and on the stamping out policy for infected and in contact pigs. Maintenance of the early warning system through continuous testing of WB on anti-ASF antibodies, but also as active and passive surveillance on ASF virus presence, that is established and based on risk assessment, is a highly valuable tool for on time reaction in the case of possible ASF outbreak in Serbia.

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Knowledge and attitude related to African swine fever of backyard farmers, hunters and governmental veterinarians in Armenia

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Keywords: ASF, knowledge and attitude, epidemiology, control.

Introduction and objective. ASF is one of the most serious transboundary swine diseases. In some countries outbreaks have been controlled by culling infected animals in addition to strict movement bans of swine and their products. However, these measures are difficult to implement as they require well-equipped veterinary services with reliable and trained personnel. Furthermore, another important challenge is the implementation of effective passive reporting system to get the outbreaks in domestic pigs population and wildboars promptly reported respectively by farmers and hunters.

Since 2007, the disease has been regularly reported in Eastern European countries and in the Russian Federation with the latest emergency outbreaks reported in Poland and the Ukraine. Recently, through the Cooperative Biological Engagement Program (CBEP) funded by the US-Defence Threat Reduction Agency (DTRA), a novel training program was implemented in 2015 in Armenia, Ukraine, Kazakhstan and Georgia. The program aimed to raise public awareness of ASF through a sustainable education campaign and the establishment of a comprehensive regional network for the control and prevention of ASF emergencies. To better target the future training activities in Armenia and to support the designing of more effective early warning system in the country the project team implemented a survey to assess the knowledge and attitude towards ASF of backyard farmers, hunters and governmental veterinarians.

Methodology. This study was implemented in the period August-October 2015. A total of 1413 persons (participating to the training activities) were interviewed (1000 farmers, 100 hunters and 313 veterinarians) through a pre-tested questionnaire. Sections of the questionnaire aiming to assess the knowledge of target groups included questions on pathogen, species susceptibility, transmission patterns, clinical signs, prevention and control measures for African swine fever. The questionnaire for farmers included a specific section aiming at assessing the attitude towards the first reaction in case of ASF introduction into the farm. Questionnaires administrated differed and reflected the expected level of knowledge from the different target groups. Descriptive analyses were performed by means of univariate and multivariate regression analysis. “Knowledge” and “attitude” scores were calculated and, in the farmers’ survey, their correlation assessed by means of Spearman’s rank correlation test.

Results. Our findings generally show that all target groups have an acceptable level of knowledge regarding disease characteristics. As expected, most veterinarians (81.2 %) knew about ASF, though there were some gaps about its aetiology and epidemiology. A large proportion of farmers (73.2 %) is overall knowledgeable about ASF even though both farmers and hunters were less informed about ASF transmission, clinical signs and prevention measures. Forty per cent (40%) of farmers would not report to the veterinary services any suspicious signs of disease. About a third does not consider feeding animal garbage a risky

behaviour. About half of the hunters had a weak understanding of the role of wild-boars in ASF transmission. They were uncertain wild boars could transmit the disease to domestic pigs. The Spearman's rank correlation test in the farmers survey revealed a strongly positive linear correlation between knowledge and attitude ($r=0.62$, $p<0.001$) suggesting that the more knowledgeable a target group is with regards a specific disease, the more likely it is that the reaction toward preventing or controlling that disease is more proactive and effective

Conclusion. This survey assessed and identified gaps in the knowledge of target groups on the disease and on the means required to prevent the introduction and spread of disease in pigs, including reporting to veterinary authorities—a critical requirement for establishing an effective early warning system. This information is also important to design more targeted awareness campaigns and training activities.

Non-invasive sampling of wild boar – Applicable for ASF virus detection?

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Keywords: wild boar, pathogen sampling of wild animals by baits, non-invasive sampling, African swine fever.

African Swine Fever virus (ASFV) has become endemic in wild boar population in some areas of the Baltic states of the EU. Samples for diagnostic purpose are usually taken from hunted or dead animals. As in specific situations hunting may be prohibited, the idea of non-invasive sampling of wild boar became an option of sample retrieval. An approach of non-invasive sampling of wild boar was developed by Mouchantat et al. (2014) and proved to be experimentally suitable for nucleic acid detection of Foot and Mouth disease, Classical and African Swine Fever in saliva of wild boar. Furthermore, antibody-detection in non-invasively sampled wild boar saliva is possible.

The process of collection of wild boar saliva from the field, the nucleic acid detection in the laboratory and the applicability of this method in ASF-endemic areas will be presented and discussed.

Participatory epidemiology – a way to support the successful design of surveillance strategies and control measures

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Keywords: acceptability, hunters, participatory epidemiology, surveillance, wild boar.

Surveillance measures can only function, if they are accepted by the key players in the system. Acceptability, which describes the willingness of persons to contribute, is often analysed using participatory methods. Participatory epidemiology facilitates the active involvement of key players in the processing of epidemiological issues.

In the present study, we used participatory methods to evaluate the functionality and acceptability of the Classical swine fever (CSF) surveillance system in wild boar in Germany, which is highly dependent on the participation of hunters. Diagrams, proportional piling and scoring tools were introduced to investigate different aspects relating to the role of the hunters within the surveillance system for CSF in wild boar. The acceptability of alternative surveillance strategies was analysed with the help of visualisation tools. By conducting focus group discussions, potential vulnerabilities in the system were detected and feasible alternative surveillance strategies identified.

Trust in the current surveillance system is high, whereas the acceptability of the operation of the system is medium. Analysis of the acceptability of alternative surveillance strategies showed, how risk-based surveillance approaches can be combined to develop strategies that get sufficient support and functionality. Furthermore, some surveillance strategies were clearly rejected by the hunters. Thus, the implementation of such strategies may be difficult.

Methods of participatory epidemiology can be used to evaluate the functionality and acceptability of existing surveillance plans among hunters and to optimize plans regarding their chances of successful implementation. Our study showed benefits and additional insights, which can result from the integration of participatory methods in the evaluation of surveillance. Participatory methods can be used to consider and include views and needs of important key figures during the design and the implementation of surveillance strategies.

CSF constituted an example and the applied methods can be readily adapted to the surveillance of African swine fever (ASF). Including participatory epidemiology and therefore the motivations and views of hunters, provides a huge chance to support the efforts of managing ASF.

African swine fever and its way through Asia and towards Europe (Epidemiology and risk assessment in Ukraine)

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Keywords: African swine fever, epidemiology, surveillance, domestic pigs, wild boars.

Introduction and significance. 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. Also the *Ornithodoros* ticks demonstrate the potential of virus distribution. Disease was occurred in Caucasus region (Georgia, Armenia, Azerbaijan) firstly (2007), and has been transmitted transboundary to Russian Federation (2008). The first outbreak of African swine fever was reported in Ukraine (the human-factor associated introduction from Russian Federation) in 2012. The ASF outbreaks were reported in Belarus (2013), Lithuania, Latvia and Poland (2014), Estonia (2015). About 1000 disease outbreaks were detected in last years on the Eurasian nooareal of the disease. Economical losses are calculated in 100th mln. US dollars (i.e. stamping out, quarantine measures, international trade restrictions etc.).

Current data for disease distribution in Ukraine, and treats of ASF introduction to EU. The disease is the significant problem for Ukrainian pig industry and entire economy as Ukraine is agrarian state. 113 cases of ASF were described in Ukraine since 2012. Most "domestic" cases were associated with weak level of the biosecurity in small farms and backyards. Now there exist 2-3 compartments of the disease potential risk zones in the North-central, Northeastern and Eastern parts of Ukraine. The last compartment I strongly associated with ATO-zone, located in Lugansk and Donetsk regions of Ukraine, where the epidemiology of disease is unknown, and risks of infected animals migration is very high (4-5 balls of the risk). Till 2015 ASF drift into Ukraine as transboundary disease in particular - due to illegal imports of food and migration of external populations of wild boar. Wild boar factor was the reason of the disease distribution from Russia and Belarus to Ukraine, Baltic States, and Poland, where disease cases has been occurred in the wildlife. Now ASF is a problem of internal populations of wild boar with trend to its rooting among soft ticks on Southern Ukraine (populations were described by NSC IECVM Scientists in 2014-2015).

Conclusion: ASF represents high risks for EU member states associated with international trade, wild boars migration (4-5 balls), and moderate (2-3 balls) risk levels associated with soft ticks areal enlargement to the North. The disease control require creation of the buffer zones from Russia and Belarus using veterinary community capacities in Ukraine, Poland and Baltic States.

**Analysis of the threat of spread of African swine fever
in Ukraine’s wild boar population:
current knowledge and future CBEP-
Ukraine sponsored research**

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Listed as high priority pathogens by the World Organization for Animal Health (OIE), African swine fever virus (ASFV) and classical swine fever virus (CSFV) cause epizootically and economically topical animal diseases. Following the first reported case of an ASF outbreak in Ukraine in July 2012, The Defense Threat Reduction Agency (DTRA) initiated the project entitled “Analysis of the threat of spread of African swine fever and classical swine fever in wild boar populations in Ukraine” (TAP-3). This year-long project was conducted within the Cooperative Biological Engagement Program (CBEP) in Ukraine and concluded in May 2014. TAP-3 was focused on laboratory diagnostic of ASF and CSF using ELISA and PCR. Within the project, 685 sera and 648 tissue samples were collected from wild boars in 8 oblasts of Ukraine (Crimea, Donetsk, Zaporizhya, Luhansk, Odesa, Sumy, Kharkiv, and Kherson). All samples tested at SSRILDVSE were negative for ASF using ELISA and PCR, respectively. Although no tissue samples tested positive via PCR for CSF, 47 serum samples tested positive for CSF by ELISA, representing 6.86% of the total number of wild boars sampled.

Since 2012, the number of reported ASF outbreaks in Ukraine has increased, and as of October 2016 there have now been 123 confirmed ASF incidents registered in 17 Oblasts (households - 103; wild boar populations – 19; infected object - 1), including 61 outbreaks in 2016 (59 in the households and 2 in the wild boar population). In order to strengthen the accuracy and effectiveness of ASF/CSF diagnostics in the country, and to provide Ukrainian scientists with the ability and infrastructure to quickly and accurately monitor ASF and CSF movement within Ukraine, DTRA has continued to support the Ukrainian surveillance and diagnostic network and fund research to expand on the work initiated within TAP-3. The newest ASF project (TAP-6) commenced on 1 September 2016 and aims to analyze the distribution of ASFV and CSFV among wild boar populations inhabiting regions of Ukraine that border the Russian Federation, Belarus, and Poland, and to evaluate the risk of transmission to domestic pigs in the country. In addition to ELISA and PCR, monitoring targeted wild boar populations in Ukraine for ASF and CSF will be accomplished by using genomic-based biosurveillance methods and trainings on real-time PCR, molecular analysis, phylogenetic analysis, and complex sequence data analysis. Data collected over the next year will provide an improved scientific basis to optimize current interventions and develop new tools and strategies to reduce the risk of ASFV transmission to domestic pigs. Ultimately, this CBEP-sponsored biosurveillance effort will facilitate the development of ASF and CSF control strategies, which will ultimately contribute to limiting the spread of both infectious agents.

Considering the continuous spread of ASF in Ukraine, and the fact that Ukraine’s State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) maintains samples from more than 120 ASFV outbreaks, currently under development is an additional CBEP-sponsored research to develop a better understanding of the evolution and spread of ASFV in Ukraine and across Eastern Europe.

This project, which will be known as UP-9, will study the DNA genomes of ASFV isolates collected from domestic pigs and wild boars infected during outbreaks in the country between 2012-2016. Full-length sequences generated from these samples will be used in phylogenetic analyses that include isolates previously detected in the Russian Federation, Poland, Baltic, and the Caucasus. These analyses will provide information regarding the source and location of ASFV introduction for individual outbreaks, the rate of spread within the country, and the rate of evolutionary change. The project will engage subject matter experts from the EU and USA to help Ukrainian scientists strengthen the country's especially dangerous pathogen detection and response networks, as well as build their international scientific relationships.

**Development and implementation of public outreach,
biosurveillance, and policy development activities
for African swine fever within the cooperative biological engagement
program in Ukraine**

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In light of the significant threat posed by African Swine Fever (ASF) in the region, The Defense Threat Reduction Agency's (DTRA) Cooperative Biological Engagement Program (CBEP) conducted a year-long regional collaborative project entitled "African swine fever (ASF) Regional Public Outreach" (TAP-4) that concluded in December of 2015. The goal of this project was to develop a comprehensive and sustainable regional network between Armenia, Georgia, Kazakhstan, and Ukraine, that through a public outreach program, would raise awareness and provide education on the control and prevention of ASF outbreaks. Developed through this project was a sustainable education campaign aimed at delivering training to enhance public awareness about ASF through: (i) identifying key personnel who could effectively implement necessary methods for reducing the spread of ASF; (ii) creating and disseminating educational and training materials to key personnel; (iii) educating key personnel on how to develop and implement a public awareness campaign; (iv) educating key personnel on the best strategies for mitigating the risk of ASF; and (v) developing a regional joint ASF working group. Country representatives participated in four regional meetings where they were trained as trainers before implementing the outreach program in their respective countries. Metabiota facilitated efforts in Ukraine to identify and educate individuals that work with swine to both recognize the clinical and epidemiological patterns of ASF, and to understand 1) common sources and routes of exposure, 2) preventative measures, 3) how to recognize symptoms, and 4) how to respond to suspected ASF cases. A total of 4 trainers (two from the Institute of Veterinary Medicine (IVM) of the National Academy of Agrarian Sciences (NAAS) and two from the State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)) were trained over the course of the project and subsequently trained 14 regional trainers. Pre- and post-ASF knowledge exams were developed and improvements in trainee knowledge as a result of the training was demonstrated. Educational materials (flyers and posters) were also developed, printed and distributed to farmers, and rural populations (100,000 flyers), as well as to veterinary hospitals (1500 posters). This project demonstrated an approach for conducting and applying a public outreach program in Ukraine that can be used to raise awareness and help mitigate future outbreaks of ASF and other diseases. While the project successfully concluded, some limitations in the implementation of the outreach classes were identified. For example, due to the large size and population of Ukraine relative to its neighbors, farmers, foresters, and hunters in many regions did not benefit from the educational campaign. Therefore, there yet exists a strong need to further develop National programs to educate consumers and limit the spread of ASFV in Ukraine and across Eastern Europe.

Based on the strong linkage between the spread of ASF and poor farm biosecurity and ineffective monitoring of consumer products and trade networks, currently under development is an additional CBEP-sponsored project aimed at developing a "Regional Field-to-Table Risk Assessment of the spread of African swine fever virus (ASFV) across Ukraine" in wild fauna and via consumer trade routes. This project, which will be known as UP-10, will seek to provide insight into the development of effective ASF quarantine

strategies on farms and in wild boar populations, prevent the spread of ASFV through consumer trade routes and other human activity, and inform policy and public outreach strategies. The project will engage subject matter experts from the EU and USA in order to help Ukrainian scientists and policymakers strengthen the country's response to ASF and prevent this virus from becoming established as an endemic disease within the territory of Ukraine.

Determination of sensitivity and timeliness of surveillance strategies using a simulation model

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Keywords: sensitivity, timeliness, surveillance, simulation model, wild boar.

Wild boar play an essential role in the epidemiology of African Swine Fever (ASF). They serve as a reservoir and may thus spread the disease not only within the wild boar population but also into commercial pig holdings. It is therefore vital to monitor the disease status in wild boar populations, which is only possible through effective surveillance. Sensitivity and timeliness, i.e. the ability to detect an outbreak rapidly, as attributes of surveillance systems should thus be regularly evaluated.

In the present study, a simulation model was developed to determine sensitivity and timeliness of surveillance strategies. On the basis of hunting and infection data obtained from a real surveillance system, the two parameters were calculated for different surveillance strategies for classical swine fever (CSF) in wild boar. A risk-factor analysis was performed and in addition to the current surveillance strategy in Germany, further risk-based surveillance strategies were developed and investigated.

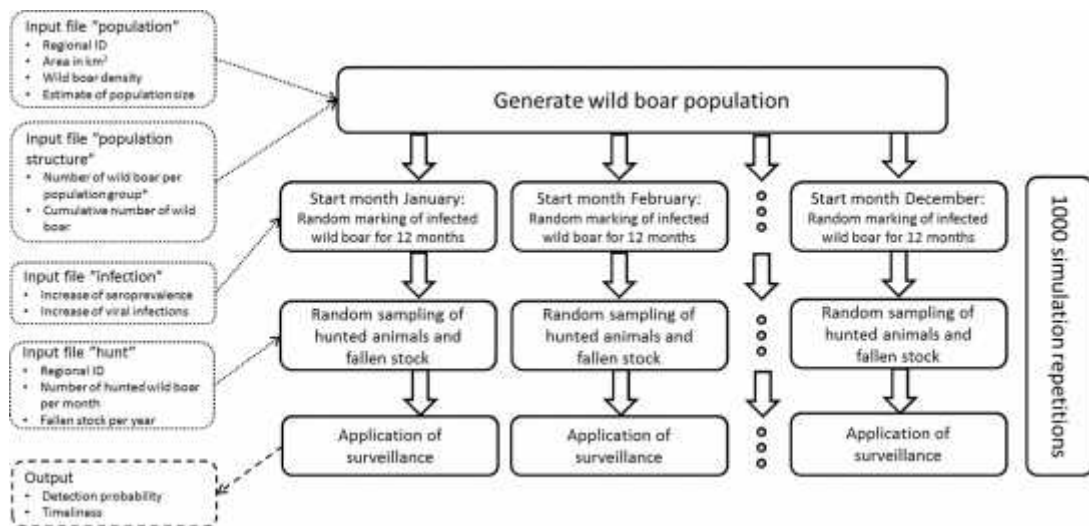


Figure 1. Structure of the simulation model to calculate sensitivity and timeliness.

The currently implemented surveillance strategy for CSF in wild boar in Germany resulted in satisfactory values for sensitivity and timeliness. Risk-based sampling yielded the best timeliness. However, sampling only animals detected through passive surveillance showed insufficient results.

The model can be readily adapted to ASF data and therefore used as a template to investigate the sensitivity and timeliness of different surveillance strategies for ASF in wild boar. Based on these results, surveillance may be improved and designed more efficiently to increase the chances for an early detection of ASF in wild boar.

Posters

Understanding the dynamics of ASF spread at the interface between wild boar and domestic swine

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Keywords: disease modelling, outbreak control, preparedness, interface.

A previously published model of foot-and-mouth disease (FMD) spread model, DTU-DADS (Boklund et al 2013; Halasa and Boklund 2014), has been adapted to African Swine fever (ASF). The ASF model, currently being adjusted to the Swedish livestock structure, models disease spread among domestic swine considering direct contacts, indirect contacts, and local spread. The model is parameterized to consider only the pig industry population, accounting for all herds in the country, and modelling contact based on animal movement data and studies investigating the movements between farms of veterinarians, service trucks and other farm visitors. Model structure and parameterization are aimed primarily at evaluating and comparing the effect of different control measures to handle a possible outbreak of ASF in the domestic population, in order to inform preparedness.

The model starts from one infected domestic farm, and makes no assumption as to how that farm got infected. While it is plausible that wild boars could be a source of infection to the first infected farm, triggering the epidemic, the model currently does not account for ASF spread in the wild boar population. That stratum of the swine population in a country is ignored based on the assumptions that: (i) ASF spread among the wild boar population would not impact the dynamic of spread within the domestic population, driven mainly through direct and indirect contact in the industry network; and (ii) the probability of a second introduction event (ASF transmission from wild boars to domestic pigs) within the duration of the first epidemic is negligible, and we can assume that a second transmission event would result in a new epidemic, rather than amplification of the first.

To support – or challenge – these two assumptions, a full characterization of the dynamics of disease spread between wild boar and domestic pigs at the interface between the populations is needed. This will be aimed specifically at informing the modelling of control strategies in the domestic population.

This characterization will be made in two steps: a literature review will be carried out to draw all the possible pathways by which wild boars could impact the dynamics of ASF introduction and spread into the domestic swine population, documenting what is known and unknown about these routes. Then these pathways will be characterized for the specific reality of Sweden, through local data collection and expert elicitation using the ASF-STOP network. A preliminary map of the pathways will be presented in this poster, which will serve as an opportunity to gather further input from experts, informing the next stages of the work.

Comparison of different sample preparation methods intended for rapid identification of African swine fever under field condition

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Keywords: field diagnosis, real-time PCR, sample preparation.

African swine fever (ASF) is devastating disease affecting pigs. It is caused by a large, complex virus, African swine fever virus (ASFV). The viral genome is a double-stranded DNA molecule ranging from 170 to 190 kb, and encodes 150 to 167 genes or open reading frames (ORFs). A gene, p72 is highly conserved among different strains or genotypes, and therefore can be used as a target for molecular identification by real-time PCR methods. Many sample types are appropriate as virus may be present in many parts of the body. ASFV is found in blood samples, which in infected animals can contain high titers of ASFV. Although suitable for diagnosis, blood does have to be prepared properly so that blood components will not inhibit the PCR assay. Commercial reagents or kits that are used in preparation of samples largely rely on equipment such as centrifuge in the laboratory settings. With the development of portable real-time PCR instruments, it is possible to perform diagnosis very close to farms of concern, as demonstrated by our previous field detection of ASFV using a real-time PCR cyclor TCOR4 and TCOR8 (Tetracore Inc. USA). However, the primary bottleneck of field diagnosis of infectious diseases, as identified during our recent field studies in Uganda, is sample preparation, particularly for blood samples. The objective of this study was to compare 3 different sample preparation methods combined with 2 real-time PCR assays on both a stationary platform Bio-Rad CFX system and a portable cyclor TCOR8, for molecular detection of ASFV p72 gene in blood samples. The results generated from this pilot study will be used to guide field work.

Study of the dynamics of wild boar's population in Albania (Sus Scrofa)

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Keywords: wild boar, population, Albania, Sus scrofa, dynamic.

Our study includes the study of wild boars (*Sus Scrofa*) population dynamics in Albania during these last 25 years. The study consists of information gathered by the Ministry of Agriculture, Albania in addition to all the methods applied in the monitoring process of this dynamic of the population. There are also several described factors related to the reduction and the increase of the density. Except this, there is a description of the variation of the dynamic as well, by applying comparative statistics methods. Our study reaches some conclusions which are considered extremely valuable for the Ministry of Environment. It helps in applying new methods regarding the estimation of wild boar's population (*Sus Scrofa*). The methods are applied in European Union places and are confidential for next studies that will have to do with monitoring of different diseases/ population estimation. This is the first study ever made in Albania for this species and it ensures a very important piece of information for both study institutes and disease monitoring ones.

The wild boars population characteristics in the Republic of Serbia

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Keywords: wild boars, hunting ground, Serbia.

In the Republic of Serbia, a certain number of wild boars is controlled and reared on enclosed hunting grounds, while a number of free-ranging population is mainly unknown. In some regions, especially near the waterways, domestic pigs are kept outdoors on the pasture, which provides favourable conditions for disease transmission. The aim of this research was to present the available data about the population characteristics of wild boars in the Republic of Serbia. According to available data, in the Republic of Serbia the population of wild boars was approximately estimated on 30.000,00 heads and the population density ranges from a minimum of 0.2/km² to over 20/km². In the country there are about 300 hunting grounds with wild boars and their surface ranges from 20 to 1000 km². There are 28 fenced hunting areas which encompass a total surface of 218km². The hunting grounds are managed by two public enterprises, four National parks in which hunting is allowed and 5 hunting grounds managed by Ministry of Defence. In the south of the country (Vojvodina Province), there is one large public enterprise with 17 hunting grounds, one National park (Fruska gora), one private hunting ground and in total 86 hunting associations. In central part of the country (central Serbia) there is one large public enterprise with 44 hunting grounds, and in total 156 hunting associations. Officially, the hunting season last for all wild boars categories from 1st July to 31st December. Generally, the population of wild boars in an enclosed hunting grounds is controlled while the number of free-ranging population is mainly unknown. One of the characteristics of outdoor swine production in some regions of Serbia is raising free-roaming domestic pigs, where they share forest habitat with wild boar population. It can be assumed that direct contacts between wild boars and domestic pigs kept in outdoor farms occur occasionally. In some regions, especially near the river banks, domestic pigs are kept outdoors on the pasture, which provides favourable conditions for infection transmission. Domestic pigs move freely in the woods, thus getting in contact with wild boars. However, after summer pasture, domestic pigs are returning into the pens. It is especially important that owners of the free-roaming animals in the same time have backyard pigs. Considering the existence of free-roaming domestic pigs located in forest habitat, the control of wild boars population is very important. The inveterate tradition to keep domestic pigs at free range and the consequent contacts with the wild boars could be in the future considered the major cause of outbreaks and disease persistence. Avoiding close contact between wild boars and domestic animals is of logical importance in disease control and eradication programmes. Having in mind this fact, the special attention should be given to active surveillance of wild boars population in the areas where close contact with domestic swine is possible. The measures should include the serological monitoring of wild boars and free-roaming domestic swine, even the prohibition of extensive grazing, pathological examination of the trunci deriving from shot wild boars. It is important to improve surveillance strategies for pathogens shared between wildlife and domestic animals and to increase disease awareness of hunters, farmers and veterinary practitioners.

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Preventive measures to minimize the risk of African swine fever spread during wild boar hunting

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Keywords: ASF, wild boar, hunting, preventive measures.

African swine fever (ASF) is a severe viral disease of domestic and wild pigs. In Europe, there are currently two main clusters of infection, one in Sardinia caused by strains of ASFV belonging to genotype I, the second in the East part of Europe caused by strains of ASFV belonging to genotype II. It appears that in Sardinia wild boar have a limited role in the spread of ASF whilst in the Baltic countries and Poland wild boar are involved in the spread of the disease, especially at the interface with the backyard's sector. Hunting is practiced in the majority of the forested areas of North East Europe and wild boar are one of the more intensively hunted ungulate species. Wild boar are susceptible to ASFV and they show similar clinical signs and mortality to domestic pigs. The dynamic of the infection in the wild boar shows the same pattern observed in domestic pigs. When wild boar die, infected carcasses, if not promptly removed, remain in the environment and they can, directly or indirectly, infect other susceptible pigs, continuing the epidemiological cycle of the disease. Wild boar can also contribute to spread the virus during the infectious period of the disease, since they eliminate the virus into the environment throughout their excretions and secretions. Considering that a large amount of ASFV is shed during the infectious period of the disease and that the virus is rather resistant into the environment, especially if protected by organic material, it can be expected that in the affected forests, the viral contamination of the environment to be rather high. Indeed, hunting wild boar implies blood contamination of the soil, transportation of dead animals to the dressing facility (when dressing is not performed directly on the field), dressing animals, offal discharge, meat dissection and its conservation. A high viral contamination of the environment increases the likelihood of virus transmission to domestic pigs.

In the framework of a control strategy for ASF, hunting is the sole practical mean to collect samples from wild boar. However, wild boar hunting can be a dangerous practice, if appropriate preventive measures are not adopted to minimize the risk of further spread of the disease. Due to the limited density dependent spread of ASFV, hunting should primarily be seen as a tool to decrease the environmental load of the virus rather than at mechanistically decrease wild boar population size.

Estimation of wild boar movement based on rabies antibody detection in Finland

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Keywords: ASF, surveillance, wild boar.

In Finland, ASF surveillance of wild boar started in 2010 and since 2014 number of samples has increased significantly. So far, ASF has never been detected in Finland.

Finnish wild boar population density is at its highest in the Southeast Finland next to Russian Federation border and there is a regular movement of wild boar across the border. In the border area oral rabies vaccination campaigns for small predators is conducted every year. We tested rabies antibodies from wild boar in order to estimate the movement of wild boar from the Southeast Finland to the interior of Finland.

Presence verification of classical swine fever in Albania

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Keywords: CSF; antigen; PK 15; confirmation.

Classical Swine Fever, also known as Hog Cholera is an infectious viral disease. The outbreak of CSF has serious consequences on the trade of pigs or their products. To realize its nearly 3 - year study, 1400 samples were collected from pigs and 16 samples wild boars, which were tested by ELISA test (Prio - Check - CSFV Ag), based on the principle of double antibody sandwich (DAS) with focus early detection of antigen and identification of disease prior appearing clinical signs. Control is primarily aimed at rural areas, but without excluding concentrated swine growth complexes. For virus isolation was performed inoculation of pathological material in the cell line PK 15 (Porcine Kidney 15), sensitive to CSF virus. Confirmation of classical swine fever in the EU Reference Laboratory, confirmed the absence of CSF antigen in suspected materials. The study goes on to perform a thorough analysis on the status of this disease in our country.

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Modelling the spread of African swine fever in Germany

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African swine fever (ASF) is caused by the African swine fever virus (ASFV) of the family *Asfarviridae*. It is characterized by hemorrhagic fever and can occur as a peracute, acute or chronic disease. The virus affects both domestic pigs and wild boar with a high case fatality rate. ASFV is very stable in blood and tissues of infected animals- including meat and meat products, but also carcasses, and in the environment. ASF has historically been endemic in African countries, where it also can be transmitted via ticks, occurs since 1978 in Sardinia and has in recent years established in the Caucasus region and in Eastern Europe. In the past, outbreaks in Europe without wild boar involvement were controlled with conventional measures such as culling and movement restrictions. However, with cases in the wild boar population, as observed in Sardinia, Poland, the Baltic countries and other affected East-European States, control and eradication of ASF prove to be difficult. No optimal strategy has been identified so far. Furthermore, some questions remain unanswered, like the importance of carcasses of infected wild boar as a virus reservoir in the environment.

The economic consequences of an introduction of ASF into a free country might be severe. As in countries already affected by the disease, trade restrictions, which have to be established and maintained, will contribute most to the financial losses.

Several studies found that Germany is at a high risk of ASF introduction. The aim of the presented project is thus to use stochastic models to simulate the potential spread of ASF in domestic pigs and in wild boars in Germany. The results of the models will improve the understanding of the epidemiology of ASF. Spatio-temporal models will employ stochastic methods to reflect the biological diversity of the disease as much as possible. Furthermore, the different mechanisms in disease spread in wildlife and livestock are taken into account.

The spread of ASF in domestic pigs is mainly determined by animal movements between farms, as well as risk contacts (persons) or introduction from wild boar. Consequently, in the livestock model, the spread of the disease is simulated via contacts and transports from an infected herd to other susceptible herds. In the wild boar population, the spread of ASF is mainly influenced by group structures and contacts between individuals. Infection probabilities in the wild boar simulation model are thus dependent on factors such as infected animals in a susceptible wild boar's sounder, but also the presence of infected animals in the vicinity.

Using the outcome of the simulations, the expected epidemiological and economic consequences for Germany in case of an ASF introduction are assessed. Furthermore, control and management options for the control of ASF will be evaluated using the models.

Workshop

Advances in vaccinology

Development of capripoxvirus vaccines and potential to transfer knowledge to the development of African swine fever vaccines

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Keywords: vaccine; capripoxvirus; vector; African swine fever.

Capripoxviruses are the cause of sheep pox, goat pox and lumpy skin disease (LSD) of cattle. These diseases continue to be important livestock diseases that pose a major threat to the livestock industry in many regions in Africa and Asia, and more recently, Europe (LSD), similar to African swine fever (ASF). Currently, several live attenuated vaccines are available and used in endemic countries to control capripoxviruses. The lumpy skin disease virus (LSDV) KS-1 vaccine provides cross-protection against both sheep pox and goat pox. However, when used in highly stressed dairy cattle to protect against LSD, the vaccine can cause clinical disease. In order to develop safer vaccines effective against all three diseases, a pathogenic strain of LSDV (Warmbaths [WB], South Africa) was attenuated by removing a putative virulence factor gene (IL-10-like) using gene knockout (KO) technology. This construct (LSDV WB005KO) was then evaluated as a vaccine for sheep, goats and cattle against virulent capripoxvirus challenge. In sheep and goats, the vaccine appeared to be safe, and did not cause disease, although it induced minor inflammation at the injection site similar to that caused by other attenuated sheep pox and goat pox vaccines. In cattle, the LSDV WB005KO construct caused severe post-vaccinal reactions at the site of inoculation. Neutralising antibodies to capripoxvirus were detected in sheep, goats and cattle, post-vaccination. Following challenge with virulent capripoxvirus, vaccinated sheep, goats and cattle were found to be completely protected and exhibited no clinical disease. These findings suggest that this novel KO strain of LSDV has potential as a vaccine to protect livestock against sheep pox and goat pox, however it requires additional attenuation to be used in cattle. The genes encoding protective antigens for *peste des petits ruminants* and Rift Valley fever have been inserted in the LSDV WB005KO construct and the resulting multivalent vaccines are currently being evaluated in sheep and goats. The similarities between capripoxviruses and ASF virus with regards to both viruses having multiple immune modulatory genes that can be removed to induce attenuation provides potential for transferring this knowledge to the development of ASF vaccines.

Global Alliance for research on African swine fever virus (GARA): Criteria for ASFV vaccine development

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The Global Alliance for research on African swine fever virus (GARA) inaugural workshop was held at Plum Island in April 2013. One working group, including researchers from the ASFV vaccine field, representatives from veterinary vaccine companies and funders. This group assessed the relative importance of different criteria for ideal vaccines and used the quantitative Kemper-Trego (KT) decision model to assess ASF vaccine candidates for which published information was currently available as of April 2013. During this vaccine breakout session, criteria and weights in the model were modified for the purpose of assessing experimental ASF vaccines. Criteria assessed included efficacy, safety, time to onset and duration of immunity, requirement for single or multiple shots, shelf life, cost to implement and capacity for mass administration. The results from this workshop will be presented. In addition a summary of the Gap analysis from this meeting and progress against these gaps at the Pretoria GARA meeting in September 2014 will be presented.

What do we have to explore in ASFV-cell interactions to develop a vaccine?

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The development of a protective vaccine against African swine fever virus (ASFV) has been a largely unmet need. First, the failure of basic classical approaches that gave immediate results in other viral infections forced a twist in vaccine research in ASFV. The lack of knowledge of key aspects of the virus replication cycle in the host cell, the porcine macrophage, has been one of the shortcomings for the development of a protective and safe vaccine. In fact, some recent advances in experimental vaccines eliciting protection were the result of increased knowledge on viral gene function, especially related to genes involved in the innate immune response against the virus. Nevertheless, there is a long way for these experimental vaccines to be used, and this necessarily comes across the development of a consistent cell line to produce the vaccine. Moreover, the viral receptor/s on the macrophage are still unknown and this would change greatly the state of the art in this disease and the research towards the development of several intervention strategies.

ASFV tropism and genes interfering host cellular pathways: actors for ASF vaccine development

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Keywords: ASFV, Virus-Host Interaction, viral restriction factors, vaccine, immune response.

We have previously shown (non published data) that attenuated ASF virus (ASFV) NHV/P68 (NHVwt), produced in porcine alveolar macrophages (PAM), fully protected vaccinated pigs against lethal challenge with homologous (Lisbon 59) and heterologous (Armenia) virulent strains. A disadvantage was the adverse reactions found in vaccinated animals, mostly related to chronic form of ASF. Trying to avoid these side effects we constructed several deletions mutants, lacking of genes involved in the control of proinflammatory response, such as COX-2 or IFN- γ . However, these recombinant viruses, which were mainly generated in COS cells, showed lower patterns of protection that the parental virus generated in PAM. ASFV is a large, enveloped, double stranded DNA virus with a marked tropism for cells of the monocyte-macrophage lineage. Although monkey cell lines such as COS-1,7 allow the infection of ASFV strains, a suitable porcine cell line able to efficiently support ASFV infection is necessary to develop models for cell-host interaction and vaccine purposes. To achieve this, four existing porcine cell lines from monocyte-macrophage origin (IPAM WT, IPAM-CD163, C 2+, WSL-gifted by FLI-) have been tested in order to set up their phenotype, ASFV infection susceptibility and viral production. Results showed that whereas the porcine cell lines analyzed were all susceptible to ASFV infection, none was as efficient as PAM in terms of levels of virus production. Current experiments are focusing on cellular factors putatively related with the ability of porcine cell lines to support an ASFV productive infection in order to establish a suitable model of study.

On the other hand, we also focused on the viral protein CD2v, encoded by ASF virulent strains, previously described by our group to interact and localize together with the molecular adaptor AP-1 during ASFV infection. The repercussion of this event on infectivity is still unknown, and we hypothesized whether other cellular factor, such as tetherin, which has been described to be related with AP-1, might be involved and possibly represent an anti-viral mechanism against ASFV. It is noteworthy that tetherin (BST2), located at the plasma membrane, is an antiviral protein induced by several types of interferon. The antiviral activity of tetherin resides mainly in its ability to restrict the release of enveloped viruses. We further hypothesize that ASFV could harbor anti-tetherin resistance during virulent strains infection through the control of AP-1 localization, perhaps based on a CD2v-dependent mechanism.

Quo vadis African Swine fever vaccine development?

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Despite intensive, decade-long research towards development of a vaccine against ASFV by a number of laboratories worldwide an efficacious - which means potent, cross-protective and safe - product is still not within reach although the whole repertoire of classical vaccine development has been applied and a multitude of approaches using e.g. naturally occurring or conventional live-attenuated viruses, engineered live-attenuated viruses, adjuvanted inactivated virus preparations, subunit vaccines, DNA-vaccines etc. have been investigated. New developments using targeted multiple gene deletion mutants and implementation of emerging technologies like comparative and functional viral genome studies, analyses of the viral and infected target cell proteomes and application of the CRISPR-Cas methodology for engineering of viral or host genomes now opens new possibilities with great promise for generation of novel ASFV control measures.

Studies on the role of ASFV E2 ubiquitin-conjugating enzyme in viral replication

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Keywords: ASFV-E2 ubiquitine conjugating enzyme; qPCR; siRNA; immunofluorescence; antiviral therapy; vaccine development.

African swine fever virus (ASFV) is the etiological agent of a highly contagious fatal acute haemorrhagic viral disease of pigs that currently has no treatment or vaccination protocol and it threatens the pig industry worldwide. This condition highlight the need for more detailed studies on the role of ASFV proteins involved in viral DNA replication and transcription. ASFV putatively encodes for an E2 ubiquitin conjugating enzyme (ORF I215L) and in this work we showed that ASFV I215L is actively transcribed since early times of infection throughout the infection course, using Vero cells infected with ASFV-Ba71V. Our siRNA studies against ASFV I215L, using this *in vitro* model, also showed that pI215L plays a critical role in viral DNA replication and gene expression, with transfected cells presenting lower viral transcripts, reduced viral yields (90%) and a reduction in the ASFV genome copies (at least 1 log) when compared to the control group. Further, a serum against the viral protein was produced to measure by western blot the pI215L levels from early times of the infection up to 24 hpi, and to assess by immunofluorescence the pI215L intracellular localization. The results obtained indicate that pI215L has an essential role during viral genome replication and transcription, suggesting that this enzyme can be used as a potential target for drug and vaccine development against ASF.

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Binding parameters of a functional histone-like protein encoded by African Swine Fever Virus

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Keywords: ASFV-A104R, Electromobility shift assay, qPCR, siRNA, immunofluorescence, vaccine development.

African swine fever virus (ASFV) causes a highly lethal disease in swine, against which there is neither effective vaccine nor treatment. ASFV is a nucleocytoplasmic double-stranded DNA virus, whose genome codes for several proteins involved in viral replication and transcription, including a putative histone-like protein (ORF A104R). In bacteria, this type of enzymes is involved in genome replication and segregation. In our study, we demonstrated that pA104R binds to oligonucleotides having different length, in an ATP-independent manner and over a wide temperature, pH, and salt ranges, with a binding site size of 16-17 nt. We showed that the positive charge of the highly conserved arginine 69 is relevant for the DNA-binding activity of pA104R, in contrast to the proline residue at position 74. Interestingly, pA104R also showed a DNA supercoiling activity when is incubated with relaxed plasmid pBR322 DNA, but only in the presence of ASFV-topoisomerase II (pP1192R).

In ASFV-infected Vero cells, pA104R shows a late expression pattern and accumulates in the host nucleus and cytoplasmic viral factories, suggesting a role of this enzyme in viral DNA metabolism. Finally, siRNA targeting the A104R transcripts reduces the transcription of late viral genes and viral progeny production. Altogether, our results demonstrate that pA104R modulates the topological state of the viral DNA, probably during different steps of ASFV cycle (e.g. DNA replication and compaction), suggesting that the generation of ASFV deletion mutants lacking ORF A104R might be used as a good strategy for the development of a DISC vaccine against ASF.

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Workshop

Researchers link European industrial partners

African swine fever diagnosis – state-of-the-art, recent advances and gap analysis

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Keywords: diagnosis, direct and indirect methods, alternative sampling, future techniques.

Rapid and reliable diagnosis is of paramount importance when it comes to early warning, timely intervention, and monitoring of transboundary animal diseases such as African swine fever (ASF). In general, reliable tools for the direct and indirect diagnosis of ASF exist. These assays include virus isolation on porcine macrophages, polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), immuno-blotting, and indirect immunostaining techniques. However, up to very recently, a majority of these test systems was based on “in-house” protocols and comparability was only guaranteed through the use in inter-laboratory comparison tests.

The current situation in Eastern Europe and methodology-oriented research projects such as RAPIDIA-Field have improved the situation and several ELISA, qPCR and also LFA kits have been brought to the market. Yet, there is room for improvement and the need for additional, fully validated but also pragmatic assays.

The optimal assay should allow detection of all ASF virus genotypes and variants, detection of infected animals with high sensitivity and specificity, should be validated according to the OIE guidelines, should be easy to handle, allow swift interpretation, should be available at reasonable costs at all times, should be adaptable to high-throughput application, and should include (at least in theory) a DIVA potential. It is clear that not all characteristics can be met at the same time and for this reason, diagnostic workflows should be established that take into account the advantages and disadvantages of the implemented assays. This may include the use of pen-side approaches but also alternative and/or non-invasive sampling strategies. The latter are of special interest for the diagnosis of ASF in the wild boar population. In this context, oral-fluid based methods, swab samples, and baiting strategies were recently investigated and showed potential.

With regard to gaps, virus isolation techniques are still lacking a robust cell line that is able to allow sufficient replication of field virus strains. Comparative trials and screening is currently implemented to overcome this problem. Moreover, antibody detection from alternative matrices could help to improve monitoring actions in remote areas and in the wild life setting.

Diagnosis has a strong link to the understanding of host-virus-interactions, pathogenesis, and host biology. In this regard, improved knowledge is dearly needed, e.g. with regard to antibody and virus kinetics in survivors but also virus persistence in the environment. Also for the latter, sensitive diagnostic tests are needed to obtain basic data.

A second link is disease epidemiology where the evolution of strains needs further investigations. In this context, harmonized genetic markers and agreement on genome regions for analyses are needed.

In conclusion, our tool box of diagnostic tests has grown considerably over the past years but there is still a need for harmonization, situation-adapted diagnostic workflows, and general knowledge of disease biology that helps us in further adjusting our methodologies.

Mitigation of health conflicts at the wildlife-livestock interface: know-how transference

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Keywords: monitoring, population control, shared infections, vaccination.

The control of diseases shared with wildlife requires two aspects: 1) the development of strategies that reduce pathogen transmission between wildlife and both domestic animals and human beings. 2) The implementation of these strategies through technology transference in order to reach the stakeholders. Diseases shared with wildlife are multi-host infections where wildlife plays a significant role, and have an impact on human health, economy, and wildlife management/conservation. Options for disease control must be cost-effective in order to be implemented in real settings. Establishing a proper surveillance and monitoring system (disease and populations) is a must before even making any decision to whether or not to intervene and how. Disease control can be achieved by different means, including: preventive actions, host population control (random or selective culling), habitat management or reproductive control, and vaccination or treatment. Ideally, tools from several fields should be combined in an integrated control strategy, and stakeholders have to be involved in the process from the beginning. SABIOTEC is aimed at transference of the abovementioned tools in order to mitigate the health conflicts at the wildlife-livestock interface. We apply innovation from research in many aspects such as improving biosecurity in cattle farms, monitoring wild ungulates populations and diseases, among other aspects. In parallel, SABIOTEC develops new suitable diagnostic tools for wildlife as well as new disease control tools, such as a new immunostimulant against TB for wild boar.

Opportunities for industrial interactions on ASFV vaccines

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Currently no commercial vaccines are available for African swine fever virus although several experimental approaches are being followed to advance vaccine development. The collaboration with industrial partners at an early stage is beneficial to ensure experimental approaches take account of important criteria for commercial development. The opportunities for industrial involvement in ASFV vaccine development include the development of live attenuated vaccines and cell lines to support replication of these, identification and delivery of subunit vaccines using different approaches, the application of adjuvants and development of DIVA (differentiation of infected and vaccinated animals) diagnostics and novel vaccine technologies. These will be summarised.

Implementation of Control and biosecurity measures to avoid African Swine Fever spread in pig premises

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Keywords: ASF, pigs, biosecurity, control measures.

African swine fever (ASF) is one of the most serious diseases of pigs that can severely affect and disrupt regional and international trade in animals and animal products with a serious socio-economic impact on pig farming system.

ASFV of genotype II, was introduced in Georgia in 2007 and since then ASF is still reported in the Russian Federation where, in certain areas, the disease became endemic representing a constant threat for the neighbouring countries. In 2013, from the Russian Federation, the virus reached Belarus and Ukraine and later, in 2014, it spread to Lithuania, Poland, Latvia and Estonia affecting mainly wild boar and backyard's pigs. Based on the characteristics of the virus and on the epidemiological findings, the introduction into the Baltic countries and into Poland was most probably from Belarus. The ASFV strain that is currently circulating in the Eastern European countries and Baltic States is a highly virulent and highly lethal strain, which has 100% sequence homology with the ASFV identified in Belarus in June 2013.

The current ASF situation in the Eastern part of Europe represents a serious threat to the EU livestock sector, particularly if the infection pressure remain high at the Eastern border of the EU.

No vaccine or drugs are available to prevent ASF infection. Therefore, it is extremely important to prevent the introduction of the disease in free areas and to reduce, as much as possible, the persistence of the virus in the infected ones. Prevention and early detection play a key role in the control strategy for ASF and enhancing early detection would also improve the efficacy of the disease control measures.

The basic elements of biosecurity derive from the knowledge of the epidemiology of the disease, the duration of pathogen excretion in infected animals, the main routes of excretion, survival of the pathogen in the environment and its routes of infection. Some basic principles of biosecurity apply to all farming systems and all diseases. However, in order to better address preventive and control measures, the main practical biosecurity measures need to be tailored to the targeted disease and to the farming systems in which they are to be implemented. Worth to mention that backyards with poor biosecurity in place are currently playing an important role in the maintenance and spread of ASF in the eastern European Countries. Indeed, in this sector of the pig production system feeding pigs with kitchen waste is common practice and the main biosecurity measures are not easy to implement, due to the minimal investment in infrastructure typical for this type of pig production system. However, there is a set of basic preventive measures applicable also in backyard holdings and, if they are properly implemented, they are effective in minimizing the risk of ASFV spread.

The final responsibility of controlling ASF belongs to the veterinary authority. However, in risk areas, pig producers have to understand the risk posed by the presence of the disease and they have to adopt all the necessary precautionary measures to protect their own herds. To achieve this, veterinary services shall provide basic information to pig holders through appropriate communication campaigns and by promoting the adoption of preventive measures.

SAFOSO role in projects on ASF and at the interface between science, policy makers and industries

Marco de Nardi

SAFOSO, Switzerland

SAFOSO (www.safoso.com) is a Switzerland-based private company offering global services in consultancy, capacity building and research in the field of animal health and food safety. Our ultimate goal is to protect public health through the improvement of animal health. We have a small, international team, which currently allows us to offer our services in 7 languages. SAFOSO offers expertise in a wide range of areas: risk assessment, monitoring and surveillance, disease control, antimicrobial resistance, animal health economics, information systems, knowledge and dissemination and evaluations. In addition SAFOSO is responsible for the secretariat of TAFS forum (<http://www.tafsforum.org/index.html>), an independent Swiss based forum that bring together scientists, industry, regulators and consumers to facilitate the understanding of controversial and emerging issues relating to the safety of food derived from animals. The focus of the presentation will be on those activities, in research and capacity building projects, that aimed at disseminate research outputs and at strengthening the interface between science and policy makers. The role of SAFOSO in TAFS will also be described.