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Epidemiological analysis of the two first cases of African swine fever in wild boar in Poland

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Summary

On February 14 and 17, 2014, two cases of African swine fever (ASF) were diagnosed in Poland in dead wild boar, near the border with Belarus, about 900 meters and 2000 meters from the border, respectively. The carcasses were located about 15 km from each other. These cases of ASF were discovered as the result of active surveillance and monitoring along the Eastern border of Poland in the population of wild boar since 2011, in connection with the suspicion of ASF near Kaliningrad (Russia), and later the detection of ASF in Ukraine, Belarus and Lithuania. During the mentioned action in Poland 16 771 wild boar and 2819 domestic swine were investigated using laboratory tests. All results obtained till February 14, 2014, were negative.

Both mentioned positive cases of ASF were diagnosed using RT-PCR and one additionally using ELISA. In the first case bone marrow was investigated and genetic material of ASFV was identified. In the second case, spleen, kidney and lung were used with the same result. Using ELISA in the serum low level of antibodies specific for ASFV was demonstrated. During 58 days since the ASF diagnosis of the second wild boar, despite of intensive surveillance, monitoring and laboratory investigations, no increase of normally occurring number of diseased pigs or dead wild boar were found.

During 5 days, after diagnosis of the two cases of ASF in wild boar specimens of blood taken from 623 domestic swine, from 118 farms located in the "infective zone", close to the Eastern border of Poland, were investigated, using PCR and ELISA. All of them were negative.

Simultaneously, decision was taken about active search for dead wild boar in the region with the radius of about 40 km, where the positive two wild boar were found. Specimens from 1033 wild boar were taken and investigated, using RT-PCR and ELISA. From the same region 2868 serum samples from domestic swine were also taken and tested with the mentioned methods. All the investigated samples were negative.

Taking into account all the obtained results after finding of the second case of ASF in the wild boar, one can state that the two cases of ASF were not the source for additional cases or outbreaks of ASF in Poland.

On the basis of the performed epidemiological analysis of all data and the evaluation of the epidemiological situation in the region, as the most possible source of ASFV for the two wild boar found in Poland should be taken the population of wild boar in Belarus.

Keywords: African swine fever, wild boar, two cases, Poland epidemiological analysis

African swine fever (ASF) is an extremely dangerous, incurable, infectious and highly contagious viral disease of domestic pigs of all breeds and wild boar. The probable reservoirs of the virus are African bush pigs, warthogs, and ticks of the *Ornithodoros* genus (species: *Ornithodoros erraticus* and *Ornithodoros moubata*). The last mentioned species act as vectors in the transmission of the ASF virus (ASFV). Other animal species are not susceptible to ASFV infection.

The disease is characterized by clinical signs and autopsy lesions that resemble those of the acute form of classical swine fever – some of the most important are high fever, considerably enlarged spleen, extensive hemorrhages, and mortality of up to 100%. The symptoms and autopsy lesions can also be less noticeable. Sporadically, especially in countries endemic with ASF, cases of symptomless carriership of ASFV are also reported (8).

Since the treatment of infected animals is prohibited and there are no vaccines against ASF, the disease is controlled exclusively by administrative methods, that is, by slaughtering and destroying all infected herds, as well as herds located in the vicinity of disease foci. For this reason, the occurrence of ASF causes extremely serious economic losses related to massive deaths of animals, slaughter and eradication costs, indemnity payments, and, above all, the suspension of the sale and export of pigs and food produced from uncooked pork.

The first case of ASF was diagnosed and described by Montgomery in Kenya in 1921 (7). In Europe, the disease appeared for the first time in 1957, after it had been brought into Portugal from Angola (3). The ASF virus spread from Portugal to Spain and then to other European countries, which was systematically reported by the World Organization for Animal Health (OIE). According to annual OIE reports, the disease appeared in France in 1964, in Italy in 1967, in the USSR in 1977, in Malta in 1978, in Belgium in 1985, and in the Netherlands in 1986. In the Iberian Peninsula, ASF remained endemic until 1995 in Spain and until 1999 in Portugal. (8).

In the 1970s and 1980s, the acute form of the disease also occurred in the Caribbean (the Dominican Republic, Haiti, and Cuba) and in South America (Brazil) (8).

According to the OIE, from the eradication of ASF in the Iberian Peninsula to June 2007, ASF remained restricted to the countries of sub-Saharan Africa and, in Europe, to Sardinia.

Since June 2007, the epidemiological situation across the eastern border has posed a serious threat of ASF for Poland.

On June 6, 2007, the OIE published the first report of the appearance of the disease in Georgia. The first cases were recorded there on April 22, 2007. By the end of 2007, foci of ASF were discovered in Abkhazia, Armenia, Chechnya, South Ossetia, and Nagorno-Karabakh. In 2008, according to the OIE, the disease continued to spread in the Caucasus. Its appearance was confirmed in Azerbaijan, North Ossetia-Alania, in the Orenburg Oblast on the Ural river, and in Ingushetia. Thus, within one year of its first appearance, the ASF virus spread over distances of up to 1200 km, which was confirmed by laboratory tests. In 2008, there were already 65 foci of ASF in Russia. From 2009 to 2013, the epidemiological situation with regard to ASF showed no improvement. According to official data, a total of 598 foci of the disease have been confirmed in the Russian Federation since the beginning of the epidemic. In 2013, two foci of ASF were detected in Belarus. One ASF focus and one case of the disease were reported in Ukraine, respectively, in 2012 and 2013. In January 2014, two cases of ASF were also recorded in Lithuania (6, 9).

Because of this precarious situation behind the eastern border of Poland, Polish veterinary authorities took a series of measures to monitor the epidemiological situation in the country with regard to the occurrence of ASF.

The aim of this paper is to present the actions of veterinary services that led to the detection of the first cases of ASF in wild boar in Poland, as well as the measures that have been taken to prevent the spreading of ASF in the country.

Material and methods

On February 7, 2011, the appearance of ASF was suspected near Kaliningrad in Russia. Since the Kaliningrad Oblast borders on Poland for 212 km, the Chief Veterinary Officer (CVO) of Poland called an alert for veterinary services in five counties of the Warmińsko-Mazurskie Voivodeship. A total of 332 samples collected in this region were examined for the presence of the ASF virus (ASFV) at the National Reference Laboratory for ASF at the National Veterinary Research Institute (Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy) in Puławy, and all of them proved negative (4).

Subsequently, in view of ASF presence in various areas of the Russian Federation and the well-founded suspicion of ASF in Belarus, the CVO of Poland also called an alert for the 40-km wide strip of land along the eastern border of the country, in the Podlaskie, Warmińsko-Mazurskie, Lubelskie, Podkarpackie, and Mazowieckie voivodeships. As part of the alert procedures, samples of blood, lymph nodes, tonsils, kidneys, spleen, lungs, and bone marrow from 19590 animals, including 2819 domestic pigs and 16771 wild boar, were collected and examined at the National Reference Laboratory for ASF from 2011 to February 14, 2014 (Table 1).

Tab. 1. The number of animals examined for ASF at the National Veterinary Research Institute in Pulawy

Number of animals examined			
2011 – 17 Feb. 2014		18 Feb. – 16 Apr. 2014	
pigs	wild boar	pigs	wild boar
2819	16 771	1033	2868
Total: 23 491*			

Explanation: *including the examination of 1 sample from a pig-boar crossbreed

From the diagnosis of the first case of ASF in the dead boar (on February 14, 2014) to April 16, 2014, a total of 1033 samples from domestic pigs and 2868 samples from wild boar (including 265 from dead boar) were examined.

The identification of the genetic material of ASFV was performed by real-time PCR (RT-PCR) (2), accredited according to the criteria of the European Union (EU). ASFV-specific antibodies were detected with a commercial ELISA kit (by Inmunologia y Genetica Aplicada, S.A., Spain). Doubtful and/or positive ELISA results were additionally verified by immunoblotting and/or immunoperoxidase tests (5). All of the tests were performed in accordance with the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (10) at the National Reference Laboratory for ASF in Puławy.

Results and discussion

Until February 14, 2014, the results of all laboratory tests on the samples from 2819 pigs and 16 771 boar (Table 1) were negative. On that day, a positive result, indicating the presence of ASFV DNA, was obtained by RT-PCR in examining bone marrow collected from a front leg of a wild boar that had been found dead. Thus the laboratory detected the first case of ASF in Poland, about 900 m from the border with Belarus.

On February 17, 2014, the National Reference Laboratory for ASF in Puławy obtained another positive result for ASFV, confirming the second case of ASF in Poland. In this case, samples of a blood clot, spleen, kidney, and lungs were examined. The genetic material of ASFV was detected by RT-PCR, and an ELISA test was used for detection of ASFV-specific antibodies in the serum obtained from the blood clot. The RT-PCR test revealed a large amount of the genetic material in the tissues examined, whereas the ELISA test showed a low level of ASFV-specific antibodies.

Positive samples were transferred to the European Union Reference Laboratory for ASF in Valdeolmos in Spain on February 18, 2014. Using PCR, the laboratory confirmed the results obtained at the National Reference Laboratory for ASF in Puławy. ASFV was isolated from a spleen sample collected from the second dead boar in the third passage on March 25, 2014. Molecular examination identified the isolated ASFV as genotype II. According to the OIE, the same genotype was detected in January 2014 in Lithuania and in 2013 in Belarus.

The epidemiological investigation established that the frozen carcass of the first dead boar infected with ASFV was found near the village of Grzybowszczyzna (in Szudziałowo Commune, Sokółka County, Podlaskie Voivodeship), about 900 m from the border with Belarus on February 3, 2014, by a farmer, who kept 6 pigs of different ages on his farm. The farmer reported the fact to the Veterinary Inspectorate, which collected samples and, on February 13, 2014, submitted them for examination to the National Reference Laboratory for ASF in Puławy. The boar was a male weighing about 50 kg.

The second dead boar was found in the forest area between the villages of Kruszyniany and Ozierany Wielkie in the Sokółka County, Podlaskie Voivodeship, on February 15, 2014, about 2 km from the border with Belarus. It was a female weighing about 100 kg.

The two carcasses were found about 15 km apart.

The CVO was informed of the positive results obtained for the presence of the genetic material of ASFV and ASFV-specific antibodies, on February 15 and 18, 2014, respectively.

In the course of epidemiological investigations following the first two cases of ASF, measures were taken to evaluate the epidemiological impact of the presence of ASFV in the above-mentioned areas, beginning with the examination of pigs on the farm belonging to the farmer who informed about the first dead boar and on all other pig-breeding farms within a radius of 10 km.

Within five days of diagnosing the two cases of ASF, blood samples collected from 623 pigs from 118 farms in 57 localities were examined. All of the samples were negative.

At the same time, a search for dead wild boar was conducted within a radius of about 40 km of the first two ASF cases. Moreover, all wild boar shot within 40 km of the Polish border with Belarus and Lithuania were subjected to laboratory tests.

In total, from February 18 to April 16, 2014, (58 days following the confirmation of the second case of ASF) 1033 samples from domestic pigs and 2868 samples from wild boar were examined – all of which proved negative.

All of the test results obtained before and after the diagnosis of ASF in the two dead boar found near the border with Belarus were negative. These results, combined with the fact that there was no increase in the mortality of wild boar or in the morbidity and mortality of domestic pigs on farms located within a 40-km radius of the ASF cases and along the border with Belarus and Lithuania, suggest that the diagnosed ASF cases did not transmit infection in other boar or pigs in Poland. This is surprising, since wild boar generally live in large groups, including 15 to 30 animals.

The fact that the two boar infected with ASFV, which must have been disseminating the virus, fed together with other boar, susceptible to infection, should have caused the disease and death in a larger number of animals, especially as the presence of antibodies specific for ASFV was confirmed in the second boar. This means that the animal lived for at least 6-8 days after infection and disseminated ASFV for a few days. It should be recalled that animals infected with ASFV begin to disseminate the virus about 2-3 days after infection, before the appearance of specific antibodies (8). Affected animals usually die before the antibodies have developed. However, a certain percentage of pigs and boar live long enough after infection for the antibodies to be detectable. The above data refer to the second dead boar (8).

Despite the intensive search for dead animals and the examination of a large number of samples, not a single positive result was obtained, nor was there any increase in the morbidity or mortality of domestic pigs and boar in the potentially infected area during the 58 days following the confirmation of the second case of ASF (from February 18 to April 16, 2014). The above facts support the conclusion that the two infected boar did not spread ASFV to other boar or domestic pigs. Poland, therefore, appears to be currently free of ASF.

With regard to the source of ASFV, the combined results of serological, virological and molecular examinations before and after the detection of the two

ASF cases in Poland suggest that ASFV probably originated from behind the eastern border of the country. Considering the location of the Polish cases of ASF, the source of the disease was most probably the boar population in Belarus. According to official OIE data, the closest foci of ASF in Belarus were located near Grodno, 170 km from the Polish border, and near Vitebsk, about 450 km from the Polish border. In late January 2014, ASF was also diagnosed in two wild boar in Lithuania, in the Alytus County close to the Neman river, and the two cases were 12 and 70 km distant from the Polish border. Thus this region, as well, could theoretically have been the source of the ASF cases detected in Poland. However, according to what is known about the habits of wild boar, even under favorable conditions, these animals do not usually migrate further than 20-30 km, which means that the above-mentioned foci and cases of ASF in Belarus and Lithuania are not likely to have been the sources of ASFV detected in the two boar found in Poland near the border with Belarus. In view of the above facts, the only reasonable explanation of the two ASF cases in Poland is the hypothesis that the two boar came from Belarus, although it is impossible to specify the exact place of origin, time, or cause of their transfer to Poland.

The situation in Belarus both in the population of domestic pigs and, even more so, in the population of wild boar, is not entirely clear. Although there are no official data on the prevalence of ASF in the Belarusian population of wild boar, the fact that boar are intensively shot throughout the country, may indicate that the official data do not reflect reality. It is worth adding that according to unofficial information, by March 2014, the population of wild boar in Belarus was almost completely eliminated.

With regard to the shooting of wild boar in Belarus, a publication by the EFSA (1) suggests that their mass extermination within a relatively short time is highly inadvisable, as it actually favors the spreading of ASF. This policy results in the migration, or flight, of wild boar, including the carriers of ASFV, to the areas where they are not shot on such an extensive scale.

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