

DOI 10.2478/pjvs-2014-0097

Original article

Epidemiology of African Swine Fever in Poland since the detection of the first case

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Abstract

The purpose of this paper is to provide characteristics of the spread of African Swine Fever (ASF) in Poland from February to August, 2014. The samples from dead wild boar and domestic pigs were submitted to the National Veterinary Research Institute, National Reference Laboratory for ASF in Pulawy, Poland, for testing by PCR and ELISA methods. In the studied period, fourteen cases of ASF in wild boar and two outbreaks in backyard pigs were confirmed. In addition to the results of laboratory tests performed in 2014, the article describes the ASF surveillance programme in wild boar and pigs in Poland carried out in 2011-2013. The spread of ASF in Poland is compared with the epidemiological situation in Lithuania, Latvia, Belarus and the Russian Federation.

Key words: African swine fever, Poland, epidemiology

Introduction

The pig production in Poland has never been so threatened, as it is today (beginning with February, 2014), as a result of the emergence of African Swine Fever (ASF), the most dangerous disease in wild boar and pigs. The description of the disease and characterization of the first cases in wild boar in Poland has been presented elsewhere (Markowska-Daniel 2010, OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012, Markowska-Daniel and Kozak 2013, Truszczyński and Pejsak 2014, Pejsak et al. 2014, Truszczyński and Pejsak 2014).

The aim of this study is to present epidemiological data related to the occurrence of ASF in Poland and to provide characteristics of the disease and its spread since the first confirmed detection (14th of February, 2014) up to the end of August, 2014.

Based on the World Organization for Animal Health (OIE) report as of 6 June 2007, the current ASF pandemic started in Poti – a port city in Georgia, located on the Black Sea coast, from which it spread across Georgia. The first ASF symptoms in this country were observed in April 2007, but the confirmatory diagnosis was completed in June 2007, following laboratory tests performed at the Institute for Animal Health, Pirbright, UK, the reference laboratory for ASF. The disease subsequently spread to Armenia, the Russian Federation, Belarus, Ukraine, Lithuania, Poland, Estonia and Latvia, and in the latter country a significant dynamics of spread has been observed since July 2014 (Markowska-Daniel 2008, Markowska-Daniel 2010, Truszczyński and Pejsak 2014).

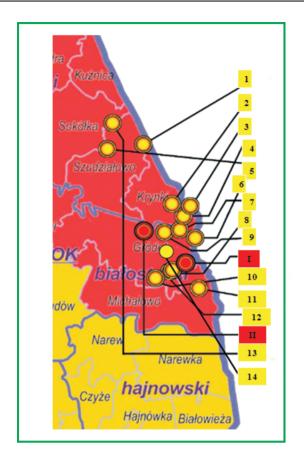




Fig. 1. ASF in Poland: 14 cases in wild boar (yellow) and 2 outbreaks in pigs (red).

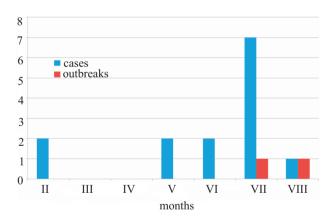


Fig. 2. The number of cases and outbreaks of ASF in Poland in 2014.

Materials and Methods

The following samples collected from wild boar and pigs were used for the detection of genetic material of ASF virus (ASFV): blood, bone marrow, lymph nodes, kidneys, lungs and spleens. For the detection of specific antibodies to ASFV, sera from dead or shot wild boar or live pigs were used. These samples, as well as detailed data collected during epidemiological investigations, were obtained from the county veterinary inspectors. General principles on

the collection and transport of samples for ASF diagnosis performed at the National Reference Laboratory (NRL) for ASF at the National Veterinary Research Institute (NVRI) in Pulawy are available at www.piwet.pulawy.pl.

The NRL uses a variety of diagnostic techniques to detect specific antibodies, as well as DNA of ASFV. These methods are used depending on the type of sample. The real-time polymerase chain reaction (real time PCR) developed by Fernandez-Pineiro (2012) is the most frequently used method to detect DNA of ASFV. For serological diagnosis, ELISA and immunoperoxidase test (IPT) are used while the latter assay is used as a confirmatory test (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2012, Markowska-Daniel and Kozak 2013).

Results

In the period from February 14 to August 31, 2014, 14 cases of ASF in wild boar and 2 outbreaks in pigs were diagnosed. All cases and outbreaks occurred in the Podlaskie voivodship, in the Białystok and Sokółka county (Fig. 1), indicating a slow spreading dynamics. The ASF cases were detected exclusively in dead wild boar, found near (up to maximum 10 km) the border with Belarus.

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2011-2013 2011 2012 2013 wild boar wild boar wild boar wild boar pigs pigs pigs pigs 244 1282 11 693 13 063 Number of animals 88 208 1672 2124 15 187 Total:

Table 1. Number of samples from pigs and wild boars tested for the presence of ASF in 2011-2013.

Table 2. Number of samples and results of diagnostic tests carried out in 2014 (between January 1 - August 31).

	Species					
N. d. 1	pigs			wild boar		
Method	1 6 1	result		1 6 1	result	
	number of samples -	+	-	number of samples	+	_
Molecular (PCR)	13 221	6	13 215	8609	32	8577
Serological (ELISA)	1126	0	1126	2807	4	2803
Total: number of tests	14 347	6	14 341	11 416	36	11 380
Total: number of tested animals	13 229	6	13 223	8609	32	8577

The analysis has shown that the highest number of ASF cases/outbreaks occurred in July, 2014 (Fig. 2).

The details of the laboratory test results for ASF performed at the NRL, NVRI Pulawy, in 2011-2013 (the period preceding the emergence of ASF in Poland) and 2014 are shown in Table 1 and Table 2, respectively.

Positive results were only found in samples collected in 2014, while no ASFV DNA was detected in 2011-2013. In 2014 (as of August 31), 6 pigs and 32 wild boar tested positive for the presence of DNA of the ASFV. The presence of antibodies was detected in sera collected from 4 wild boar. It is worth noting that Poland is an example of a country in which the number of samples tested virologically and serologically in the framework of the "Programme of the Chief Veterinary Officer aiming at early detection of ASFV infections and broadening the knowledge about risk associated with the emergence of ASF on the territory of the Republic of Poland" was the highest in Europe.

The timeline of events related to ASF cases in wild boar and outbreaks in pigs during the period from February 14 to August 31, 2014, is presented below.

The 1st case was confirmed at the NRL on February 14, 2014, following detection of DNA of ASFV in samples from a wild boar found dead near the village of Grzybowszczyzna, Szudziałowo municipality, Podlaskie voivodship, approximately 900 meters from the border with Belarus.

The second case was detected on February 17, 2014, in specimens from a dead wild boar found on February 15, near the village of Kruszyniany (Krynki municipality, Sokółka county), close to the border

with Belarus, about 15 km from the location of the previous case.

On May 21, wild boar carcass (a 3-year-old sow) were found in the Świsłocz river passing along the Polish-Belarusian border, near the village of Rudaki, in the Krynki municipality, Sokółka county, Podlaskie voivodship, approximately 4.5 km from the site in which the second infected boar had been found. The results of diagnostic tests confirmed that the wild boar was infected with ASFV and therefore this event constituted the 3rd case of the disease.

The 4th case of ASF was confirmed after detection of ASFV in the carcasses of three wild boar (approximately 3 to 4-year-old sow and two piglets) found on May 29, 2014, near the village of Łosiniany, Krynki municipality, Sokółka county, Podlaskie voivodship, about 3 km from the border with Belarus and from the location of the 3rd case. Samples for laboratory investigations were dispatched on May 29, and the presence of genetic material of ASFV was confirmed on the same day.

The next dead wild boar that was diagnosed positive for ASFV (5th case) was found on June 24 in the village of Słoja, Szudziałowo municipality, Sokółka county.

The 6th case involved 4 dead wild boar found between 24-30 of June, on the riverside of the Świsłocz river, in the vicinity of the village of Bobrowniki (Gródek municipality, Białystok county). The diagnostic tests revealed the presence of viral DNA and in the case of one animal – a low level of antibodies specific for ASFV.

The carcasses of 6 wild boar that made up the 7th case of ASF were found on July 4, 2014, in the forest,

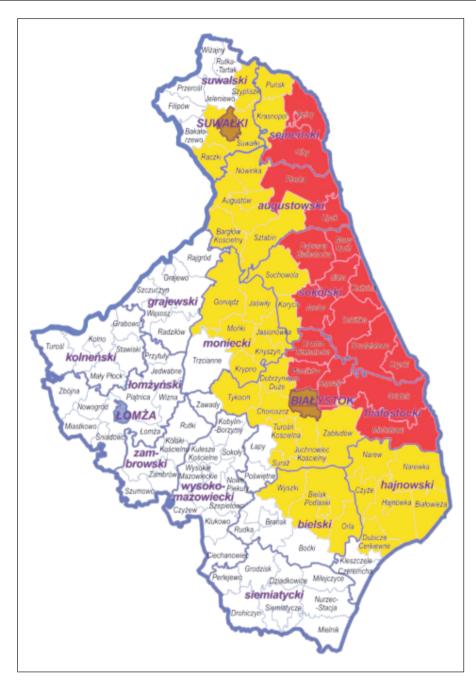


Fig. 3. Restriction (red colour) and protection (yellow and maroon colour) areas.

Table 3. Pig population in the restriction and protection areas.

	Number of pigs	Number of herds	
Podlaskie voivodship	515 468	11 831	
Restriction area	44 019	2 204	
Protection area	170 971	3 271	
Total	214 990	5 475	

in the village of Łużany (Gródek municipality, Białystok county), approximately 4 km from the border with Belarus. The virus was detected in all tested animals. The 8th case of ASF was found on July 8, 2014, near

the village of Wiejki in the Gródek municipality, about 6 km from the border with Belarus.

The 9th case involved a group of 5 dead wild boar found on July 15, 2014, in the forest, about 1.5 km



from the village of Skroblaki (Gródek municipality) and approximately 3.5 km from the location of the 7th case and 7.5 km from the Poland/Belarus border.

The next wild boar carcasses positive for ASFV (10th case) were found on July 29, 2014, in the village of Wiejki (Gródek municipality), in the forest, approximately 10 km from the border with Belarus.

The 11th case of ASF was discovered on July, 29, 2014, in the forest near the village of Jałówka, Michałowo municipality, Białystok county, about 3 km from the border.

Three dead wild boar that made up the 12th case of ASF were found between July 30 and August 11, 2014, in the neighborhood of the village of Wiejki (Gródek municipality), 7 km from the border with Belarus.

On August 9, 2014, the 13th case of ASF was identified and positive PCR results were obtained in samples collected from 3 dead wild boar found between August 8 and 21, 2014, in the village of Horczaki Górne, Szudziałowo municipality, 5 km from Poland/Belarus border.

The 14th case of ASF was diagnosed on August 26, 2014 in a wild boar found dead in the village of Kolonia Mostowlany (Michałowo municipality) approximately 3.5 km from the border with Belarus.

Overall, positive results were obtained in the case of 32 animals of different age, found alone or in small groups (maximum 6 individuals). The longest distance between a positive ASF case and the border with Belarus was 10 km. Following sample collection, all carcasses were disposed under a supervision of veterinary authorities. All holdings within the radius of 3 km and 7 km were visited by a state veterinarian in order to perform a census of all pigs, clinical examination and epidemiological inquiry.

The first outbreak of ASF in domestic pigs was diagnosed on July 22, 2014, in a backyard holding of 8 pigs located in the village of Zielona, Gródek municipality, Białystok county, 2.5 km from the border with Belarus. The epidemiological inquiry revealed that on July 17, three pigs had died and had been buried by the owner which suggested that the virus had been introduced to the premise at least a few days earlier. As more pigs became sick on July 19, the owner informed state veterinary inspection. Samples were collected by the inspectors from 2 dead and 3 sick pigs and laboratory tests performed at the NRL gave positive results for ASFV. After receiving the official report, 3 pigs remaining on the holding were immediately killed, samples were collected (PCR results for ASF were positive) and carcasses were destroyed under official supervision. Control measures foreseen in the law concerning the occurrence of infectious diseases of animals, including cleaning and disinfection, were also implemented.

The second outbreak of ASF in domestic pigs was identified on August 6, 2014, in a holding with only 1 pig in the village of Józefowo, Gródek municipality, approximately 13 km North from the location of the 1st outbreak. The ASF-affected holdings were located close to the forest with very poor biosecurity. The laboratory results obtained by the NRL in Pulawy were confirmed by the EU Reference Laboratory for ASF in Valdeolmos, Spain.

Following confirmation of ASF outbreaks, control measures laid down in the Council Directive 2002/60/EC (2002) and in the Regulations of the Minister of Agriculture and Rural Development (2014) were implemented (stamping out, carcass disposal, cleaning, disinfection, destruction of feedingstuff, bedding and manure, establishment of protection and surveillance zones with a radius of 3 i 10 km, respectively). Inspection of all holdings within a radius of 10 km from each ASF-positive case was carried out. Pigs kept in farms located inside the zones were killed and carcasses were disposed. Altogether, 346 pigs from 92 holdings were killed.

After detection of cases and outbreaks of ASF, two areas had been established in accordance with the Commission Implementing Decision 2014/178/EU (2014). The first "restriction area", adjacent to the border with Belarus and encompassing the counties of Sokółka and Białystok, and "protection area", next to the restriction area (Fig. 3).

There are 44 019 pigs in 2204 herds and 170 971 pigs in 3271 herds in the restriction and protection area, respectively (Table 3).

Discussion

The ASFV strains isolated from cases and outbreaks in Poland belonged to the same genotype as strains recovered from the biological material derived from wild boar in Belarus and Lithuania (Gallardo at al. 2014). The analysis of nucleotide sequences in ASFV strains detected in Poland revealed the presence of an insertion of 10 nucletotides identical to that found in an ASFV strain identified in Belarus in 2013. However, the insertion was not found in the Russian strains (Gallardo et al. 2014). This finding indicates that the virus was introduced to Poland from the territory of Belarus. Based on the aforementioned results one can conclude that there is a possibility of an occurrence of genetic variation among ASFV strains currently circulating in Europe and Russia.

According to the latest observations in the context of further spread of ASF, the major threat is associated with the growing population of wild boar in the eastern part of the country (Fig. 4), in which the virus

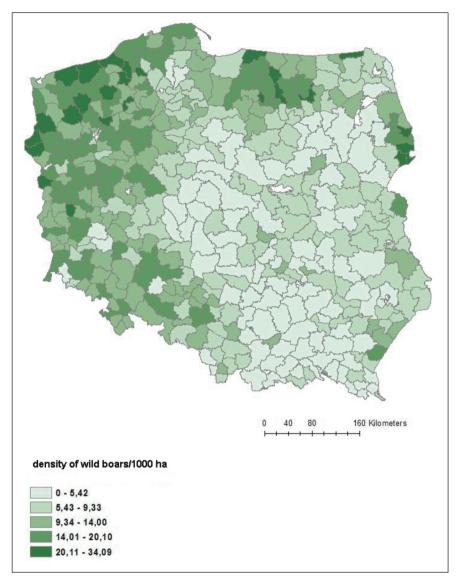


Fig. 4. Wild boar density in Poland (Central Statistical Office of Poland 2013).

has been proven to circulate (14 cases of ASF detected in Poland till end of August 2014).

A similar situation has been observed in countries bordering the EU from the East, where a number of cases in wild boar is higher than a number of outbreaks in pigs. A significant diversity regarding the density of wild boar also poses a threat of further spread of ASF. The density of more than 2 wild boar/km² favors a sustainable circulation of the virus in the wildlife reservoir. The excessive density of a wild boar population is mostly caused by a low hunting activity in the restriction area.

A comparison of the epidemiological situation with respect to ASF between Poland and Latvia, Lithuania, Belarus and Russia shows that different scenarios can be envisaged. For example, the first case of ASF in Latvia was diagnosed on June 25, 2014.

In the subsequent two months 61 more cases in wild boar and 39 outbreaks in pigs have been confirmed. The cases and outbreaks have been registered both, in the North and South of this relatively small country. As far as Lithuania is concerned, the number of cases and outbreaks is distinctly lower but the economic losses are immense, which is, among other things, associated with the occurrence of ASF in a large farm in Ignalin of about 20 000 pigs.

The situation concerning the spread of ASF in the Russian Federation and Belarus is difficult to objectively assess as the data are incomplete. However, the report from 2011 (Zaberezhny 2011) indicates that ASF has established itself in these countries as endemic, which makes the control and eradictation of the disease extremely difficult. The spread of ASF in the countries described above is connected with



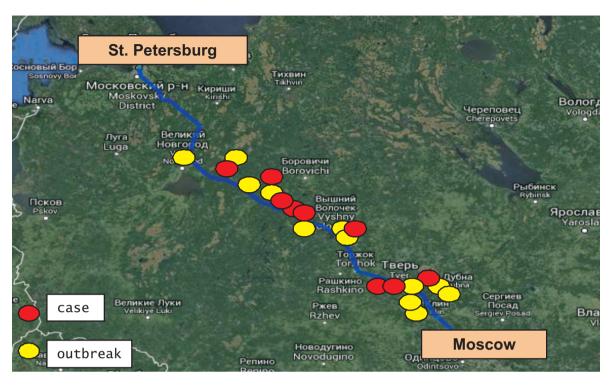


Fig. 5. Road transport and near-road trade activity as an important vector of ASF.

a human activity and a long-distant transport of ASFV-contaminated meat and pig-derived products, which makes humans the major vector of spread.

Means of transport are considered as an important route of ASF spread. The analysis of geographical locations of ASF outbreaks clearly indicates that most of them are situated in close proximities to major transport routes (Fig. 5), which is true not only to Russian Federation but generally.

To summarize, the ASF situation in Poland has been substantially different from the described above. Since the first detection of ASF in February 2014, the disease has not spread beyond 4 municipalities in 2 counties. When studying the probability of the ASFV dispersion to further areas one can assume that, like in other countries, human-associated movement and trade of ASF-contaminated pork and wild boar meat can be pointed as the most likely cause of ASF spreading.

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