

Prevalence of antibodies to Aujeszky's disease virus in wild boar in Poland, between 2011 and 2014: a retrospective study

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Received: May 19, 2017

Accepted: November 2, 2017

Abstract

Introduction: Aujeszky's disease virus (ADV) infects a wide range of animals, including members of the *Suidae* family, *i.e.* domestic and wild pigs, as well as wild boar. Since wild boar are a potential ADV reservoir and a source of infection for domestic pigs, the aim of the study was to evaluate ADV antibody prevalence in the Polish wild boar population, during the years 2011 to 2014. **Material and Methods:** Wild boar blood samples were collected during three consecutive hunting seasons; *i.e.* 2011/2012, 2012/2013, and 2013/2014, and tested for ADV antibodies by ELISA. **Results:** ADV antibodies were detected in samples from all tested voivodships. The average seroprevalence reached 32.2%. Seroprevalence, over the examined hunting seasons, was 27.4% in 2011/2012, 32.4% in 2012/2013, and 35.5% in 2013/2014. The highest percentage of seroreagents was detected in four voivodships, situated along the western border of Poland, *i.e.* Zachodnio-Pomorskie (ZP), Lubuskie (LB), Dolnośląskie (DS), and Opolskie (OP). This area is positively correlated with the highest density of the wild boar population and the highest wild boar hunting bag. **Conclusion:** The results of this study confirm that the wild boar population may still pose a threat to domestic pigs, which is of special importance at the final stage of Aujeszky's disease eradication programme in Poland.

Keywords: wild boar, Aujeszky's disease, antibodies, disease surveillance, Poland.

Introduction

Aujeszky's disease (AD) is caused by Suid herpesvirus 1 (SuHV1, syn. Aujeszky's disease virus – ADV or pseudorabies virus – PRV) which belongs to the *Herpesviridae* family, subfamily *Alphaherpesvirinae*, genus *Varicellovirus* (9). The only natural hosts for ADV are members of the *Suidae* family; *i.e.* domestic pigs (DP) as well as wild swine, including European wild boar (WB), and feral swine, typical in the USA and some regions of Europe (21). Considering domestic pigs, the main reservoir and source of ADV are pigs during the incubation period, diseased and latently infected (21). Aujeszky's disease represents one of the most economically important diseases in domestic pigs, causing substantial direct and indirect losses in the pig industry. Direct losses are due to reproductive failures, the mortality rate reaching 100% in piglets up to 14 days of life, and growth retardation resulting in a prolonged

fattening period. Indirect losses, observed in weaning and finishing pigs, are related to secondary bacterial infections and additional costs of medication (21). Also, the appearance of the disease causes serious restrictions or even the prohibition of international pig movement and trade (5, 19).

As mentioned above, wild swine are equally susceptible to ADV infection as domestic ones. Therefore, these wild animals should be considered as a potential ADV reservoir and source of infection for domestic pigs (21). This threat is especially important in countries, regions, or pig holdings free from ADV and, therefore, AD monitoring among wild swine is strongly advisable (25, 34). This is particularly important in Poland, where the AD eradication programme has been carried out since 2008 (22). Therefore, the object of this study was to evaluate the antibody prevalence of ADV in the Polish wild boar population, during the years 2011 to 2014.

Material and Methods

Sera. A total of 27,548 WB blood samples from the whole territory of Poland, collected and examined between 2011 and 2014, under the auspices of classical swine fever monitoring by the Regional Veterinary Diagnostic Laboratories of the Veterinary Inspectorate, were delivered to the Department of Swine Diseases of the National Veterinary Research Institute in Pulawy. The blood samples were collected during three consecutive hunting seasons; *i.e.* 2011/2012, 2012/2013, and 2013/2014. From the first hunting season, 7,078 samples were received and 7,021 (99.2%) of them were tested. In the next hunting season, a total of 10,155 blood samples were delivered, but only 10,079 (99.3%) of them were tested. In the last hunting season, 10,315 blood samples were delivered and only 10,163 (98.5%) were analysed. Limitations of 0.8%, 0.7%, and 1.5%, respectively, resulted mostly from the poor quality of the samples (haemolysis and autolysis) which yielded them unsuitable for ELISA testing.

ELISA. An ELISA Pseudorabies Virus gPI Antibody Test Kit (IDEXX PRV/ADV gI, IDEXX Laboratories, USA) was used, according to manufacturer's instructions.

Results

The results of the examination of WB blood samples, collected during the three consecutive hunting seasons are shown in Table 1 and Figs 1, 2, and 3, respectively.

Regarding hunting season 2011/2012, data indicated that serologically ADV positive WB were detected in all 15 voivodships examined (Table 1 and Fig. 1). No WB blood samples were received from Zachodnio-Pomorskie. The highest percentage of seropositive WB was found in Opolskie – 44.2%, followed by Dolnośląskie – 38.1%, Lubuskie – 31.3%, Podlaskie – 30.2% and Wielkopolskie – 29.9%. The highest percentages of ADV seropositive WB were detected mainly in three western voivodships (Opolskie, Dolnośląskie, and Lubuskie). In the remaining voivodships, the percentage of WB infected with ADV ranged from 7.0% (Świętokrzyskie) to 28.5% (Kujawsko-Pomorskie). An average percentage of ADV seroreagents detected in the hunting season 2011/2012 was 27.4% (Table 1).

Table 1. The results of ADV antibody detection in WB between 2011 and 2014

Voivodship (abbr.)*	Hunting season						Total	
	2011/2012		2012/2013		2013/2014		Total	
	No. of samples		No. of samples		No. of samples		No. of samples	
	tested	positive (%)	tested	positive (%)	tested	positive (%)	tested	positive (%)
Dolnośląskie (DS)	663	253 (8.1%)	1,026	411 (40.1%)	897	354 (39.5%)	2,586	1,018 (39.4%)
Kujawsko-Pomorskie (KP)	428	122 (28.5%)	827	250 (30.2%)	662	225 (34.0%)	1,917	597 (31.1%)
Lubelskie (LU)	851	230 (27.0%)	390	110 (28.2%)	877	361 (41.1%)	2,118	701 (33.1%)
Lubuskie (LB)	553	173 (31.3%)	931	274 (29.4%)	1,006	311 (30.9%)	2,490	758 (30.4%)
Łódzkie (LD)	280	75 (26.8%)	297	92 (31.0%)	192	43 (22.4%)	769	210 (27.3%)
Małopolskie (MA)	210	37 (17.6%)	296	67 (22.6%)	114	59 (27.6%)	620	163 (26.3%)
Mazowieckie (MZ)	430	111 (25.8%)	161	58 (36.0%)	512	166 (32.4%)	1,103	335 (30.4%)
Opolskie (OP)	285	126 (44.2%)	495	204 (41.2%)	312	129 (41.4%)	1,092	459 (42.0%)
Podkarpackie (PK)	343	69 (20.1%)	489	120 (24.5%)	509	138 (27.1%)	1,341	327 (24.4%)
Podlaskie (PD)	610	184 (30.2%)	186	41 (22.0%)	595	229 (38.5%)	1,391	454 (32.7%)
Pomorskie (PM)	949	205 (21.6%)	1,167	289 (24.8%)	1,171	389 (33.2%)	3,287	883 (26.9%)
Śląskie (SL)	306	76 (24.8%)	525	105 (20.0%)	389	116 (29.1%)	1,220	297 (24.3%)
Świętokrzyskie (SK)	85	6 (7.0%)	122	11 (9.0%)	151	30 (33.1%)	358	47 (13.1%)
Warmińsko-Mazurskie (WN)	884	211 (23.9%)	561	150 (26.7%)	361	112 (31.0%)	1,806	473 (26.2%)
Wielkopolskie (WP)	144	43 (29.9%)	345	115 (33.3%)	4,45	134 (30.1%)	934	292 (31.3%)
Zachodnio-Pomorskie (ZP)	-	-	2,261	966 (42.7%)	1,861	794 (42.7)	4,122	1,760 (42.7%)
Total	7,021	1,921 (27.4%)	10,079	3,263 (32.4%)	10,163	3,610 (35.5%)	27,154	8,774 (32.3%)

* - according to the Toponymic Guidelines of Poland for Map Editors and Other Users, 4th revised edition, Head Office of Geodesy and Cartography, Warszawa 2010

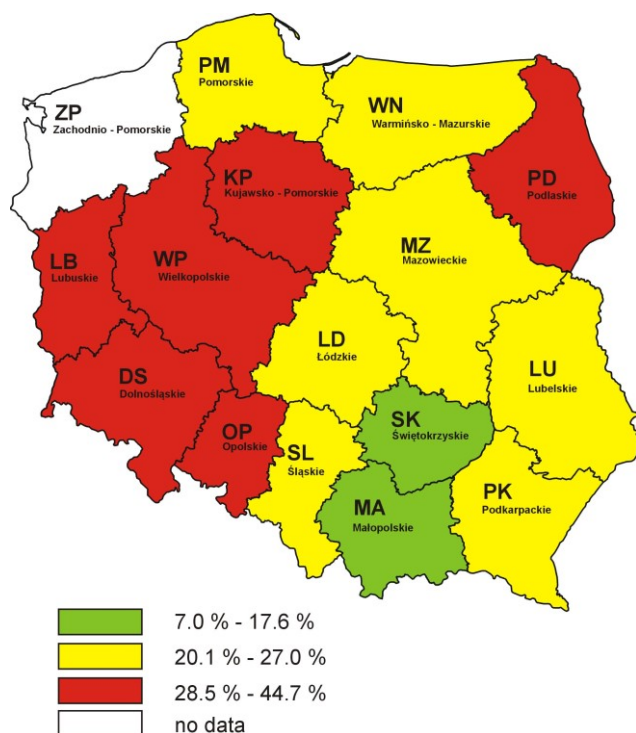


Fig. 1. ADV seroprevalence in WB in Poland, in the hunting season 2011/2012

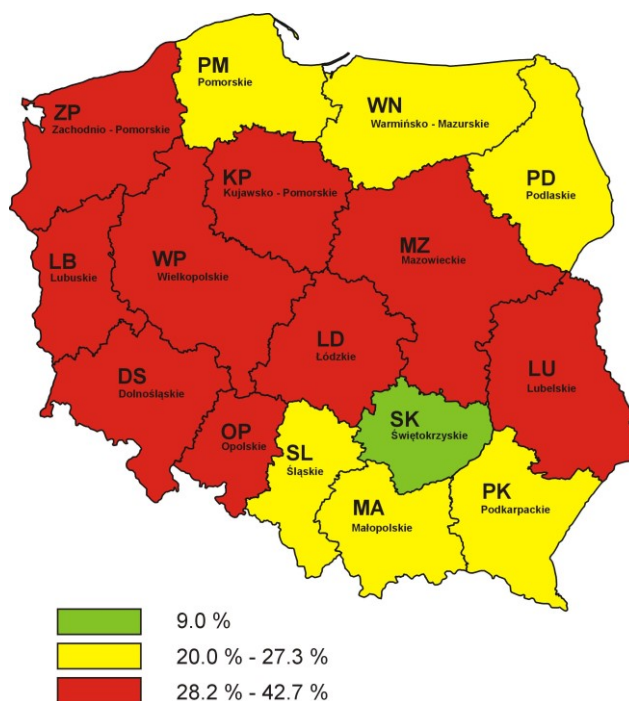


Fig. 2. ADV seroprevalence in WB in Poland, in the hunting season 2012/2013

In the following 2012/2013 hunting season, ADV seroreagents were present in all 16 Polish voivodships (Table 1 and Fig. 2). The highest percentage of ADV seropositive WB was found in Zachodnio-Pomorskie (42.7%), followed by Opolskie (41.2%), Dolnośląskie (40.1%), Mazowieckie (36.0%), and Wielkopolskie (33.3%). In Łódzkie, Kujawsko-Pomorskie, Lubuskie, Lubelskie, and Warminsko-Mazurskie the percentage of seroreagents was 31.0%, 30.2%, 29.4%, 28.2%, and 26.7%, respectively. In the remaining voivodships, the percentage of WB seropositive for ADV ranged from 9.0% (Świętokrzyskie) to 24.8% (Pomorskie). An average percentage of ADV seropositive WB detected in the hunting season 2012/2013 was 32.4% (Table 1).

The results of the examination of WB blood samples collected in the hunting season 2013/2014 indicated that ADV seroreagents were detected in all 16 voivodships (Table 1 and Fig. 3). The highest percentage of WB infected with ADV were found in Zachodnio-Pomorskie (42.7%) and in Opolskie (41.4%). The percentages of ADV seroreagents in Lubelskie, Dolnośląskie, Podlaskie, Kujawsko-Pomorskie, Pomorskie, Świętokrzyskie, Mazowieckie, and Warminsko-Mazurskie were 41.1%, 39.5%, 38.5%, 34.0%, 33.2%, 33.1%, 32.4%, and 31.0%, respectively. In Lubuskie, Wielkopolskie, Śląskie, Małopolskie, Podkarpackie, and Łódzkie, they were 30.9%, 30.1%, 29.1%, 27.6%, 27.1%, and 22.4%, respectively. The average percentage of seropositive WB detected in hunting season 2013/2014 was 35.5% (Table 1).

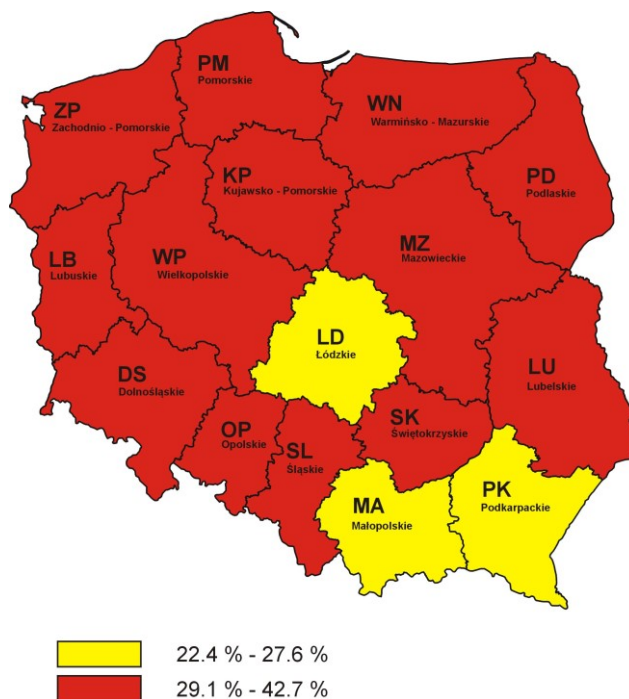


Fig. 3. ADV seroprevalence in WB in Poland, in hunting season 2013/2014

Total results of the examination of WB blood samples collected during the three consecutive hunting seasons are summarised in Table 1 and Fig. 4. An average percentage of ADV seropositive WB in

a hunting season amounted to 32.2% and ranged from 13.1% (Świętokrzyskie) to 42.7% (Zachodnio-Pomorskie).

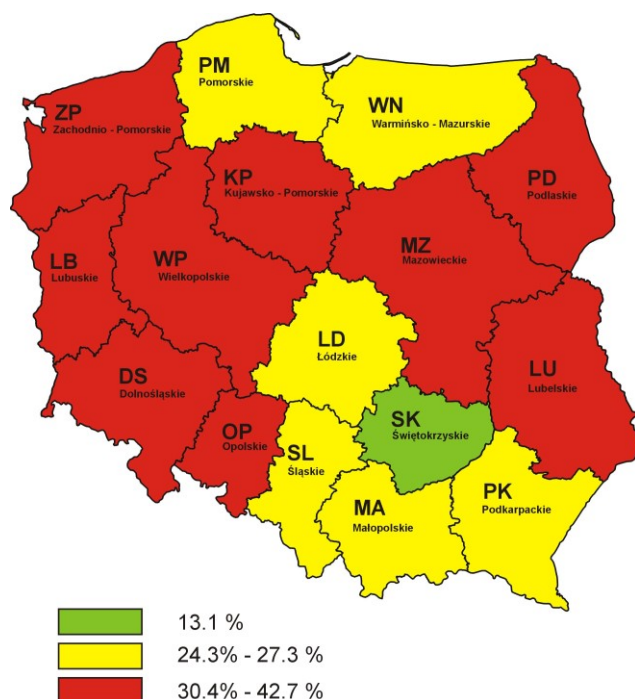


Fig. 4. ADV seroprevalence in WB in Poland, between 2011 and 2014

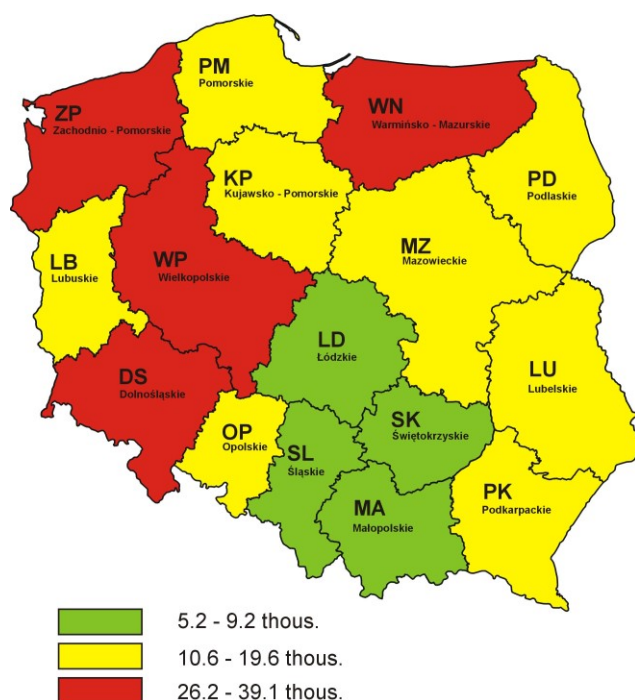


Fig. 5. Wild boar population (approx.) in Poland, between 2011 and 2014

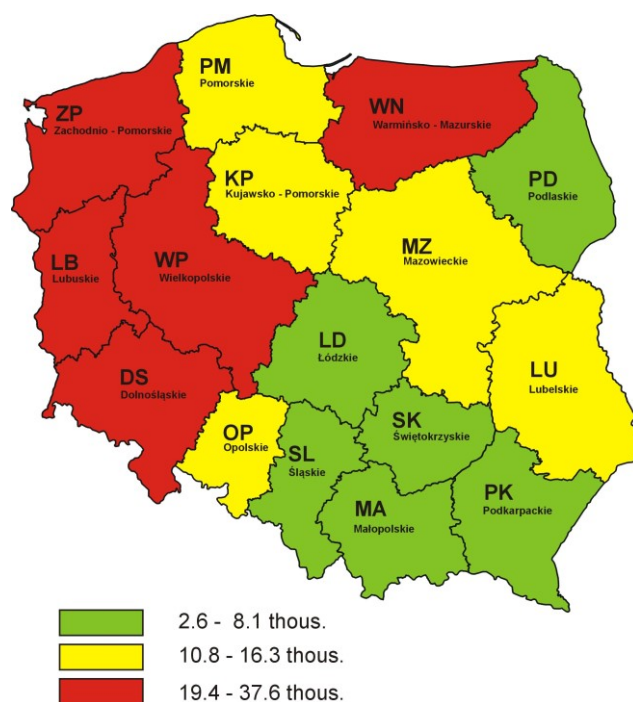


Fig. 6. Wild boar hunting bag (mean) in Poland, between 2011 and 2014

Discussion

The results of the present study should be compared to the results of a similar investigation conducted in Poland during the years 1997 to 2000 (20). This previous study revealed an average of 14.52% (12.9% – 18.6%) seropositive WB. Such animals were detected in 15 out of the 16 voivodships examined (20). The comparison of these results with the current ones, presented in this paper, reveals more than a double increase in the percentage of ADV seroreagents among WB, detected in all 16 voivodships, which indicates that ADV infection is endemic in the WB population of Poland.

The data concerning seroprevalence of ADV seroreagents show the intensive rate of infection with this virus, mostly in the western voivodships. Interestingly, these results correlate well with WB population density (Fig. 5) and WB hunting bag (Fig. 6).

The aforementioned increase in ADV seroprevalence is most likely connected with an increase in the WB population (25). During the last 14 years the population of WB in Poland has increased by about 140.6%, from 118,300 in the 2000/2001 hunting season to 284,600 in the 2013/2014 hunting season (12). At the same time, hunting bag increased by about 160.2%, from 93,000 in the 2000/2001 hunting season to 242,000 in the 2013/2014 hunting season (12). WB population increase could be explained by several reasons, e.g. climate change (shorter and milder winters), modifications to agricultural land use, and supplementary feeding during winter. Also, year-to-year intensification of hunting pressure has had an influence on the increased, uncontrolled reproduction of WB

(i.e. 2–3 litters per year) observed not only among multiparous sows, but also among one-year-old gilts (25).

The high ADV infection rate of WB determined during the surveyed hunting seasons in four voivodships situated along the western border of Poland, i.e. in Zachodnio-Pomorskie (ZP), Lubuskie (LB), Dolnośląskie (DS), and Opolskie (OP), should be underlined. The high percentage of ADV seropositive WB detected in the 2011/2012 hunting season in these voivodships was confirmed by the results of the examination of WB blood samples collected in the following hunting seasons of 2012/2013 and 2013/2014 (Table 1).

Interestingly, in the hunting season 2012/2013, a higher percentage of ADV seroreagents, in the range of 1.2% – 10.2%, was detected in 12 voivodships, which resulted in a 5% higher average seroprevalence of ADV, in comparison to the 2011/2012 hunting season (Table 1). A slight decrease in 1.9% – 8.2% of ADV seroprevalence was only found in four voivodships (Table 1).

However, alarmingly, in the hunting season 2013/2014, a significant increase in ADV seropositive WB was observed in six voivodships, compared to the previous hunting season. The aforementioned increase was detected in Świętokrzyskie (SK) (+24.1%) and then in Śląskie (SL) (+19.1%), Podlaskie (PD) (+16.5%), Lubelskie (LU) (+12.9%), and Pomorskie (PM) (+8.4%). Moreover, a slight increase in ADV seroreagents (0.2% to 5.0%) was detected in six more voivodships. Finally, in Zachodnio-Pomorskie (ZP) the rate of infection was the same as in the previous hunting season, whereas a decrease in ADV seroprevalence, ranging from 0.6% to 8.6%, was found in the four remaining voivodships.

The problem of an exploding increase in the WB population has been observed in Europe since the 1950s, referring to, among other places, France (1), Spain (3), Switzerland (18), Italy (25), Germany (27), Croatia (29), Czech Republic (35), and Slovenia (39, 43). A similar trend has been observed in the USA (6) where, over the last 10 years, the feral swine population has increased four-fold and is already distributed across 39 of the 50 states (25).

The endemic prevalence of AD in WB, as observed in Poland, after two series of investigations, performed at 15 year intervals (20, 40) was also found in other European countries, as well as in the USA (25). Large-scale investigations conducted in Germany over many years have allowed the detection of 0.4% to 16.5% ADV seropositive WB on average, depending on the region or “land” examined (10, 25, 27). Pannwitz *et al.* (27) indicate that the percentage of WB infected with ADV along Germany’s eastern border with Poland, can exceed 45%. These estimates have been made on the basis of observations carried out since the 1990s, showing an increasing seroprevalence, ranging between 20% and 30%. The cited authors suggest that the reason for high AD prevalence could be the migration of ADV-infected

WB across the bordering Oder River, in both directions; i.e. from Germany to Poland and back to Germany (27). In fact, there was no investigation of WB regarding ADV seroprevalence along the Polish - German border before 1997. However, the results of the present study show a high percentage of ADV infected WB, averaging from 30.4% in Lubuskie (LB) to 42.7% in Zachodnio-Pomorskie (ZP) (Table 1 and Fig. 4).

Infections of WB with ADV in East Germany appear to be dynamic, over time, with variance of the percentage of seroreagents and areas of their prevalence. The disappearance of AD in some areas and its movement into new, non-infected areas was also observed (41). The so-called “pockets of infection” were found to have moved in the western direction, at a distance of about 3.3 km per year (41). A similar conclusion was drawn by Denzin *et al.* (10) who detected an average of 4.47% ADV seropositive WB. Furthermore, in the years 2009 to 2011, the number of such animals doubled, reaching 9.05% (10).

In Spain, different serological studies carried out in various regions, revealed from 0.8% to 60.6% of seropositive WB (3, 32, 33, 45). Interestingly, ADV seroprevalence among the free-living WB ranged from 21.4% to 42.4%, and among WB living in fenced areas the average seroprevalence was 66.1% (32, 45).

A similar comparison of the ADV infection rate among WB living in free and/or in fenced areas was performed in Austria. The percentage of seroreagents during the years 2010 to 2011 among the free-living WB amounted to 37.9%, and among the WB in fenced areas it was 69.6% (37), with an average of 55.2% (37). During the years 2011 to 2012, the percentage of WB infected with ADV was estimated at 22.8% (38).

Studies performed in former Yugoslavia during the 1980s revealed the percentage of ADV-infected WB from fenced areas to be 74.4%, and 26.8% from open areas (13).

The comparison of the aforementioned data from Spain, Austria, and former Yugoslavia indicates that ADV circulates very intensively among WB held in pens, resulting in a higher seroprevalence than among free-roaming WB, with a much lower rate of ADV infection.

In Croatia, during the winter 1999 hunting season, the rate of ADV seropositive WB was 54.4% (47). Later, Roic *et al.* (29) found an average seroprevalence of 38.5% during the years 2005 to 2010. During 2005 and 2006, 28.1% of seroreagents were detected and during 2009 and 2010 the number doubled, reaching 57.4%. In Slovenia, in the period 2003 to 2004, the percentage of ADV-infected WB reached 26%–31% (43, 44). Later, in the 2010/2011 hunting season, the seroprevalence increased to 45.1% (39).

In Italy, data collected in various studies on the WB population indicated that ADV seroprevalence ranged from 20.0% to 51.0% (17, 23).

In France, the first investigation on WB involving AD was undertaken in 1979 and no seroreagents were

found (2). The next ADV serosurvey, carried out during the years 1991 to 1998, revealed approximately 3.3% of seroreagents among free-living WB, and 1.18% among WB held in fenced areas (1). The latter result is significantly lower than that detected in the following years in Austria or in Spain (32, 37, 45). However, taking into account the dynamics of ADV spread among WB, together with the increasing population of these animals over recent decades, the cited results reflect the actual situation in France, during the period 1991 to 1998 (1). An investigation carried out in France between 2000 and 2004 revealed a much higher percentage of ADV seropositive WB, reaching 53% (25).

In the Netherlands, the first serological investigation of WB infected with ADV, undertaken in 1994, in only one of the country's regions, revealed 2.6% seroreagents (8). The next ADV serosurvey carried out during the period between 1996 and 1999 indicated an unequivocal lack of ADV infections among the WB population (11).

In Belgium, neighbouring Germany and the Netherlands, serological screening, conducted during 2004 and 2005 detected 15% – 22% of ADV seropositive WB (25). In Switzerland, the percentage of seroreagents ranged from 2.4% to 6.9%, depending on the region investigated. In this country, an increasing population of WB was also observed (18). A similar trend was found in the Czech Republic, where the mean percentage of ADV seroreagents based on the results of a serological investigation carried out between 2004 and 2005, was estimated at 29.9% (35). In Russia, a similar serosurvey has shown 32.5% – 39.5% of ADV seropositive WB, depending on the region of investigation (25).

As appears from the aforementioned data, in those European countries where serological investigations targeting ADV seroprevalence among WB populations have been performed, the occurrence of WB infected with ADV has been confirmed, although in different percentages.

A similar problem to that observed in Europe also exists in the USA. An investigation dedicated to ADV infection among feral swine started in the mid-1970s and has continued up until now (6, 7, 14, 15, 28, 30, 31, 36, 42, 46). The percentage of ADV seropositive feral swine differed between years and depended, among other things, on the state or region of investigation. It was therefore difficult to estimate ADV seroprevalence for the whole country. These studies were performed mainly in the southern or south-eastern states of the USA, where the feral swine population seems to be the biggest. As a consequence, it was found that the percentage of ADV seroreagents among the tested animals could be as high as 100% (7). On the other hand, wide-scale serological investigations carried out in 35 states, where feral swine are present, allowed the detection of 18% of animals infected with ADV in 25 states (28). Moreover, based on many years of serosurveys, it was found that ADV could

have been endemic in certain regions for as long as 32 years (7, 28).

Wild swine infected with ADV were also detected in Brazil, Tunisia, and Iran. The percentage of seroreagents amounted to 25.2%–100%, 63%, and 42.7%, respectively (4, 25). Investigations in South Korea allowed the detection of 35.7% of ADV seropositive WB (16).

The presented results, originating from different European countries, the USA, Brasil, Tunisia, and Iran, indicate a high percentage of WB and/or feral swine infected with ADV, repeatedly higher than the values detected in Poland between 2011 and 2014. Interestingly, the results of an ADV serosurvey conducted in our country between 1997 and 2000 and in the present study indicate that ADV has remained endemic in the WB population in Poland for at least a dozen years. In Germany, this period was estimated at 23 years (25) and, in the USA, for as long as 32 years (28). Furthermore, it is evident that the WB population is still growing, not only in Europe, but also in the USA. This tendency will result in further spread of AD and an increase in WB infected with ADV (25). Taking into account the aforementioned spreading of AD among WB populations and the tremendous progress in the elimination of AD, as well as its complete eradication from many European countries and the USA, the potential risk of ADV transmission from infected WB to susceptible farm animals has become a matter of growing concern (25).

Based on serological and molecular studies, it is suggested that ADV infection in DP and WB represents epidemiologically distinct infection cycles (15, 26, 27, 30). On the other hand, experimental investigations have shown that the risk of ADV transmission from WB to DP cannot be excluded, as infected WB can shed the virus in a sufficient amount to infect susceptible swine (15, 25). The earlier suggestion that the venereal route is the main one for ADV transmission among the WB population (30, 31) was verified recently, on the basis of the results of intensive experiments. It has been proven that venereal transmission of ADV is not the only one, but both venereal and oral/nasal shedding represent the two major routes of ADV transmission (14, 36).

Taking into account the aforementioned data on the one hand, and the reasonably high biosecurity level of commercial pig farms in agriculturally developed countries on the other, it seems that the risk of spill-over infection with ADV from WB to DP appears to be high only for backyard holdings or outdoor-reared swine (6, 14, 25). Such cases are very rare and have only been documented in France and the Czech Republic (25). In 2003, however, Germany was declared an ADV-free country. In spite of a high seroprevalence of ADV, particularly in East Germany, no spill-over of this virus has been detected in DP (10, 24, 27). A similar situation can be observed in Spain, where an average percentage of ADV-infected WB between 2000 and 2010 amounted

to 49.6% and ADV seroprevalence among DP, during intensive AD eradication, decreased from 70% in 2003 to 1.7% in 2010 (3). Similarly, Croatia, where the percentage of ADV seropositive WB increased from 28.1% during the hunting season 2005/2006 to 57.4% in the hunting season 2009/2010, is officially AD free (29). In Austria, free of AD since 1997, the detected percentage of seroreagents amounted, on average, to 55.2% (37), in the Czech Republic – with the same AD-free status – 29.9% of ADV seropositive WB were found, and in Slovenia, free of AD since 2010, 26%–45.1% of ADV seroreagents were recorded (39, 43, 44). In France, free of AD (except for a few departments), WB infected with ADV were also found (1, 25).

In the USA, where AD eradication has been carried out for over 15 years (14), ADV is spreading uncontrollably among feral swine, including the population of these animals in such states as Nebraska (45) situated in the north, far away from their traditional, natural habitat in the southern part of the USA.

Overall, there has been a noticeable increase in the percentage of ADV-infected WB in the past two decades, not only in Europe but also in the USA and many other countries, which might have been induced by the permanent increase in the populations of these wild animals (25). In view of this situation, the constant monitoring of AD among WB populations seems to be a necessity (34, 42). It is especially important in the countries that are close to completing their AD eradication programmes, or which are officially recognised as AD-free (25). There are two approaches on how such a serosurvey should be performed. One of them is based on monitoring at bi- or even triennial intervals, during which sufficient information on the spread and dynamics of ADV infection in WB populations would be obtained (25). Such serosurvey has been conducted in East Germany over several years with invaluable results (27, 41). Of course, for statistical evaluation, the following should be considered: sample size, size of the area of study, and practical aspects (*i.e.* the feasibility of sample collection) and the detection of other diseases (25).

The second approach assumes that populations of WB, already known to be infected with ADV, are of no interest, because of the endemic circulation of this virus, which could last as long as 32 years (7, 28). Alternatively, special attention should be devoted to WB populations with an unknown epizootic status regarding AD (7).

In conclusion, it should be stated that the presented studies to a great extent fall within the first approach mentioned above. The results of this study show a considerable increase in the percentage of WB infected with ADV, compared to the results of the survey conducted during 1997 to 2000 (20, 40). Over the last dozen years, the ADV infection rate has doubled in Polish WB population. Moreover, ADV seropositive WB have been detected across the whole country. This unequivocally indicates the necessity for the

continuation of this kind of serosurvey, at bi- or even triennial intervals, aiming to assess the spread and dynamics of ADV infection among WB. The issue is especially important in the present situation, when Poland is applying to be officially recognised by the EU Commission as an AD-free country.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This study was supported by the National Centre of Research and Development (Poland) grant no. 12-0126-10 and by project no. S/215, “The role of wild boar in the epidemiology of Aujeszky’s disease”, conducted within the statutory activity of the National Veterinary Research Institute in Pulawy, Poland.

Animal Rights Statement: None required.

Acknowledgements: The authors would like to thank Ewa Piekutowska-Nózka and Stanisława Boreczek for their skilled technical assistance.

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