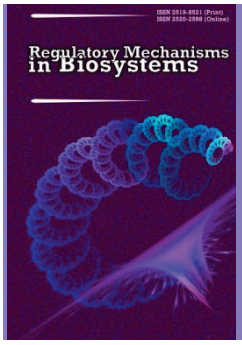

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2022, 13(1), 46–52
doi: 10.15421/022206

Spread of Aujeszky's disease among wild boars and domestic pigs in Ukraine

V. V. Ukhovskyi*, **, L. Y. Korniienko*, A. V. Pyskun*, O. M. Chechet*, Z. M. Drozhzhe*, I. M. Polupan*, G. B. Aliekseieva*, O. A. Moroz*, O. M. Romanov***

**State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
***Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine*
****Institute of Veterinary Medicine, NAAS of Ukraine, Kyiv, Ukraine*

Article info

Received 11.01.2022
Received in revised form 02.02.2022
Accepted 03.02.2022

State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Donetska st., 30, Kyiv, 03151, Ukraine. Tel.: +38-066-789-85-19. E-mail: uhovskiy@ukr.net

Bila Tserkva National Agrarian University, Soborna st., 8/1, Bila Tserkva, 09117, Ukraine. Tel.: +38-066-789-85-19. E-mail: uhovskiy@ukr.net

Institute of Veterinary Medicine, NAAS of Ukraine, Donetska st., 30, Kyiv, 03151, Ukraine. Tel.: +38-067-981-52-26. E-mail: romanov.oleksandr.mykolajovych@gmail.com

Ukhovskyi, V. V., Korniienko, L. Y., Pyskun, A. V., Chechet, O. M., Drozhzhe, Z. M., Polupan, I. M., Aliekseieva, G. B., Moroz, O. A., & Romanov, O. M. (2022). Spread of Aujeszky's disease among wild boars and domestic pigs in Ukraine. Regulatory Mechanisms in Biosystems, 13(1), 46–52. doi:10.15421/022206

Aujeszky's disease, also known as pseudorabies is an acute infectious viral disease in a variety of mammals caused by Suid herpesvirus 1, a member of the subfamily Alphaherpesvirinae and the family Herpesviridae. This virus causes significant economic losses in agriculture of numerous countries. In particular, in pig farming, the main losses are currently associated with restrictions of international trade, as well as the cost of vaccination or slaughter of infected animals. The main reservoirs species of this disease are considered to be wild boars and domestic pigs. So, in this article the authors performed a comparative retrospective analysis of the prevalence of Aujeszky's disease among these species in Ukraine over 2009–2020 by systematizing and analyzing the epizootic data and results of serological studies. As a result of the conducted research, there is a clear trend that shows the reduction in the number of locations in Ukraine (farms, households, etc.) with domestic pigs affected by Aujeszky's disease. Thus, in 2009 four such farms were found, and in 2018 – only one. During 2014–2016 and 2019–2020, affected farms and locations were not registered for this disease. In total, 21 such points with infected pigs were registered during the analyzed period. As for wild boars, during the analyzed 12 years, locations with animals affected by Aujeszky's disease were registered only in 2013 and 2014 (two and one, respectively). It should be noted that all of those with infected wild boars were found in the AR Crimea. For the twelve years, the number of tested sera samples from domestic pigs amounted to 378,678 (7.4% were positive) and from wild boars – 9,052 (19.8% were positive). As a result of comparative geographical analysis of the obtained data on the detection of specific humoral antibodies to the Aujeszky's disease virus among both species for 12 years in the regions of Ukraine, there is no established clear correlation between the spread of the disease among wild boars and domestic pigs. Thus, the highest seroprevalence rates among wild boars were observed in the northern and northeastern parts of Ukraine, and in domestic pigs, on the contrary, in the southern and central parts of this country. It should be noted, that since 2014 the number of serological tests among both species for carriers of Aujeszky's disease has significantly decreased.

Keywords: Pseudorabies; Suid herpesvirus 1; PRV; epizootic process; serological studies; *Sus scrofa*; swine.

Introduction

Aujeszky's disease, also known as pseudorabies is an infectious acute viral disease in a variety of mammals (such as dogs, cats, cattle, sheep, rabbits, foxes, minks, etc.), that is caused by Suid herpesvirus 1 (SHV-1) (subfamily Alphaherpesvirinae) (OIE, 2018). This pathogen affects all species of farm animals. In particular, pseudorabies is an extremely important problem for fur farming and pig breeding (He et al., 2019).

In all species of animals infected by SHV-1 there are signs of brain and spinal cord damage, the respiratory system may be affected and, in addition, there is severe itching and scratching of the places of virus penetration on the skin. The latter sign is not observed only in pigs. In particular, the lowest rate of mortality is registered among this species, but in piglets this indicator can reach to 100% (Korniienko et al., 2000). Later, resistance increases, and mature swine tend to survive and remain infected for the rest of their lives (there is a persistence of the virus). In other animal species, regardless of age, the disease is fatal ("epizootic deadlock of the pathogen") (Beran et al., 1980; Di Marco et al., 2021).

In swine, the disease can manifest itself as a typical slow infection with persistence of the virus (Romero et al., 2001). Among domestic pigs, clinical symptoms of central nervous system damage are registered in 1–2-month-old piglets. Respiratory symptoms (dyspnea, rhinitis) are observed in the fattening livestock. Reproductive insufficiency can be registered in adult sows. At the same time, clinical signs of the disease in wild boars are practically not manifested. However, they can be a constant reservoir of Aujeszky's disease virus in nature (Corn et al., 2004). In swine with a latent form of infection, the pathogen persists mainly in the trigeminal ganglia (mainly in domestic pigs), sacral ganglia (mainly in wild pigs) and tonsils (Lu et al., 2021).

Pigs are the only natural hosts of the pseudorabies virus (PRV), although classical strains are found in many species of mammals (Pedersen et al., 2013; Di Marco et al., 2021). Due to the eradication programs, classic PRV strains have been eradicated among swine in most developed countries (Vannier et al., 1997; Stegeman, 1997; van Nes, 2001; Müller et al., 2003). However, this virus remains endemic in Eastern and South-Eastern Europe, Latin America and Asia (Sun et al., 2016; OIE, 2018).

The leading routes of transmission in domestic pigs are aerogenic and alimentary. Transmission of the virus can also occur through the conjunctiva. Swine with the latent form of the infection and persistence of the virus bring the pathogen into uninfected herds. In addition, this virus can be transplacentally transmitted. Contact transmission during mating is considered to be a leading form of spread of the virus among wild boars (Panyasing et al., 2018). Air, water and feed can be contaminated by this virus (Romero et al., 2003; Pomeranz et al., 2005; Lu et al., 2021).

Wild boars, like domestic pigs, can be sources and reservoirs of the SHV-1 pathogen, and it is detected in this species in those countries where eradication of Aujeszky's disease has already been carried out among domestic pigs (Corn et al., 2004; Vengust et al., 2005; Lari et al., 2006; Mahmoud et al., 2011). Wild boars seropositive to Aujeszky's disease virus were detected in the 98 districts of the United States, the overall prevalence was 27.7% of the total number of animals examined (in 4,293 animals from 15,494) (Pedersen et al., 2013). In many European countries, pseudorabies among dogs is constantly registered after they are fed with meat and offal from killed wild boars (Cano-Terriza et al., 2019; Ciarello et al., 2022). Studies have shown that Aujeszky's disease virus is secreted from the genitals of wild boars and can be transmitted through mating (Charrier et al., 2018). However, as early as 1997, it was shown that although *Sus scrofa* can be a reservoir of the Aujeszky's disease virus in the natural environment and can transmit it transplacentally, by contact or alimentary means (cannibalism), they are carriers of highly attenuated strains (Hahn et al., 1997).

It is possible to isolate a PRV from the pathological material on transplanted cultures of cells of pig's origin. Its antigen is detected by immunoperoxidase or immunofluorescent staining, in the neutralization reaction with specific sera or monoclonal antibodies (OIE, 2018). According to the recommendations of the World Organization for Animal Health, polymerase chain reaction (PCR) can be used to confirm the presence of the virus in the material. Developed PCR kits allow vaccine viruses to be differentiated from field ones (DIVA-strategy) (Cheng et al., 2021). Also, the enzyme-linked immunosorbent assay (ELISA) is widely used to confirm the presence of antibodies to the PRV. ELISA with DIVA-strategy capabilities has also been developed (Ai et al., 2018; Silva-Junior et al., 2020).

Marker vaccines, represented by vaccines with natural or artificial genetic deletions in DNA, play an important role in the measures to combat Aujeszky's disease (Freuling et al., 2017; Colomer et al., 2020). The virus used to prepare these vaccines doesn't include a specific glycoprotein, such as the glycoprotein gE (g_1). Such vaccines have advantages over the conventional viral vaccines because their usage makes it possible to further distinguish naturally infected animals from vaccinated ones by comparing antibodies (Freuling et al., 2017; Kondibaeva et al., 2021). Thus, infected animals will show antibodies to the gE glycoprotein, and vaccinated (uninfected) animals will have no antibodies to this glycoprotein-marker (Mettenleiter, 2020). The use of non-marker live vaccines is not recommended, because strains of SHV-1 are capable to recombination. Thus, simultaneous application of two vaccine strains into the animal's organism can lead to their recombination with the formation of new variants of the virus, including virulent ones (OIE, 2018).

Prevention and control measures used in different countries around the world are diverse (Ukhovskyi et al., 2020). All of them are based on the anti-epizootic measures to prevent and control Aujeszky's disease, even the elimination of the virus (eradication) (Komaromi & Szabo, 2005; Freuling et al., 2017; Baron et al., 2020). Such measures often include the slaughter of all serologically responsive pigs, regardless of the intensity of the lesions (in some cases, the slaughter of the whole herd) and require constant serological control for welfare at the state level (United Kingdom); eradication by using the marker vaccines followed by serological studies in these herds for detection of field virus carriers and slaughter of the positive animals (Ukraine, most countries of Europe, USA); use of the traditional live and inactivated vaccines to prevent clinical manifestations of the disease (Asia, some countries of Europe). The first country that conducted eradication of Aujeszky's disease on its territory was the United Kingdom (in 1989). Countries such as Denmark (1991), Switzerland (1993), Sweden (1995), Austria (1996), Luxembourg (1999), Germany (2000), Greece (2001), Belgium (2001), and Netherlands (2004) have reported the eradication of this disease on their territory in different years (Korniienko et al., 2020). Japan and the United States announced the elimination of Aujeszky's disease in 1993. The incidence analysis in recent years shows that some countries which have eradicated pseudorabies have re-detected the PRV on their territory again (Austria, Germany, Japan, and the USA) (OIE, 2018). The registration of this disease on the territory of countries that have declared its eradication, especially among wild boars, confirms the thesis that this species is the main reservoir of the pathogen in the wild (Korniienko et al., 2020).

For Ukraine, according to the current "Instruction on measures for the prevention and eradication of Aujeszky's disease in farm and fur animals" (2008), live vaccines from only one strain of the virus should be used in any given administrative-territorial unit. An important role in the system of measures is played by the serological methods of diagnosis, including neutralization tests (NR) and enzyme-linked immunosorbent assay (ELISA). The "Program for the eradication of Aujeszky's disease among swine in Ukraine over 2008–2012" had a significant impact on the recovery of farms affected by PRV from this disease.

Taking into account the urgency of the problem regarding control and prevention measures of Aujeszky's disease in the world and, in particular, in Ukraine, the aim of our study was to conduct a comparative retrospective analysis of this disease among wild boars and domestic pigs in Ukraine over 2009–2020 by systematizing and analyzing the epizootic data and results of serological studies.

Materials and methods

Spreading and seroprevalence of Aujeszky's disease in domestic pigs and wild boars was analyzed according to the reports of regional laboratories of the State Service of Ukraine on Food Safety and Consumer Protection and obtained data of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv, Ukraine) (reporting form 1-Vet. and 2-Vet.) for the period 12 years.

In order to determine the presence of specific humoral antibodies to SHV-1, 9,052 blood sera samples from wild boars were collected after shooting in the hunting seasons of 2009–2020 from various hunting grounds and forestries in all regions of Ukraine. Also, 378 678 blood sera samples from domestic pigs were collected from different farms and households in all regions of Ukraine.

Studies on the presence of specific humoral antibodies to the PRV in the sera samples of wild boars and domestic pigs were performed by the enzyme-linked immunosorbent assay by using two commercial kits: ID Screen® Aujeszky gE Competition (manufacturer ID.vet, France) and Pseudorabies Virus gpI Antibody Test Kit (manufacturer IDEXX, USA). These kits are discriminatory in that they distinguish between infected and latently infected animals among vaccinated swine by using the marker gE-negative vaccine against Aujeszky's disease.

Seroprevalence for each oblast was calculated as the number of positive samples divided by the sample quantity in oblast. Calculating binomial confidence intervals (BCI) was conducted by using the method Clopper-Pearsor exact with confidence level – 0.95 and was performed for seroprevalence estimates using the R epitools package (Ausvet, version 2020, Australia) (https://epitools.ausvet.com.au). That website is free and it is intended for use by epidemiologists and researchers involved in estimating disease prevalence or demonstrating freedom from disease through structured surveys, or in other epidemiological applications. Information about the total number of locations / farms affected by Aujeszky's disease was obtained from the State Statistics Service of Ukraine (http://ukrstat.gov.ua).

Positive incidences by oblast were mapped in the software Quantum GIS 3.16.0 (International Quantum GIS Project, version 2020, Germany), which is free on the website (www.qgis.org/ru/site/forusers/download.html). The vector layers for the borders of Ukraine's regions were downloaded from the site www.diva-gis.org/data. Quantile classification with 5 classes of the data was chosen. With this classification, an equal number of oblasts fall into each class.

Data is presented in the article without taking into account the occupied territory of the Autonomous Republic of Crimea, the city of Sevastopol and part of the temporarily occupied territories of Donetsk and Luhansk regions.

Results

In total, 21 locations (farms, households etc.) affected with Aujeszky's disease among domestic pigs were registered during the analyzed period in Ukraine. Thus, in 2009 such locations were registered in Vinnytska (1 location), Donetska (1), and Cherkaska (2) regions. In 2010, they were

registered in Vinnytska (2 locations) and Donetska (1) regions. Over 2011, four affected locations were found – two in Vinnytska and one each in Donetska and Mykolaivska regions. The following year, there was only one such location in the Zaporizka region. In 2013, three affected farms were registered in the AR Crimea. In 2017, a single affected location was registered in Zaporizka and also Khersonska regions. In 2018, only one such location was found in Khersonska region. During the period of 2014–2016 and 2019–2020, locations affected with Aujeszky's disease were not registered in Ukraine.

For wild boars, during the analyzed period, locations affected with Aujeszky's disease were registered only in 2013 and 2014, respectively, two and one. It should be noted that all of them were found in the AR Crimea.

Regarding serological studies, for the period 2009–2020, 9,052 sera samples from wild boars were tested for the presence of antibodies to pseudorabies and 1,788 positive reactions were obtained, which is 19.8% (BCI, 18.9–20.6%) of the studied samples. The generalized and systematized results of blood sera from wild boars studies are presented in Table 1 and Figure 1.

Table 1

The results of blood sera from wild boars studied for the presence of specific humoral antibodies to Aujeszky's disease virus according to the reports of regional laboratories and SSRILDVSE

Years	Number of tested samples	Number of positive samples	Percentage of responding animals	Binomial confidence interval
2009	1,301	195	15.0	13.09–17.05
2010	1,214	224	18.5	16.31–20.75
2011	257	36	14.0	10.01–18.86
2012	1,852	344	18.6	16.83–20.42
2013	820	178	21.7	18.93–24.69
2014	1,935	480	24.8	22.90–26.79
2015	106	49	46.2	36.49–56.18
2016	2	0	0.0	0
2017	407	51	12.5	9.47–16.14
2018	912	183	20.1	17.51–22.81
2019	246	48	19.5	14.75–25.02
2020	0	0	0.0	0
Total	9,052	1,788	19.8	18.94–20.59

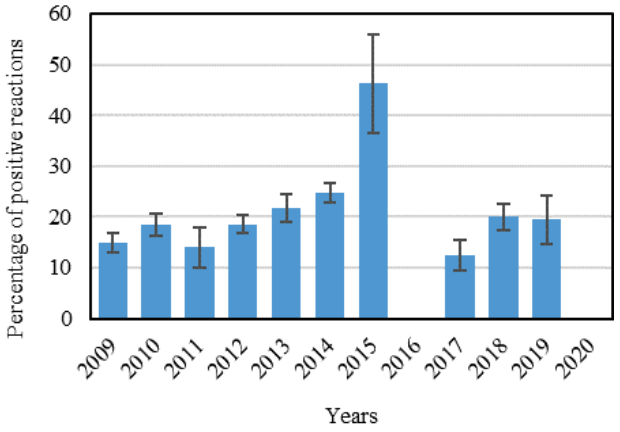

Fig. 1. Dynamics of the seroprevalence among wild boars for Aujeszky's disease virus by years according to the reports of regional laboratories and SSRILDVSE

As shown in Figure 1, seroprevalence among wild boars in Ukraine for the analyzed period was the highest in 2015 – 46.2% (BCI, 36.5–56.2%). The high percentage of positive reactions in this year is due to the fact, that only 106 samples from seven regions of Ukraine were studied and in two of them (Odessa and Poltava) a significant percentage of positive reactions was detected – 72.4% and 65.4% respectively. At the same time, the lowest rate of this indicator was in 2009, 2011 and 2017 – 15.0% (BCI, 13.1–17.1%), 14.0% (BCI, 10.0–18.9%), and 12.5% (BCI, 9.5–16.1%), respectively. In 2016, sera samples with humoral antibodies to PRV were not detected. This is due to the very low number of samples tested – only two sera samples from the Kherson region were tested during this year. In 2020, there were no studies regarding seroprevalence of PRV among wild boars. Overall, from 2009 to 2019, the seroprevalence rate for Aujeszky's disease virus among this species was about the same, except in 2015.

Blood sera samples for our investigation were selected from all the regions of Ukraine. For the twelve years period, the largest numbers of samples were tested in 2012 and 2014 – 1,852 and 1,935 samples, respectively, and the smallest in 2016 – only two samples. Also, a small number of samples were tested in 2015 and 2019 years – 106 and 246 samples, respectively. Data on the scope of serological diagnosis for the detection of specific humoral antibodies to SHV-1 among wild boars in terms of Ukrainian regions are presented in Figure 2.

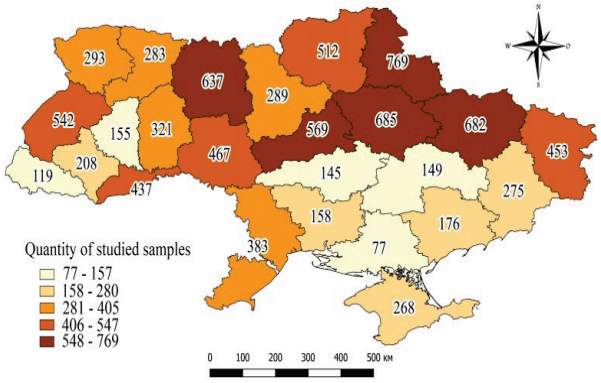

Fig. 2. Quantity of wild boars' sera samples tested for Aujeszky's disease virus in Ukraine over 2009–2020

Systematized data indicate that the monitoring investigation covered all regions of Ukraine. During the period 2009–2020, the largest number of sera samples from this species of animals was studied in seven areas: Sumska (769 samples), Poltavska (685 samples), Kharkivska (682 samples), Zhytomyrska (637 samples), Cherkaska (569 samples), Lvivska (542 samples), and Chernihivska (512 samples) regions. The smallest number of studies was conducted in the following areas: Zaporizka (176 samples), Mykolaivska (158 samples), Ternopilska (155 samples), Dnipropetrovska (149 samples), Kirovohradska (145 samples), Zakarpatska (119 samples), and Khersonska (77 samples) regions. The results of serological monitoring among wild boars for the twelve years period regarding the detection of specific humoral antibodies to the pseudorabies virus in terms of Ukrainian regions are presented in Figure 3.

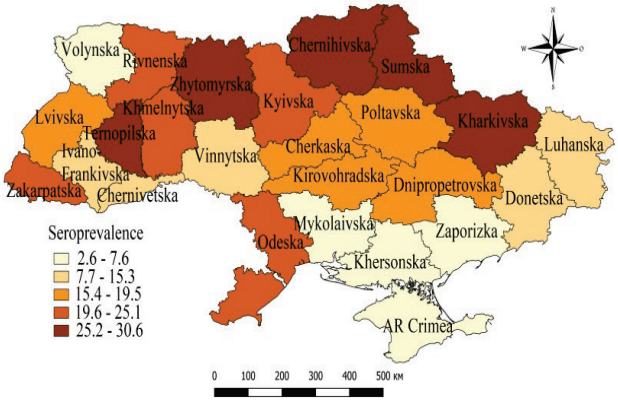

Fig. 3. Cartographic analysis of seroprevalence of wild boars' for Aujeszky's disease virus in Ukraine over 2009–2020

In general, the seroprevalence rate for this disease in Ukraine during the analyzed period ranged from 30.6% (in Sumska region) to 2.6% (in Khersonska region). As can be seen from the data shown in Figure 4, the highest rates were found in five Ukrainian oblasts: Sumska (30.6%), Kharkivska (30.2%), Chernihivska (28.3%), Zhytomyrska (27.5%), and Ternopilska (26.5%). Ivano-Frankivska, AR Crimea, Volynska, Mykolaivska, Zaporizka, and Khersonska regions had the lowest percentage of positive-response animals within range of 2.6–7.7%. It should be empha-

sized that the results of the research did not reveal any region of Ukraine where the circulation of Aujeszky's disease virus in wildlife was not observed.

Regarding serological studies among domestic pigs, in the period from 2009 to 2020, 378,678 sera samples from this species were tested and 27,897 positive reactions were obtained for the presence of antibodies to PRV, which is 7.4% (BCI, 7.28–7.45%) of total studied. The generalized and systematized results of blood sera from swine studies are presented in Table 2 and Figure 4.

Table 2

The results of blood sera from domestic pigs studied for the presence of specific humoral antibodies to Aujeszky's disease virus according to the reports of regional laboratories and SSRILDVSE

Years	Number of tested samples	Number of positive samples	Percentage of responding animals	Binomial confidence interval
2009	65,949	8,183	12.4	12.16–12.66
2010	32,913	2,789	8.5	8.18–8.78
2011	103,025	7,264	7.1	6.90–7.21
2012	84,431	5,935	7.0	6.86–7.20
2013	45,878	2,252	4.9	4.71–5.11
2014	26,925	1,216	4.5	4.27–4.77
2015	5,364	55	1.0	0.77–1.33
2016	2,948	8	0.3	0.12–0.53
2017	3,209	35	1.1	0.76–1.51
2018	3,677	152	4.1	3.51–4.83
2019	1,534	0	0.0	0
2020	2,825	8	0.3	0.12–0.56
Total	378,678	27,897	7.4	7.28–7.45

Seroprevalence among swine in Ukraine for the analyzed period was the highest in 2009 – 12.4% (BCI, 12.2–12.7%). The lowest rates of this indicator were in 2016 and 2020 – 0.3% (BCI, 0.1–0.5%) and 0.3%; (BCI, 0.1–0.6%), respectively. In 2019, sera samples with humoral antibodies to PRV were not detected. This is due to the very low number of samples tested – 1,534 samples from the four regions were tested during this year. In general, during the analyzed period there is a tendency to significant reduction in the incidence of seropositivity among pigs to the Aujeszky's disease virus.

Sera samples for the investigation were collected from the all regions of Ukraine. The largest numbers of samples were tested in 2011 and 2012 – 103,025 and 84,431 samples, respectively, and the smallest – in 2019 (only 1,534 samples). It should be noted that during the analyzed period, the volume of serological diagnostic of swine for Aujeszky's disease decreased significantly – from 103,025 samples in 2011 to 2,825 in 2020. Data on the scope of serological diagnosis for the detection of specific humoral antibodies to SHV-1 among domestic pigs in terms of Ukrainian regions are presented in Figure 4.

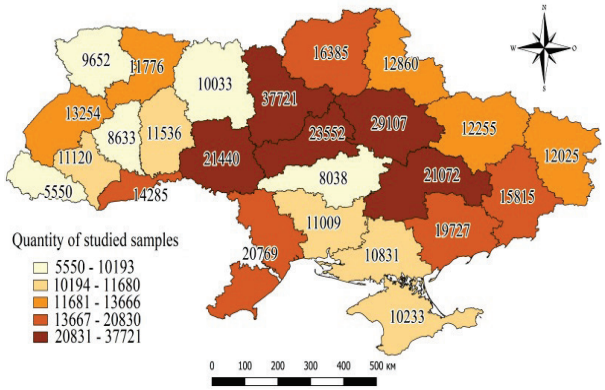

Fig. 4. Quantity of domestic pigs' sera samples tested for Aujeszky's disease virus over 2009–2020

The obtained data indicate that the monitoring studies covered all regions of Ukraine. During the whole investigation period, the largest numbers of sera samples from pigs were studied in six areas: Kyivska (37,721 samples), Poltavska (29,107 samples), Cherkaska (23,552 samples), Vinnytska (21,440 samples), Dnipropetrovska (21,072 samples), and Odeska (20,769 samples) regions. The smallest number of studies was conducted in Volynska (9,652 samples), Ternopilska (8,633 samples), Kirovohradska (8,038 samples), and Zakarpatska (5,550 samples) regions. In our opinion, it should be noted that during the analyzed period, the volume of serological tests decreased significantly, almost 67 times (from 103,205 samples in 2011 to 1,534 samples in 2019). The results of serological monitoring among swine for the twelve years period regarding the detection of specific humoral antibodies to pseudorabies virus in terms of Ukrainian regions are presented in Figure 5.

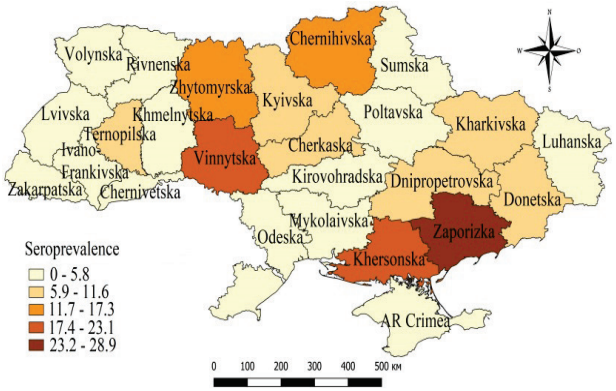

Fig. 5. Cartographic analysis of domestic pigs' seroprevalence to Aujeszky's disease virus in Ukraine over 2009–2020

In general, the seroprevalence rate for pseudorabies among domestic pigs in Ukraine during the analyzed period ranged from 28.9% (in Zaporizka region) to 0.1% (in Odeska region). The highest indicators were found in the five oblasts: Zaporizka (28.9%), Khersonska (18.6%), Vinnytska (18.0%), Chernihivska (13.2%), and Zhytomyrska (12.2%) regions. The lowest percentage of positive-response pigs was registered in the following areas: AR Crimea, Sumska, Volynska, Kirovohradska, Rivnenska, Mykolaivska, and Odeska regions (0.1%–0.7%). In only one region of Ukraine (Chernivetska region) during the investigation period, were sera samples containing antibodies to Aujeszky's disease virus not detected.

The systematized results of serological monitoring among wild boars and domestic pigs for the twelve years period regarding the detection of specific humoral antibodies to PRV in terms of Ukrainian regions are presented in Figure 6. In the vast majority of cases, no correlation is observed between the spread of Aujeszky's disease in wild boars and domestic pigs, with the exception of five areas: Chernihivska, Zhytomyrska, Kharkivska, Kyivska, and Ternopilska regions. According to the results of the territorial spread of Aujeszky's disease studies among wild boars in Ukraine, it was found that the highest incidence rates among this species are observed in the northern and northeastern parts of our country (Fig. 6). At the same time, on the other hand, the highest seroprevalence rates among domestic pigs are observed in the southern and central parts of Ukraine.

Discussion

Despite all preventive, diagnostic and therapeutic measures of veterinary specialists, infectious diseases are widespread among animals in all countries of the world and cause significant economic losses to husbandry. In particular, in pig farming, the main costs are currently associated with limitations on international trade, as well as the cost of vaccination or slaughter of infected animals (OIE, 2018). According to the scientific literature, one of the most common diseases among pigs is Aujeszky's disease (pseudorabies) (Di Marco et al., 2021). PRV remains endemic in Eastern and South-Eastern Europe, Latin America and Asia. Variant strains of this virus continue to circulate among swine in China (Sun et al., 2016). In addition, there is information that about 20.0% of wild boars in the United States are seropositive to this infection. At the same time, the prevalence of pseudorabies among this species in European countries varies up to 60.0% (Lipowski et al., 2017; Ai et al., 2018; Carr et al., 2018).

In general, PRV eradication programs include culling infected animals, vaccination by "marker" vaccines, restricting import of pigs, and isolating domestic pigs from wild boars. In most countries such programs are based on using the vaccines with deletion characteristics ('marker" vaccines) (Freuling et al., 2017; Colomer et al., 2020; Mettenleiter, 2020). The detection of infected or latently infected animals in the herds vaccinated by a gE-negative vaccine is performed by using the discriminant ELISA kits that specifically detect antibodies to PRV gE glycoprotein. If antibodies to PRV gE glycoprotein are detected in the serum of pigs vaccinated by the gE-negative vaccine, they are considered infected (Ai et al., 2018; Silva-Junior et al., 2020). gE-ELISA is almost as sensitive as screening ELISA and significantly exceeds the virus-neutralization reaction (OIE, 2018). Such ELISA kits give opportunity to identify not only infected but also latently infected animals (Silva-Junior et al., 2020). Thus, Aujeszky's disease eradication programs are based on the using of gE-negative (or other glycoprotein-negative) "marker" vaccines and appropriate discriminatory tests (Vannier et al., 1997; Stegeman, 1997; van Nes, 2001; Müller et al., 2003; Korniienko et al., 2020).

As for the spread of the disease, the presence of PRV among wild boars always increases the risk of the pathogen entering domestic pigs or other species of animals, etc. (Pedersen et al., 2013). European researchers also point to the spread of the virus through hunters (Keuling et al., 2008; Cano-Terriza, 2019). However, it should be borne in mind that in most cases, after contact with biomaterials from wild boars, domestic pigs themselves become carriers of the virus (Charrier et al., 2018). For the rest of animal species this disease is fatal ("epizootic deadlock of the pathogen") (Korniienko et al., 2020).

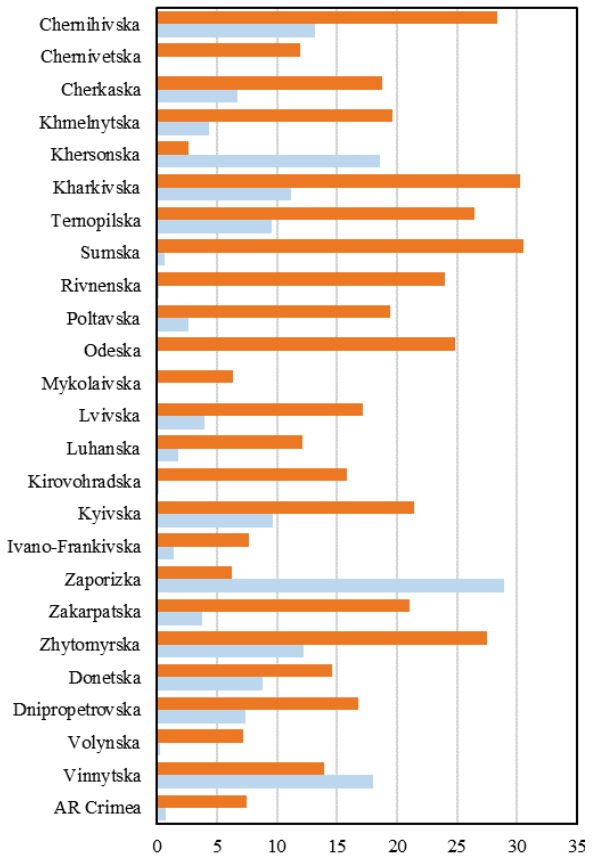

Fig. 6. Results of the seroprevalence studies among domestic pigs and wild boars for Aujeszky's disease virus by regions: orange column – seroprevalence among wild boars, blue column – seroprevalence among domestic pigs

Because PRV strains in wild boars are attenuated, these pathogens, by causing outbreaks in domestic pigs, can lead to the infection without the manifestation of the disease. This contributes to the fact that such outbreaks remain undetected for a long time and cause the further spread of the virus (Charrier et al., 2018). Seroconversion in such animals also occurs with a certain delay. The risks of transmission of the virus from wild boars to domestic pigs are a constant threat and require continuous monitoring. In addition, the PRV infects other species: dogs, cats, cattle, sheep, goats etc. (Pedersen et al., 2013; Di Marco et al., 2021). Recent studies show that the pathogen also affects humans, sometimes causing the development of encephalitis (He et al., 2019; Liu et al., 2021). In Ukraine, according to the official reports of regional laboratories of State Service of Ukraine on Food Safety and Consumer Protection and obtained data of State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv, Ukraine) (reporting form 1-Vet. And 2-Vet.) over 1994–2020 one affected location and two sick dogs were found in Khmelnytska region (1999) and one sick dog in Mykolaivska region (2009). A sick dog was also found in one of the settlements of Vinnytska region in 2015 during an outbreak of Aujeszky's disease among small ruminants. During this period, one affected location and one sick animal were registered among cattle in 1998, and seven infected cattle were found in 1999.

As no large-scale scientific research on Aujeszky's disease has been conducted in Ukraine, the aim of our study was to conduct a comparative retrospective analysis of this disease among wild boars and domestic pigs in our country over 2009–2020 by systematizing and analyzing the epizootic data and serological studies results. According to the literature, these species play a major role as reservoirs of maintaining PRV due to its constant persistence in the organism of pigs and the formation of a latent form of infection (OIE, 2018).

The results of this work have been presented since 2009, as the actual research began only in 2008, when the "Program for the eradication of Aujeszky's disease among swine in Ukraine over 2008–2012" was approved. The main elements of this program, the following measures were: specific vaccination against pseudorabies by using gE-negative "marker" vaccines; conducting serological screening for the presence of antibodies to the glycoprotein gE; introduction of a ban on the import into Ukraine of pigs seropositive to PRV; on farms that do not isolate animals with antibodies for two years, vaccination is stopped and they are given the status of free from this disease; conducting serological monitoring in the wild fauna in order to identify the reservoirs of this disease; ensuring control over the implementation of veterinary and sanitary measures during the conducting of the preventive and health measures for this disease.

As a result of the implementation of these provisions of the program, the number of registered Aujeszky's disease outbreaks among domestic pigs has significantly decreased in Ukraine. Thus, during 2008–2011 the number of locations affected by PRV ranged from three to four, and in 2012 (the last year of the program) only one such farm was registered in Zaporizka region. As a result of the conducted research, there is a clear trend that shows the reduction of the number of affected locations / farms for pseudorabies among domestic pigs in Ukraine. Thus, in 2009 four such farms were found, and in 2018 – only one. During 2014–2016 and 2019–2020, affected farms and locations were not registered for this disease. In total, 21 such points with sick pigs were registered during the analyzed period. For comparison, according to official reports, the largest numbers of such locations (farms, households, etc.) in Ukraine were registered in the early 1990s and 2000s. Thus, in 1994, the eighteen such points were identified, and in 2001 and 2002 – thirteen and fifteen, respectively. As for wild boars, during the analyzed 12 years, locations affected by Aujeszky's disease were registered only in 2013 and 2014 (two and one, respectively). It should be noted that all of them were found in the Autonomous Republic of Crimea.

In addition, we conducted an analysis of serological studies among wild boars and domestic pigs in Ukraine for the period 2009–2020. Studies on the presence of specific humoral antibodies to the PRV in the sera samples of these species were performed by the enzyme-linked immunosorbent assay that are discriminatory and recommended by the World Organization for Animal Health (OIE, 2018).

Analysis of the results of serological studies among domestic pigs showed that the realisation of plans during the implementation of the "Program for the eradication of Aujeszky's disease among swine in Ukraine over 2008–2012" lasted from 2009 to 2012. The slight underperformance of these plans was observed in 2010 (57.7%), 2011 (74.4%), and 2013 (58.0%). At the same time, a significant overfulfillment of the

plan was registered in 2009 (125.9%). After the end of the recovery program, in the period 2015–2018, the implementation of research plans was only 2.6–10.3%. Serological studies among domestic pigs were not planned in 2019–2020. The largest number of seropositive swine was found during 2009–2010 (8.5–12.4%). During the whole investigation period, 378,678 blood sera samples from domestic pigs were actually tested and 27,897 positive reactions were detected, which is 7.4% of the total number of investigated.

Analysis of the results of serological studies among wild boars for the presence of antibodies to the Aujeszky's disease virus showed that the planning and implementation of research effectively began only in 2009 (the beginning of the recovery program). By comparison, in the previous period of 2004–2008, only eleven sera samples from this species were studied. During the period of the implementation of the recovery program (2008–2012), plans were conducted within the range of 80.4–449.6%. Subsequently, in 2016–2018, we registered significant underperformance of plans, but it should be noted that research in these years was also conducted. Overall, during the whole investigation period, 9,052 blood sera samples from wild boars were tested and 1,788 positive reactions were detected, which is 19.8% of the total number of investigated.

In our opinion, it should be emphasized that during the analyzed period there is an irregular volume of serological studies among wild boars' sera for different years of research. For example, in 2014, 1,935 samples were studied, in 2016 – only two samples, and in 2020 no research was conducted at all. In general, after 2014 the number of serological tests among both species for carriers of Aujeszky's disease has significantly decreased.

Regarding the registration of the disease in these species from neighboring countries, the similar studies were conducted in Poland during 2011–2014. Thus, the seroprevalence of antibodies to PRV among wild boars in this country was 27.4% in the hunting season 2011/2012, 32.4% – in 2012/2013, and 35.5% – in 2013/2014. The total seroprevalence was 32.2% (Lipowski et al., 2017). In Russia molecular studies were performed by PCR among this species from the Moscow region and PRV carriers were identified in 2007 (Shcherbakov et al., 2007). At the same time, the literature regarding Aujeszky's disease monitoring in Belarus, Romania and Moldova dates back to the 1970s (Belykh, 1970; Demidov & Kranova, 1975; Sorodoc & Surdan, 1979).

As a result of comparative geographical analysis of the obtained data on the detection of specific humoral antibodies to the Aujeszky's disease virus among both species for 12 years in the regions of Ukraine, there is no established clear correlation between the spread of the disease in wild boars and domestic pigs. Thus, the highest seroprevalence rates among wild boars were observed in the northern and northeastern parts of Ukraine, and in domestic pigs, on the contrary, in the southern and central parts of this country.

Given the data obtained, it can be argued that the leading role in the spread of Aujeszky's disease among domestic pigs in Ukraine is played by the following factors: violation of maintenance conditions, relocation of animals from farms affected by PRV, hypodiagnostics of the disease, violation of quarantine conditions, violation of the terms of susceptible livestock vaccination, etc. In our opinion, wild boars play a secondary role in the spread of Aujeszky's disease among domestic pigs in our country.

Conclusions

An analysis of the epizootic situation regarding Aujeszky's disease in Ukraine during the years 2009–2020 showed that 21 affected locations (farms, households etc.) were officially registered among domestic pigs for that period. For the twelve years, the number of tested sera samples from domestic pigs amounted to 378,678 (7.4% were positive) and from wild boars – 9,052 (19.8% were positive). As a result of comparative geographical analysis of the obtained data on the detection of specific humoral antibodies to the Aujeszky's disease virus among both species for the whole period in the regions of Ukraine, there is no established clear correlation between the spread of the disease in wild boars and domestic pigs, with the exception of five areas: Chernihivska, Zhytomyrska, Kharkivska, Kyivska, and Ternopilska regions.

During the period of validity of the provisions "Program for the eradication of Aujeszky's disease among swine in Ukraine over 2008–2012", there were significant trends in the direction of recovery of our country from this disease. Thus, in the first years after the end of this Program (2014–2016), no affected locations were registered at all for either domestic pigs and other animal species. It should be noted that since 2014 the number of serological tests among both species for carriers of Aujeszky's disease has significantly decreased.

The authors would like to acknowledge the specialists of State Service of Ukraine on Food Safety and Consumer Protection and the State Laboratories of Veterinary Medicine in the regions for presenting the official reports and for assistance.

References

Ai, J. W., Weng, S. S., Cheng, Q., Cui, P., Li, Y. J., Wu, H. L., Zhu, Y. M., Xu, B., & Zhang, W. H. (2018). Human endophthalmitis caused by pseudorabies virus infection, China, 2017. Emerging Infectious Diseases, 24(6), 1087–1090.

Baron, J. N., Aznar, M. N., Monterubbianesi, M., Beatriz Martínez-López, B. (2020). Application of network analysis and cluster analysis for better prevention and control of swine diseases in Argentina. PLoS One, 15(6), e0234489.

Belykh, G. V. (1970). Bolezn Aueski svinej v Moldavii [Aujeszky's disease in swine in Moldavia]. Veterinariia, 10, 62 (in Russian).

Beran, G. W., Davies, E. B., Arambulo, P. V., Will, L. A., Hill, H. T., & Rock, D. L. (1980). Persistence of pseudorabies virus in infected swine. Journal of the American Veterinary Medical Association, 176(10), 998–1000.

Cano-Terriza, D., Martínez, R., Moreno, A., Pérez-Marín, J. E., Jiménez-Ruiz, S., Paniagua, J., Borge, C., & García-Bocanegra, I. (2019). Survey of Aujeszky's disease virus in hunting dogs from Spain. EcoHealth, 16(2), 351–355.

Carr, A. N., Milleson, M. P., Hernandez, F. A., Merrill, H. R., Avery, L., & Wisely, S. M. (2018). Wildlife management practices associated with pathogen exposure in non-native wild pigs in Florida, U.S. Viruses, 11(1), 14.

Charrier, F., Rossi, S., Jori, F., Maestrini, O., Richomme, C., Casabianca, F., Ducrot, C., Jouve, J., Pavio, N., & Le Potier, M. F. (2018). Aujeszky's disease and hepatitis E viruses transmission between domestic pigs and wild boars in Corsica: Evaluating the importance of wild/domestic interactions and the efficacy of management measures. Frontiers in Veterinary Science, 5, 1.

Cheng, T. Y., Henao-Diaz, A., Poonsuk, K., Buckley, A., van Geelen, A., Lager, K., Harmon, K., Gauger, P., Wang, C., Ambagala, A., Zimmerman, J., & Giménez-Lirola, L. (2021). Pseudorabies (Aujeszky's disease) virus DNA detection in swine nasal swab and oral fluid specimens using a gB-based real-time quantitative PCR. Preventive Veterinary Medicine, 189, 105308.

Ciarello, F. P., Moreno, A., Miragliotta, N., Antonino, A., Fiasconaro, M., Purpari, G., Amato, B., Ippolito, D., & Di Marco, P. V. (2022). Aujeszky's disease in hunting dogs after the ingestion of wild boar raw meat in Sicily (Italy): Clinical, diagnostic and phylogenetic features. BMC Veterinary Research, 18(1), 27.

Colomer, M. A., Margalida, A., & Fraile, L. (2020). Vaccination is a suitable tool in the control of Aujeszky's disease outbreaks in pigs using a population dynamics P-systems model. Animals, 10(5), 909.

Corn, J. L., Stallknecht, D. E., Mechlin, N. M., Luttrel, M. P., & Fischer, J. R. (2004). Persistence of pseudorabies virus in feral swine populations. Journal of Wildlife Diseases, 40(2), 307–310.

Demidov, V. A., & Kranova, V. I. (1975). Kompleksnaja immunizatsija svinej protiv chumy, bolezni Aueski i rozhi [Complex immunization of swine against plague, Aujeszky's disease and erysipelas]. Veterinariia, (9), 60–61 (in Russian).

Di Marco, P. V., Moreno, A., Castelli, A., Ippolito, D., Aliberti, A., Amato, B., Vitale, M., Fiasconaro, M., & Pruiti, C. F. (2021). Retrieving historical cases of Aujeszky's disease in Sicily (Italy): Report of a natural outbreak affecting sheep, goats, dogs, cats and foxes and considerations on critical issues and perspectives in light of the recent EU Regulation 429/2016. Pathogens, 10(10), 1301.

Freuling, C. M., Müller, T. F., & Mettenleiter, T. C. (2017). Vaccines against pseudorabies virus (PrV). Veterinary Microbiology, 206, 3–9.

Hahn, E. C., Page, G. R., Hahn, P. C., Gillis, K. D., Romero, C., Annelli, J. A., & Gibbs, E. P. (1997). Mechanisms of transmission of Aujeszky's disease virus originating from feral swine in the USA. Veterinary Microbiology, 55, 123–130.

He, W., Auclert, L. Z., Zhai, X., Wong, G., Zhang, C., Zhu, H., Xing, G., Wang, S., He, W., Li, K., Wang, L., Han, G. Z., Veit, M., Zhou, J., & Su, S. (2019). Interspecies transmission, genetic diversity, and evolutionary dynamics of pseudorabies virus. The Journal of Infectious Diseases, 219(11), 1705–1715.

Keuling, O., Stier, N., & Roth, M. (2008). How does hunting influence and spatial usage in wild boar? European Journal of Wildlife Research, 54, 729–737.

Komaromi, M., & Szabo, I. (2005). Eradication of Aujeszky's disease from a large-scale pig farm. Acta Veterinaria Hungarica, 53(4), 515–524.

Kondibaeva, Z. B., Yespembetov, B. A., Abeuov, K. B., Mussayeva, A. K., Siyabekov, S. T., Nussupova, S. T., Akmatova, E. K., Pazylov, Y. K., Maikhin, K. T.,

& Syrym, N. S. (2021). Inactivated vaccine against Aujeszky's disease. Veterinary World, 14(11), 2957–2963.
Korniienko, L. Y., Mezhenska, N. A., Moroz, O. A., Halatiuk, O. I., Tsarenko, T. M., Kovalenko, V. L., Kuhtyn, M. D., Karpulenko, M. S., Mezhenskyi, A. O., & Harkavenko, T. O. (2020). Povilni infektsijni khvoroby tvaryn [Slow infectious diseases]. Chabanenko Y. A., Cherkasy (in Ukrainian).
Lari, A., Lorenzi, D., Nigrelli, D., Brocchi, E., Faccini, S., & Poli, A. (2006). Pseudorabies virus in European wild boar from central Italy. Journal of Wildlife Diseases, 42(2), 319–324.
Lipowski, A., Szczotka-Bochniarz, A., & Pejsak, Z. (2017). Prevalence of antibodies to Aujeszky's disease virus in wild boar in Poland, between 2011 and 2014: A retrospective study. Journal of Veterinary Research, 61(4), 397–404.
Liu, Q., Wang, X., Xie, C., Ding, S., Yang, H., Guo, S., Li, J., Qin, L., Ban, F., Wang, D., Wang, C., Feng, L., Ma, H., Wu, B., Zhang, L., Dong, C., Li, X., Zhang, J., Chen, H., Yan, R., Wang., X., & Li, W. (2021). A novel human acute encephalitis caused by pseudorabies virus variant strain. Clinical Infectious Diseases, 73(11), 3690–3700.
Lu, J.-J., Yuan, W.-Z., Zhu, Y.-P., Hou, S.-H., & Wang, X.-J. (2021). Latent pseudorabies virus infection in medulla oblongata from quarantined pigs. Transboundary and Emerging Diseases, 68(2), 543–551.
Mahmoud, H. Y., Suzuki, K., Tsuji, T., Yokoyama, M., Shimojima, M., & Maeda, K. (2011). Pseudorabies virus infection in wild boars in Japan. Journal of Veterinary Medical Science, 73(11), 1535–1537.
Mettenleiter, T. C. (2020). Aujeszky's disease and the development of the marker/DIVA vaccination concept. Pathogens, 9(7), 563.
Müller, T., Bätza, H. J., Schlüter, H., Conraths, F. J., & Mettenleiter, T. C. (2003). Eradication of Aujeszky's disease in Germany. Journal of Veterinary Medicine, B, Infectious Diseases and Veterinary Public Health, 50(5), 207–213.
Panyasing, Y., Kedkovid, R., Kittawornrat, A., Ji, J., Zimmerman, J., & Thanawongnuwech, R. (2018). Detection of Aujeszky's disease virus DNA and antibody in swine oral fluid specimens. Transboundary and Emerging Diseases, 65(6), 1828–1835.
Pedersen, K., Bevins, S. N., Baroch, J. A., Cumbee, J. C., Chandler, S. C., Woodruff, B. S., Bigelow, T. T., & De Liberto, T. J. (2013). Pseudorabies in feral swine in the United States, 2009–2012. Journal of Wildlife Diseases, 49, 709–713.
Pomeranz, L. E., Reynolds, A. E., & Hengartner, C. J. (2005). Molecular biology of pseudorabies virus: Impact on neurovirology and veterinary medicine. Microbiology and Molecular Biology Reviews, 69(3), 462–500.
Romero, C. H., Meade, P. N., Homer, B. L., Shultz, J. E., & Lollis, G. (2003). Potential sites of virus latency associated with indigenous pseudorabies viruses in feral swine. Journal of Wildlife Diseases, 39(3), 567–675.
Shcherbakov, A. V., Kukushkin, S. A., Timina, A. M., Baĭbikov, T. Z., Kovalishin, V. F., Kanshina, A. V., Biadovskaia, O. P., Prokhvatilova, L. B., Ruchnova, O. I., Bakunov, I. N., & Babkin, M. V. (2007). Monitoring of infectious diseases among wild boars. Voprosy Virusologii, 52(3), 29–33.
Silva-Junior, L. C., Fontes, K., Nascimento, S. A., Rodriguez, M. C., Camargos, M. F., Freitas, A. C., Castro, R. S., & Jesus, A. (2020). Development of a DIVA ELISA for diagnosis of Aujeszky's disease using recombinant gE fused to thioredoxin as antigen. Veterinary Journal, 257, 105448.
Sorodoc, G., & Surdan, C. (1979). Thirty years of research on animal virus diseases performed in the "Stefan S. Nicolau" Institute of Virology. Virologie, 30(4), 341–346.
Stegeman, A. (1997). Aujeszky's disease (pseudorabies) virus eradication campaign in the Netherlands. Veterinary Microbiology, 55, 175–180.
Sun, Y., Luo, Y., Wang, C. H., Yuan, J., Li, N., Song, K., & Qiu, H. J. (2016). Control of swine pseudorabies in China: Opportunities and limitations. Veterinary Microbiology, 183, 119–124.
Ukhovskyi, V. V., Romanov, O. M., Borisevich, B. V., Tarasov, O. A., Ukhovska, T. M., Pyskun, A. V., Solomon, V. V., & Ayshpur, O. Y. (2020). Pathologic changes in pig organs, infected with the Aujeszky's disease. Ukrainian Journal of Ecology, 10(5), 8–15.
van Nes, A. (2001). Mathematical modelling of pseudorabies virus (syn. Aujeszky's disease virus) outbreaks aids eradication programmes: A review. Veterinary Quarterly, 23(1), 21–26.
Vannier, P, Vedeau, F, & Allemeersch, C. (1997). Eradication and control programmes against Aujeszky's disease (pseudorabies) in France. Veterinary Microbiology, 55(1–4), 167–173.
Vengust, G.,Valencak, Z., & Bidovec, A. (2005). Presence of antibodies against Aujeszky's disease virus in wild boar (*Sus scrofa*) in Slovenia. Journal of Wildlife Diseases, 41(4), 800–802.
World Organization for Animal Health (2018). Chapter 3.1.2: Aujeszky's disease (infection with Aujeszky's disease virus. In: Swenson, S. L. (Ed.). Manual of diagnostic tests and vaccines for terrestrial animals. 8th edition. Bentley Delivery Center, Bentley. Pp. 321–337.