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Original article

Vertical transmission of anti-ASFV antibodies as one of potential causes of seropositive results among young wild boar population in Poland

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Abstract

The present study attempted to elucidate possible routes leading to the achievement of seropositive results, among young (aged ≤ 1 year) wild boar population. In the years 2017-2018, the National Reference Laboratory (NRL) for African swine fever (ASF) in Poland examined nearly 27-thousand wild boar blood samples, collected during an active surveillance of ASF risk zones, for the presence of viral DNA and anti-ASFV antibodies.

Out of all the examined samples, 420 were positive. However, in more than half of them (292 samples) antibodies against African swine fever virus (ASFV) were detected, while ASFV DNA was not detected in blood. Out of all 292 seropositive/PCR-negative samples, 126 belonged to young wild boars (aged ≤ 1 year). For this reason, the NRL in Poland has examined 10 selected seropositive wild boar carcasses to confirm or exclude post-mortem lesions for ASF as well as to investigate the presence of viral DNA in the internal organs. Neither pathological lesions for ASF nor the presence of genetic material of ASFV were found in the examined wild boars. To elucidate this outcomes, following hypotheses about possible reasons of the obtained results were drawn: the presence of convalescent animals, infection of low-virulent ASFV isolate and the vertical transmission of antibodies through the colostrum.

Key words: Poland, African swine fever, ASF, immunoglobulins, antibodies, seropositive, wild boar, surveillance

Introduction

African swine fever (ASF) is one of the most dangerous disease among wild boar and domestic pigs, mainly due to enormous sanitary and socio-economic considerations (Juszkiewicz et al. 2019) caused by African swine fever virus (ASFV). The first cases of the disease in Poland were reported at the beginning of 2014 (Woźniakowski et al. 2015). Today, after five years of an escalating ASFV epizootics in Poland, nearly 25% (77,045 km²) of the country's area is covered by ASF risk areas, which are divided into three zones (General Veterinary Inspectorate 2019). It has been proven, that wild boars are the main source of ASFV and play a key role as a virus reservoir in the environment (Pejsak et al. 2018). Due to the fact that the only organisms susceptible to ASFV are animals belonging to the *Suidae* family (i.e. wild boar, domestic pigs and boar-pig hybrids), most of the ASF outbreaks in domestic pig farms may occur within an area populated by wild boars, which situation has been noted previously in several countries (Nurmoja et al. 2018, Pejsak et al. 2018).

Wild boars belong to sedentary species, which rarely change their habitation (i.e. due to shyness), females live in groups (so-called 'packs') with young boars, while male boar wander alone and join the pack only during the rutting season. The migration of wild boars contributes to spreading the ASFV, which is probably transmitted among them by direct contact. They are omnivorous, however there is no evidence for intra-species scavenging. Obviously, carcasses of infected wild boars may be considered as a source of infection, since other wild boars tend to sniff and poke them, when exploring insects and their larvae to eat (Probst et al. 2017, Podgórski et al. 2018).

From an epidemiological point of view, it seems to be extremely important to control the population of wild boars and to monitor their health. In Poland, the Government, together with the National Reference Laboratory for ASF and the Veterinary Inspection, are leading a national program for the eradication and prevention of ASF. Among many other, the procedures of the program are based on passive and active surveillance. The passive one is focused on the examination of dead animals, while active surveillance consists of the controlled culling of wild boars within ASF risk areas. During the active surveillance, the blood from culled animals is collected and examined for the presence of ASFV genetic material as well as for the presence of antibodies in serum.

Detection of the virus, based on the tested samples, is of great importance in predicting the spread of the disease and leads to intensification of biosecurity

programs, on the affected area. However, not every seropositive animal, in which antibodies were detected, will be the actual source of the virus, due to the fact that, in some of them, the virus is not detected by molecular method (PCR) in the blood or organs. In our study, we attempted to find possible routes which lead to obtaining of seropositive results during ASF active surveillance in wild boar population in Poland.

Materials and Methods

All stages of analyses, including sample preparation, DNA extraction and molecular/serological detection methods were conducted by qualified staff, under biosafety level 3 conditions (BSL-3). Post-mortem examination was conducted, according to current veterinary practice regulations.

Samples

Blood and sera from active surveillance

In total, 26,606 blood and sera samples were obtained during the controlled culling of the wild boar population, from zone II and zone III (i.e. areas invaded by ASF) during 2017-2018. All field samples were derived from local veterinary facilities (i.e. an ASFV monitoring programme) and were analysed for the presence of ASFV DNA or antibodies against ASFV, using molecular and serological methods.

Dissection samples

Ten hunted wild boars were subjected to post-mortem examination; nine young wild boars – 3 males and 6 females (less than 4-5 months of age) and one adult female boar (2 years old). Age of adult wild boar was estimated based on weight (39 kilograms), while age of young boars was estimated based on weight (9-15 kilograms) and characteristics of the coat (Fig. 1)

Blood was sent to the laboratory immediately after culling and tested for the presence of anti-ASFV antibodies and viral DNA. Subsequently, upon laboratory request, thanks to the courtesy of local veterinary authorities from the Piaseczno district, the carcasses were sent for further examination. The carcasses were examined to confirm pathological lesions, characteristic of ASF. Internal organs (i.e. the spleen, liver, kidneys, lungs, submandibular lymph nodes, tonsils and bone marrow, as well as a blood clot from the heart) were collected and examined for the presence of ASFV DNA by real-time PCR.



Fig. 1. Characteristic stripes on the coat of a young wild boar (age less than 4-5 months).

DNA detection

Internal organs were collected, about 1 g of each sample was transferred to 50 mL tubes, weighted, and a phosphate buffered saline (PBS) was added, to obtain 10% dilution (w/v) and homogenized. In the case of bone marrow, the samples were diluted additionally; 20 μ L of homogenized bone marrow was mixed with 180 μ L of PBS to avoid the influence of DNA-polymerase inhibitors. Both 10% diluted and 1% diluted sample of bone marrow were subjected to further analysis. Whole blood was directly used for extraction procedures. An aliquot of 200 μ L of each sample was used for DNA extraction, with a QIAamp DNA Mini Kit, according to the manufacturer's protocols. Real-time PCR was performed as described previously by Fernández-Pinero (Fernandez-Pinero et al. 2012).

Antibodies detection (ELISA and IPT)

Samples without anticoagulant (EDTA) were selected for serological analyses. Prior to serological examination, in order to obtain serum, blood specimens were centrifuged (1800 G, 10 min, RT). The presence of anti-ASFV antibodies was confirmed with ELISA (Ingezim PPA COMPAC, Ingenasa, Spain) performed according to the manufacturer's recommendations.

Moreover, all the positive and doubtful ELISA results were confirmed using the indirect immunoperoxidase technique (IPT), which shows higher sensitivity than ELISA.

Results

During the years 2017-2018, the National Reference Laboratory for ASF in Poland examined nearly 27-thousand wild boar blood samples under active surveillance, from zone II and zone III, for the presence of antibodies against ASFV and viral DNA. Total active prevalence was estimated at about 1.5%. Nearly 70% of positive samples, showed the presence of antibodies against the ASFV p72 protein, but simultaneously, they were negative for the ASFV genetic material. Out of all 292 seropositive and PCR-negative samples, 126 belonged to young wild boars (aged ≤ 1 year) (Table 1, Fig. 2).

When it comes to pathological findings in 10 selected hunted, seropositive wild boars no pathological lesions typical for ASF were found in the post-mortem examination, thus we excluded the clinical form of the disease, which stands in-line with no detectable virus DNA, in the examined tissues.

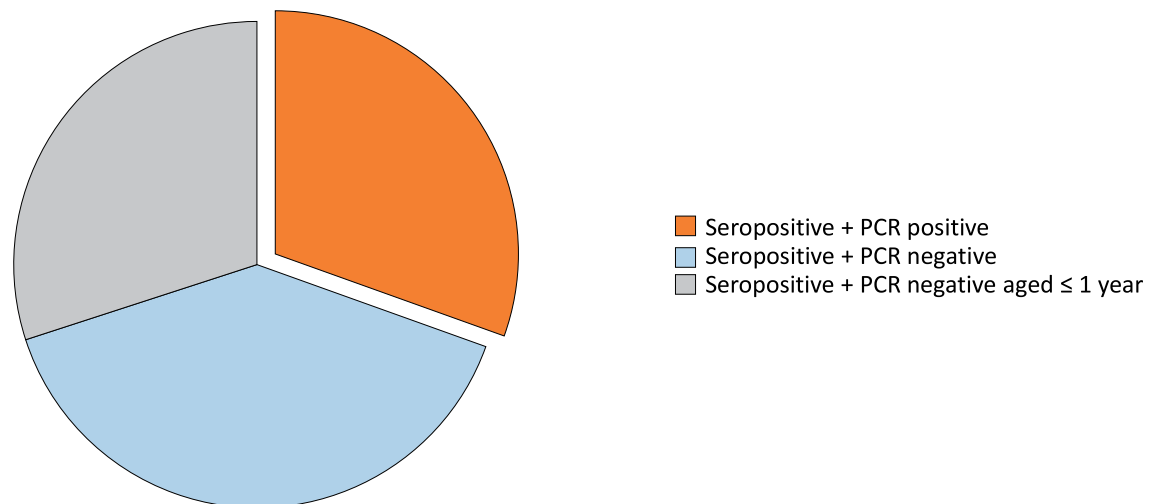


Fig. 2. Characteristics of seropositive samples collected during active surveillance in wild boars population in Poland (2017-2018).

Table 1. Serological and molecular results obtained in blood samples collected from wild boar under active surveillance (zone II and III, Poland 2017-2018).

Year	Tested	Positives (molecular and/or serological)	Prevalence	Seropositive and PCR-negative	Aged ≤ 1 year
2017	6,016	117	1.95%	95	46
2018	20,590	303	1.47%	197	80
Sum	26,606	420	1.58%	292	126

Discussion

ASFV infection may develop into peracute, acute, subacute and chronic forms of the disease. The subacute and chronic courses increase the probability for the survival of the affected animals (Sánchez-Vizcaíno et al. 2015, Sánchez-Cordón et al. 2018). Moreover in case of low-virulent isolates, causing chronic ASF, some animals may stay aviraemic, which was showed previously by Gallardo et al. (Gallardo et al. 2015). This explains the presence of adult seropositive animals with no detectable DNA of ASFV.

As regards to young wild boars, one of possible scenario may be vertical transmission of antibodies. The vertical transmission of antibodies, namely immunoglobulins G (IgG) can be explained by two pathways: immunoglobulins may be obtained through the placenta or through the colostrum (Pravieux et al. 2007). The passive transmission of IgG through the placenta is commonly known in humans and some animals, which have, respectively, a hemochorial or endotheliochorial type of the placenta. In pigs, the another one, namely epitheliochorial type of placenta is present, therefore it poses the barrier between the sow and piglet which is impermeable to immunoglobulins (Šterzl et al. 1966, Fouda et al. 2018). This fact excludes the opportunity of transferring antibodies in such a way.

However, there is still a possibility to acquire antibodies through the colostrum. The passive vaccination of suckling piglets was possible, in the case of the ASFV challenged piglets or in the case of PEDV (Schlafer et al. 1984, Park et al. 2018). IgG half-life may vary from an average of 21 days to even a few months, during the protective activity of the FcRn receptor (Chaudhury et al. 2003). Despite of vertical transmission of antibodies, an infection by low-virulent isolate of ASFV and subclinical form of the disease still cannot be excluded.

Conclusion

It is not surprising that in course of infectious diseases, even those with the highest mortality rates, survivor-animals may be observed - thus we may expect an increase in the number of seropositive results during ASF surveillance in the future. We assume young wild boars have theoretically less chances of being infected by ASFV and survive without the lesions and absence of ASFV DNA in internal organs or blood. The fact that anti-ASFV antibodies may be found in hunted, young wild boars, while viral DNA remains undetectable is still interesting and not clearly elucidated yet.

Considering the above-mentioned facts and taking into account the 292 seropositive samples without the detection of ASFV DNA, we have drawn 3 hypotheses:

1. Some of the seropositive samples were probably taken from convalescent boars.
2. Part of tested samples might be seropositive due infection of low-virulent ASFV isolate.
3. Some of the results may be a consequence of vertical transmission of antibodies, through colostrum from convalescent female boar.

African swine fever still remains one of the most important infectious disease in pigs or wild boar population. Until now, a vaccine against ASF has not been available and the only way to prevent the disease is to control and monitor the health status of susceptible animals, as well as to introduce a high level biosecurity system into domestic pig farms. Considering the results of the tested field samples, the fact that not every seropositive animal can be a source of infectious virus may be comforting in this uneven fight.

Abbreviations

ASF: African swine fever; **ASFV:** African swine fever virus; **NRL:** National Reference Laboratory; **PCR:** Polymerase chain reaction

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References

Chaudhury C, Mehnaz S, Robinson JM, Hayton WL, Pearl DK, Roopenian DC, Anderson CL (2003) The Major Histocompatibility Complex-related Fc Receptor for IgG (FcRn) Binds Albumin and Prolongs Its Lifespan. *J Exp Med* 197: 315-322.

- Fernández-Pinero J, Gallardo C, Elizalde M, Robles A, Gómez C, Bishop R, Heath L, Couacy-Hymann E, Fasina FO, Pelayo V, Soler A, Arias M (2012) Molecular Diagnosis of African Swine Fever by a New Real-Time PCR Using Universal Probe Library. *Transbound Emerg Dis* 60: 48-58.
- Fouda GG, Martinez DR, Swamy GK, Permar SR (2018) The Impact of IgG Transplacental Transfer on Early Life Immunity. *Immunohorizons* 2: 14-25.
- Gallardo C, Soler A, Nieto R, Sánchez MA, Martins C, Pelayo V, Carrascosa A, Revilla Y, Simón A, Briones V, Sánchez-Vizcaino JM, Arias M (2015) Experimental Transmission of African Swine Fever (ASF) Low Virulent Isolate NH/P68 by Surviving Pigs. *Transbound Emerg Dis* 60: 612-622.
- Juszkiewicz M, Walczak M, Woźniakowski G (2019) Characteristics of selected active substances used in disinfectants and their virucidal activity against ASFV. *J Vet Res* 63: 17-25.
- Nurmoja I, Mõtus K, Kristian M, Niine T, Schulz K, Depner K, Viltrop A (2018) Epidemiological analysis of the 2015-2017 African swine fever outbreaks in Estonia. *Prev Vet Med pii: S0167-5877(18)30361-1*.
- Park JE, Kang KJ, Ryu JH, Park JY, Jang H, Sung DJ, Shin HJ (2018) Porcine epidemic diarrhea vaccine evaluation using a newly isolated strain from Korea. *Vet Microbiol* 221: 19-26.
- Pejsak Z, Niemczuk K, Frant M, Mazur M, Pomorska-Mól M, Ziętek-Barszcz A, Bocian Ł, Łyjak M, Borowska D, Woźniakowski G (2018) Four years of African swine fever in Poland. New insights into epidemiology and prognosis of future disease spread. *Pol J Vet Sci* 21: 835-841.
- Pejsak Z, Truszczyński M, Tarasiuk K (2018) African swine fever (ASF) in wild boar. *Med Weter* 74: 743-746.
- Podgórski T, Śmietanka K (2018) Do wild boar movements drive the spread of African Swine Fever? *Transbound Emerg Dis* 65: 1588-1596.
- Pravieux JJ, Poulet H, Charreyre C, Juillard V (2007) Protection of Newborn Animals through Maternal Immunization. *J Comp Pathol*, 137 (Suppl 1): S32-4.
- Probst C, Globig A, Knoll B, Conraths FJ, Depner K. (2017) Behaviour of free ranging wild boar towards their dead fellows: potential implications for the transmission of African swine fever *R Soc Open Sci* 4: 170054.
- Sánchez-Cordón PJ, Montoya M, Reis AL, Dixon LK (2018) African swine fever: A re-emerging viral disease threatening the global pig industry. *Vet J* 233: 41-48.
- Sánchez-Vizcaino JM, Mur L, Gomez-Villamandos JC, Carrasco L (2015). An update on the epidemiology and pathology of African swine fever. *J Comp Pathol* 152: 9-21.
- Schlafer DH, McVicar JW, Mebus CA (1984) African swine fever convalescent sows: subsequent pregnancy and the effect of colostrum antibody on challenge inoculation of their pigs. *Am J Vet Res* 45: 1361-1366.
- Šterzl J, Rejnek J, Trávníček J (1966). Impermeability of pig placenta for antibodies. *Folia Microbiol (Praha)*, 11: 7-10.
- Woźniakowski G, Kozak E, Kowalczyk A, Łyjak M, Pomorska-Mól M, Niemczuk K, Pejsak Z (2015) Current status of African swine fever virus in a population of wild boar in eastern Poland (2014-2015). *Arch Virol* 161: 189-195.