https://doi.org/10.17221/50/2021-VETMED

Serological prevalence of *Leptospira* serovars among pigs in Ukraine during the period of 2001–2019

Vitalii Ukhovskyi[1], Anton Pyskun[2], Leonid Korniienko[1], Halyna Aliekseieva[2], Olexandr Moroz[1], Olena Pyskun[3], Ganna Kyivska[3], Andrii Mezhenskyi[4]*

[1]*Department of Epizootology, State Scientific and Research Institute of Laboratory Diagnostic and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
[2]*Department of Immunological Research, State Scientific and Research Institute of Laboratory Diagnostic and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
[3]*Department of Organization of Scientific and International Work, State Scientific and Research Institute of Laboratory Diagnostic and Veterinary and Sanitary Expertise, Kyiv, Ukraine*
[4]*State Scientific and Research Institute of Laboratory Diagnostic and Veterinary and Sanitary Expertise, Kyiv, Ukraine*

**Corresponding author: uhovskiy@ukr.net*

Citation: Ukhovskyi V, Pyskun A, Korniienko L, Aliekseieva G, Moroz O, Pyskun O, Kyivska G, Mezhenskyi A (2022): Serological prevalence of *Leptospira* serovars among pigs in Ukraine during 2001–2019. Vet Med-Czech 67, 13–27.

Abstract: Leptospirosis is a widespread infection among pigs throughout the world. In most cases in Ukraine, only the microscopic agglutination test (MAT) is used for the diagnosis of leptospirosis in animals. In general, during the period of 2001–2019, 2 381 163 samples of blood sera from swine were tested in our country and 85 338 positive reactions were received, which is 3.58% [binomial confidence intervals (BCI), 3.56–3.61%]. It was established that the serovars *copenhageni* – 33.91% (BCI, 33.59–34.23%), *bratislava* – 14.14% (BCI, 13.90–14.37%), *pomona* – 8.58% (BCI, 8.39–8.77%), and *tarassovi* – 7.12% (BCI, 6.95–7.30%) play a leading role in the aetiological structure of swine leptospirosis. A large number of positive reactions to several serovars was observed – 29.78% (BCI, 29.47–30.09%) of the total number of positive cases. In addition, the article presents data according to a retrospective analysis of the eight serovars circulating among pigs in Ukraine. Thus, during the nineteen year period, there was a decrease in the number of positive reactions to *bratislava*, *pomona*, and *tarassovi* and an increase in the number of positive reactions to *copenhageni*, *polonica*, and *kabura*. Mapping Ukraine's territory for leptospirosis among pigs was carried out. This allows one to identify zones with a risk of leptospirosis infections among swine. The maps show that the highest incidence rates were identified in the eastern and central parts of Ukraine.

Keywords: etiological structure; GIS; leptospirosis; mapping; microscopic agglutination test; swine

The development of swine farming, as well as other livestock industries, is hindered by many infectious diseases, including leptospirosis, which is one of the most common zoonotic infections in many countries (Allan et al. 2015).

In most cases, leptospirosis in pigs is characterised by the polymorphism of the clinical signs and pathological changes in the organs (Ngugi et al. 2019). This disease affects pigs of all ages. In mature animals, the leptospirosis is mostly asymptomatic, but

https://doi.org/10.17221/50/2021-VETMED

it may cause frequent abortions. Sick and chronically infected animals may remain carriers of causative agents for years or for life and serve as reservoirs of the infection for other animals and humans (Ellis 2015). Given all of the above, swine leptospirosis has a worldwide distribution (Bharti et al. 2003) and causes serious long-term economic losses in different countries (Zhang et al. 2020).

The recovery of animals and farms from leptospirosis, as a zoonotic disease, is a very important and actual issue also because sick animals pose a great threat to human health (Samir et al. 2015).

The aetiological structure of leptospirosis is constantly changing and it has some differences in each country (Bertasio et al. 2020; Petri et al. 2020; Poudel et al. 2020). Therefore, an effective specific prevention (vaccination) and a serological diagnosis of leptospirosis require knowledge of the prevalence and aetiological structure of the disease, namely the list of *Leptospira* serovars that cause animal disease in each region (country) (Adler 2015; Felix et al. 2020).

Recently, there has been an increasing change in the aetiological structure of leptospirosis in farm animals in Ukraine, namely: the detection of new serogroups of *Leptospira* that have not been previously registered. Thus, cases of bovine leptospirosis caused by the serovar hardjo have been recorded in Ukraine only since 2014 (Pyskun et al. 2019).

The multi-aetiological structure and the ever-increasing number of new serovars and serogroups significantly complicates the diagnosis of the leptospirosis (Guglielmini et al. 2019). Nowadays, the genus *Leptospira* consists of 20 species and includes nine pathogenic, five intermediate and six saprophytic species. There are more than 300 distinct leptospiral serovars recognised and they are grouped into 25 serogroups (Vincent et al. 2019).

The purpose of this study is to conduct a retrospective analysis of swine leptospirosis in Ukraine and to study the dynamics of the changes in the aetiological structure of leptospirosis in this species during a nineteen year period. The most common *Leptospira* serovars highlighted by serology monitoring plans among pigs in the country during the period of 2001–2019 were identified. The authors studied the territorial distribution of leptospirosis among pigs and conducted an ecological-geographical analysis of the locations with positive reactions to each of eight *Leptospira* serovars, representative of eight serogroups, which are used for the diagnosis of leptospirosis in Ukraine.

MATERIAL AND METHODS

Aetiological structure analysis of leptospirosis in pigs

The spreading and aetiological structure of leptospirosis in pigs was analysed during the period of 2001–2019 according to reports of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE), which is a Ukrainian reference centre for the study of leptospirosis. Samples were collected from pigs of commercial closed-type farms, during routine state monitoring investigations and were examined by specialists of the state laboratories of veterinary medicine of Ukraine (24 regional and more than 100 district laboratories) and SSRILDVSE. Most of the samples were not collected on the basis of clinical sings, but there was also a small part of them that have been carried out with the suspicion of leptospirosis. The monitoring investigation covers all the regions of Ukraine. The number of studies in each area depends on the number of pigs being reared in that area (proportionally: the more pigs in the area, the more samples are tested for leptospirosis). In general, the number of samples tested for leptospirosis among pigs ranged from 1.1% (2003, 2016–2019) to 2.5% (2008 and 2009) of the total number of animals of this species in Ukraine (Figure 1). The seroprevalence for each region was calculated as the number of positive samples divided by the sample quantity in the region. Calculating the binomial confidence intervals (BCI) was performed using the exact Clopper-Pearsor method with a confidence level 95% and was performed for the seroprevalence estimates using the R epitools package (https://cran.r-project.org/web/packages/epitools/epitools.pdf).

Antigens

The cultures of the reference *Leptospira* strains including eight serovars (strains): *canicola* (Hond Utrecht IV), *grippotyphosa* (Moskva V), *kabura* (Kabura), *copenhageni* (M 20), *pomona* (Pomona), *polonica* (493 Poland), *tarassovi* (Perepelicyni), *bratislava* (Jez Bratislava), were used to perform the MAT. These diagnostic strains of *Leptospira*, used in serological studies of leptospirosis in animals, were prepared by veterinary diagnostic labo-

https://doi.org/10.17221/50/2021-VETMED

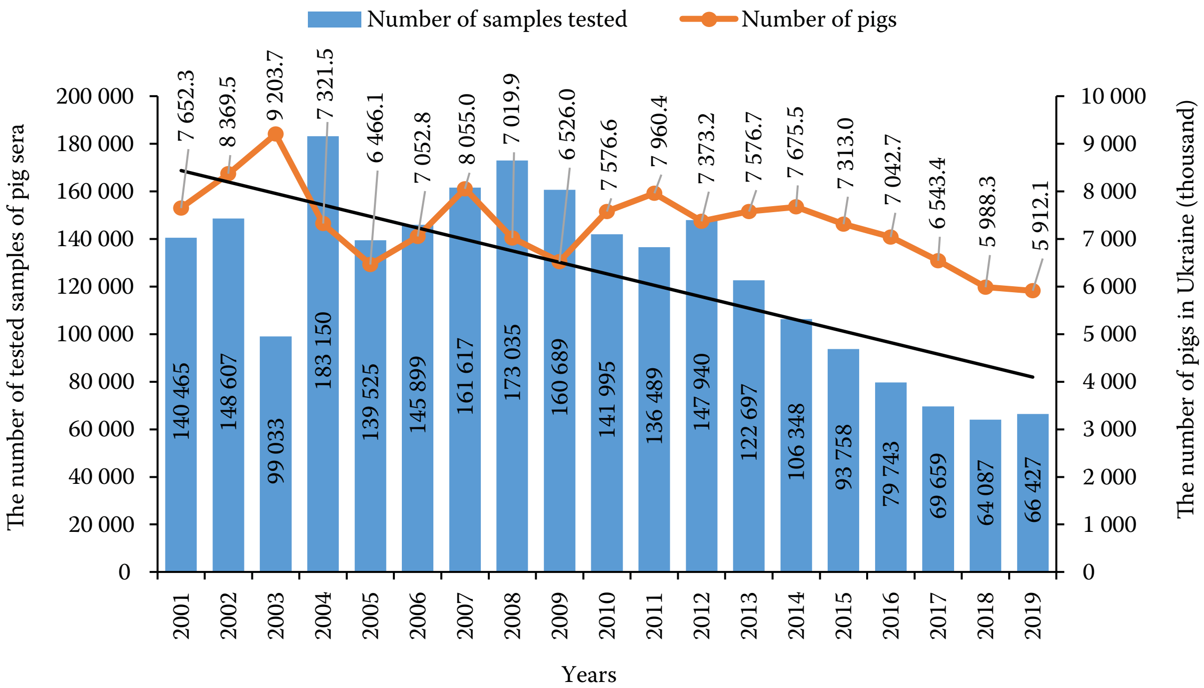


Figure 1. Dynamics of the number of studied blood sera samples from pigs with leptospirosis and the total population of this species in Ukraine (2001–2019)

ratories in Ukraine. The leptospires were cultivated in a Korthof liquid medium at 28–30 °C under aerobic conditions.

Microscopic agglutination test

The test was carried out according to the World Organisation for Animal Health (OIE) Manual of Standards for Diagnostic Tests and Vaccines (Petrakovsky 2021). Briefly, the serum samples diluted to 1 : 25 were mixed with an equal volume of each of the *Leptospira* cultures. The serum dilution (including the added antigen) used during preliminary examination was 1 : 50. For samples reacting in the preliminary examination with one or more serovars, a series of twofold dilutions was prepared to get the end point – 50% agglutination. The samples with titres equal to or higher than 1 : 50 were recognised as positive in the unvaccinated animals. According to Ukrainian veterinary regulations, the blood sera are tested in MAT at the titres of 1 : 50, 1 : 100, 1 : 500, and 1 : 2 500. Therefore, sera studies of titres of more than 1 : 2 500 were not performed (Ministry of Agrarian Policy and Food 1994). It should be noted that the swine serum samples for the studies were collected from unvaccinated animals.

In the case of a positive reaction to several serovars at once (in one sample), according to the Ukrainian instructions (Ministry of Agrarian Policy and Food 1994) for the diagnosis of leptospirosis, a positive test to the serovar to which the antibodies have been detected in the highest titre is considered. A sample is considered positive to several serovars at once (mixed reactions) in the case of the same antibody titre detection.

Mapping and spatial analysis

Data regarding the leptospirosis incidences among pigs in the three regions for 2014–2019 were not available and, thus, they were not mapped.

Data about the number of pigs in the territory of Ukraine were obtained from the website of the State Statistics Service of Ukraine (hukrstat.gov.ua).

Positive incidences by regions were mapped in the software Quantum GIS v3.16.0, that is free on the website: https://www.qgis.org/ru/site/forusers/download.html. The vector layers for the borders of Ukraine's regions were downloaded from the site: https://www.diva-gis.org/Data. A quantile classification with five classes of data was chosen. With this classification, an equal number of regions fall into each class.

https://doi.org/10.17221/50/2021-VETMED

After the systematisation of all the positive reactions, all the regions of the country were divided into five zones of infection risk: very low (from 69 to 432), low (from 433 to 1 079), medium (from 1 080 to 2 488), high (from 2 489 to 6 506), and very high (from 6 507 to 16 092).

RESULTS

The scope of the serological diagnosis among the pigs on the detection of the specific antibodies to pathogenic leptospires in Ukraine for the period 2001–2019 is presented in Figure 1.

As shown on Figure 1, during the period of 2001–2019, the largest number of sera samples were tested in 2004 and 2008 – 183 150 and 173 035 samples, respectively, and the smallest number of sera samples were tested in 2018 and 2019 – 64 087 and 66 427, respectively. It should be noted that in recent years, since 2008, the volume of the serological diagnoses of swine has constantly been decreasing, as evidenced by the trend line. Thus, the number of tested sera decreased from 173 035 samples in 2008 to 64 087 – in 2018 (the number of tested samples decreased by 63.0% for this period).

For comparison purposes, the volumes of studies on leptospirosis and the total number of the pigs in Ukraine are shown in Figure 1. The dynamics of the livestock number is presented, according to the State Statistics Service of Ukraine as of January 1 of each year. As seen from the graph, the number of pigs in Ukraine for the analysed periods also reduced. Thus, during the period of 2008–2018, when the volume of the serological diagnoses of leptospirosis began to decrease, the number of pigs in Ukraine decreased from 7 019.9 to 5 988.3 thousand heads (there was a decrease in the amount of the population by 14.7%). The systematised results regarding the detected positive reactions are presented on the Figure 2.

As shown on Figure 2, the infection of pigs during the analysed period was the highest in 2001, 2002 and 2003 – 6.45% (BCI, 6.32–6.58%), 6.16% (BCI, 6.04–6.29%), and 6.70% (BCI, 6.54 –6.86%), respectively. The lowest levels of infection were observed in 2016, 2017, and 2019 – 1.20% (BCI, 1.12–1.28%), 1.14% (BCI, 1.06–1.22%), and 1.20% (BCI, 1.12–1.29%). In 2009–2014, there was a sharp decline in infections, over the next four years (2014–2017), it was at about the same level – the indicator ranged from 1.14% (BCI, 1.06–1.22%) to 1.57% (BCI, 1.49–1.65%). In the following year, 2018, the seroprevalence increased to 2.12% (BCI, 2.01–2.23%). Overall, according to our investigation, from 2001 to 2019, there is a steady trend to reduce the incidence of leptospirosis infections in pigs.

During the period of 2001–2019, 2 381 163 samples of pigs' sera were investigated by the state veterinary diagnostic laboratories in Ukraine and 85 338 positive reactions to leptospirosis were obtained. The analysis of the results indicates the extensive circulation of leptospires among the swine

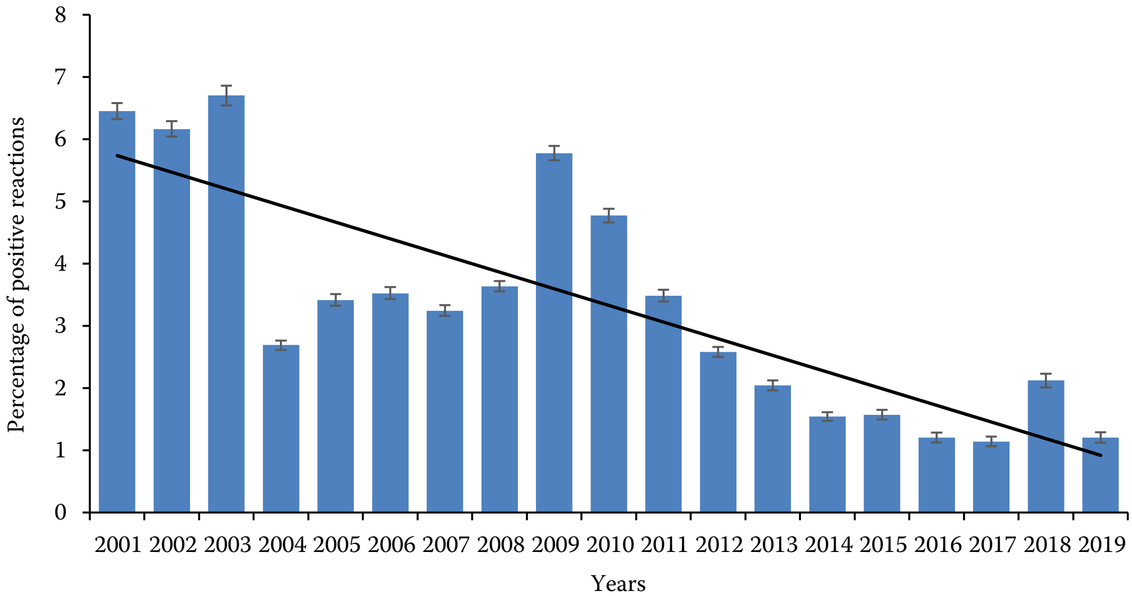


Figure 2. Dynamics of the leptospirosis infection in pigs in Ukraine (2001–2019)

https://doi.org/10.17221/50/2021-VETMED

population in Ukraine as evidenced by the percentage of positively responding pigs by MAT of 3.58% (BCI, 3.56–3.61%) of the studied samples. The majority of the positive reactions were registered at a titre of 1 : 100, slightly less at a titre of 1 : 50. Titres of 1 : 500 and 1 : 2 500 were observed in only a small number of reactions. The aetiological structure highlighted by the serology monitoring plans' serovars is shown on Figure 3.

As shown in Figure 3, the dominant serovars of *Leptospira* were *copenhageni* (33.91%; BCI, 33.59–34.23%), *bratislava* (14.14%; BCI, 13.90–14.37%), *pomona* (8.58%; BCI, 8.39–8.77%), and *tarassovi* (7.12%; BCI, 6.95–7.30%). Positive reactions with other serovars were observed less frequently: *canicola* – 3.10% (BCI, 2.98–3.22%), *grippotyphosa* – 1.86% (BCI, 1.77–1.95%), *polonica* – 0.84% (BCI, 0.78–0.90%), and *kabura* – 0.67% (BCI, 0.62–0.73%). The proportion of disease cases where antibodies to multiple serovars of *Leptospira* (mixed reactions) were detected was 29.78% (BCI, 29.47–30.09%).

In the next stage of our investigation, we analysed the locations with positive reactions of swine leptospirosis for the period of 2001–2019 to each of the eight serovars that were used in the diagnosis of this disease in Ukraine by MAT.

Dynamics of the serovar *copenhageni* seroprevalence among pigs in Ukraine

During the studied period, 28 940 positive reactions to the serovar *copenhageni* were obtained, which is 33.91% (BCI, 33.59–34.23%) of the total number of positive responding pigs. This pathogen is widespread in Ukraine among the swine population and ranks first place in a number of positive

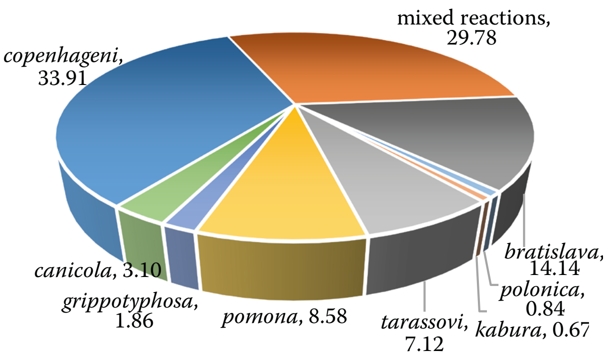


Figure 3. Aetiological structure of leptospirosis among pigs in Ukraine (2001–2019)

reactions (aetiological structure) of leptospirosis (Figure 4A).

The infections caused by the serovar *copenhageni* for the analysed period was the highest in 2019 – 70.01% (BCI, 66.70–73.18%). The lowest levels of this infection were in 2011, 2013, and 2014 – 15.92% (BCI, 14.89–16.99%), 17.66% (BCI, 16.18–19.21%), and 22.56% (BCI, 20.55–24.66%), respectively. In general, during the analysed period, we detected a difference in the percentage of infections by this serovar. Thus, from 2001 to 2019, there is a slight tendency to increase the incidence of infections in pigs with this serovar (especially in the last five years). However, in absolute terms, there was a decreasing tendency in the infection.

Dynamics of the serovar *bratislava* seroprevalence among pigs in Ukraine

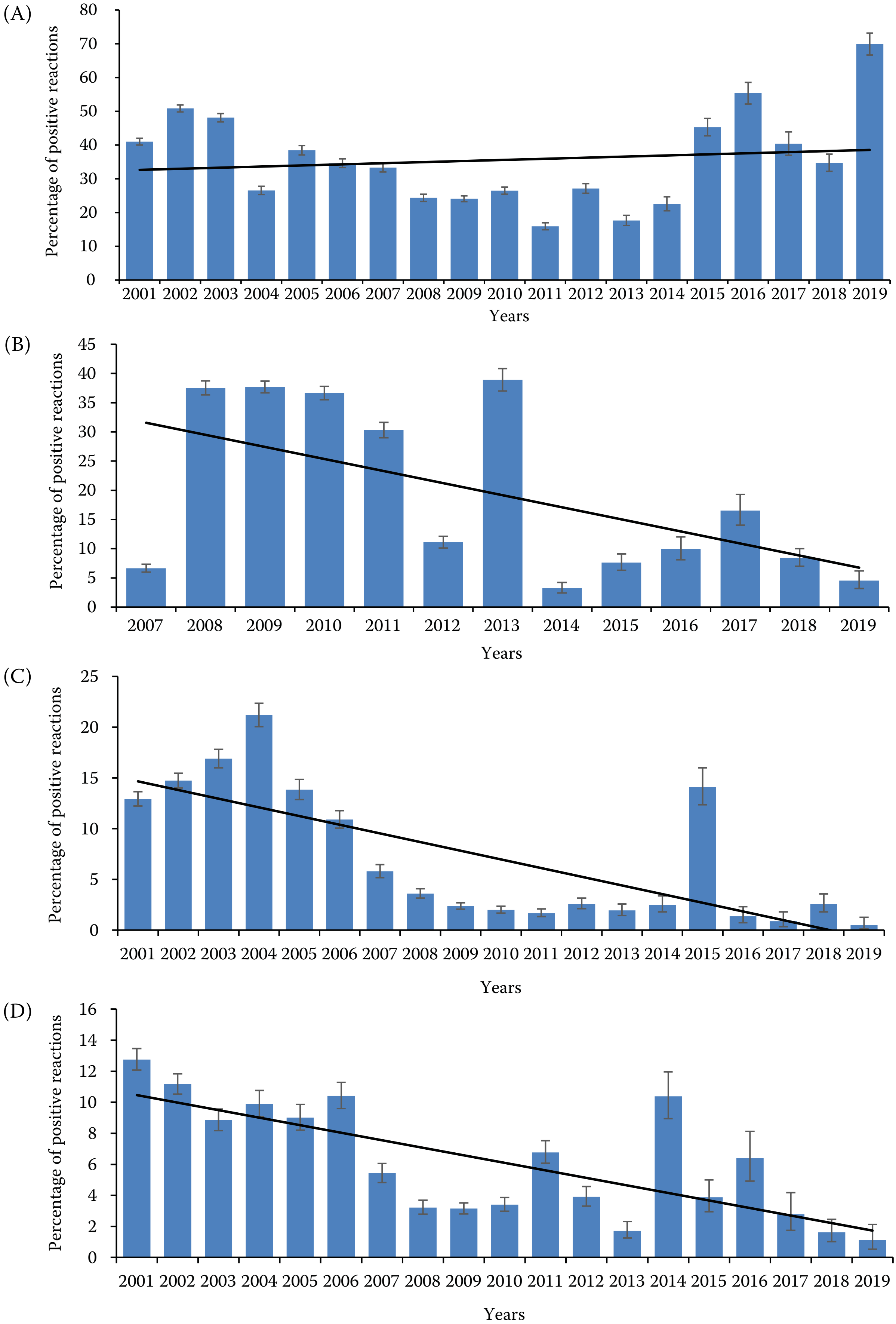
The serovar *bratislava* was added to the Ukrainian list of diagnostic strains, used for serum testing on leptospirosis, only in 2007. Overall, during the nineteen years, the state veterinary laboratories of Ukraine had received 12 065 positive reactions to this pathogen, which is 14.14% (BCI, 13.90–14.37%) of the total number of positive responding pigs. This significant infection percentage indicates that this microorganism is one of the dominant serovars of *Leptospira* among livestock in our country and it plays an important role in swine leptospirosis (Figure 4B).

The peaks of infection caused by the serovar *bratislava* were in 2008, 2009, 2010, and 2013 when these indicators were 37.53% (BCI, 36.33–38.74%), 37.69% (BCI, 36.70–38.69%), 36.65% (35.50–37.81%), and 38.91% (BCI, 37.00–40.86%) (at the beginning of the research when using this microorganism as an antigen in MAT). The least number of cases were registered in 2014 – 3.24% (BCI, 2.44–4.22%). In general, from 2007 to 2019, there was a steady decrease in the infection of pigs with this serovar. This is evidenced by the trend line of the seropositive swine numbers with this serovar.

Dynamics of the serovar *pomona* seroprevalence among pigs in Ukraine

During the studied period, 7 322 positive reactions to the serovar *pomona* were detected, which

https://doi.org/10.17221/50/2021-VETMED



https://doi.org/10.17221/50/2021-VETMED

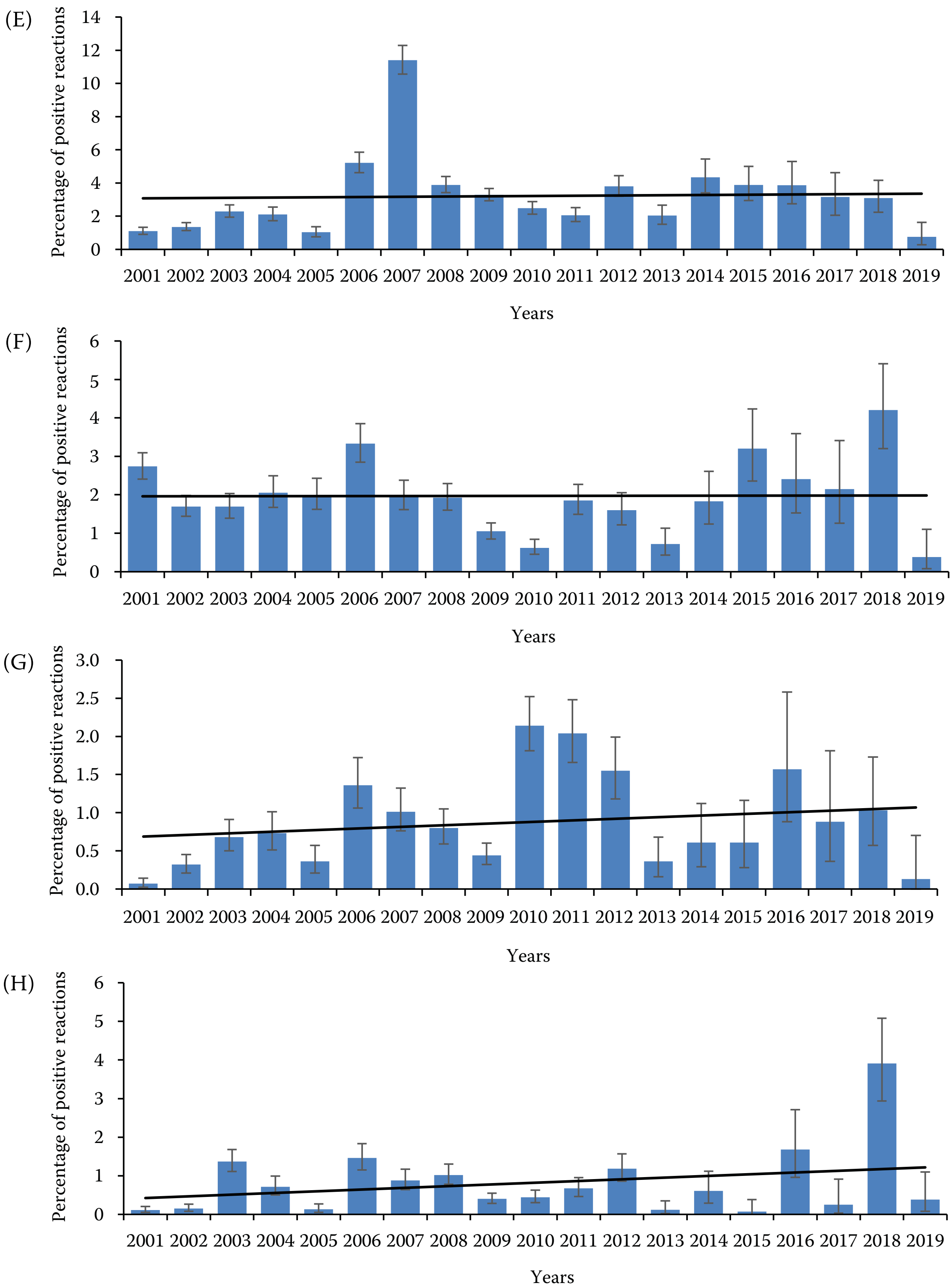


Figure 4. Dynamics of the seropositive swine number to various *Leptospira* serovars (2001–2019)
(A) Sv. *copenhageni* (serogroup *Icterohaemorrhagiae*). (B) Sv. *bratislava* (serogroup *Australis*). (C) Sv. *pomona* (serogroup *Pomona*). (D) Sv. *tarassovi* (serogroup *Tarassovi*). (E) Sv. *canicola* (serogroup *Canicola*). (F) Sv. *grippotyphosa* (serogroup *Grippotyphosa*). (G) Sv. *polonica* (serogroup *Sejroe*). (H) Sv. *kabura* (serogroup *Hebdomadis*)

https://doi.org/10.17221/50/2021-VETMED

is 8.58% (BCI, 8.39–8.77%) of the total number of positive responding pigs. As shown in Figure 4C, this pathogen plays a leading role in the aetiological structure of swine leptospirosis in Ukraine.

Thus, during the analysed period, the infection caused by this agent was the highest in 2004 – 21.18% (BCI, 20.05–22.35%), and the lowest in 2017 and 2019 when these indicators were 0.88% (BCI, 0.36–1.81%) and 0.50% (BCI, 0.14–1.28%), respectively. During the systematisation of the obtained results, two main periods were observed: the first – from 2001 to 2004, when a steady annual increase in the cases of the disease caused by this serovar was registered from 12.92% (BCI, 12.24–13.63%) in 2001 to 21.18% (BCI, 20.05–22.35%) in 2004; the second – from 2004 to 2019, there was a steady annual decrease in the cases of swine infection from 21.18% (BCI, 20.05–22.35%) in 2004 to 0.50% (BCI, 0.14–1.28%) in 2019. The exception was 2015, when the infection rate was 14.10% (BCI, 12.36–15.99%). This is due to locations with positive reactions to swine leptospirosis in 2015 in the Poltavska region.

In general, during the analysed period, we observed a decreasing tendency in the rates of infection caused by the serovar *pomona*. In absolute terms (the number of positive reactions per year), a steady decrease in infections was also registered.

Dynamics of the serovar *tarassovi* seroprevalence among pigs in Ukraine

For the analysed period, veterinary laboratories received 6 080 positive reactions to serovar *tarassovi*, which is 7.12% (BCI, 6.95–7.30%) of the total number of positive responding pigs. This *Leptospira* serovar is widespread in Ukraine among livestock and ranks fourth (in the number of positive reactions) in the aetiological structure of swine leptospirosis (Figure 4D).

The infection rate was the highest in 2001 – 12.76% (BCI, 12.08–13.46%), and the lowest in 2013 and 2019 – 1.72% (BCI, 1.25–2.31%) and 1.13% (BCI, 0.52–2.13%), respectively. In general, as in previous cases, during the period of 2001–2019, we observed a decreasing tendency in the rates of infection caused by this serovar.

In regards to the whole period, there was a significant increase in the number of cases of leptospirosis caused by serovar *tarassovi* in 2014 only, when the percentage of infected animals was 10.39% (BCI, 8.95–11.97%). This is 8.67% higher than in the previous year – 1.72% (BCI, 1.25–2.31%). Regarding the absolute indicators (the number of positive reactions per year), there was also a downward trend in the infection: from 1 156 in 2001 to nine positive samples in 2019.

Dynamics of the serovar *canicola* seroprevalence among pigs in Ukraine

During the studied period, 2 644 positive reactions to the serovar *canicola* were obtained, which is 3.10% (BCI, 2.98–3.22%) of the total number of positive responding pigs. The systematised results of our research indicate that this pathogen does not play a leading role in the aetiological structure of swine leptospirosis (Figure 4E).

The infections caused by this serovar during the analysed period was the highest in 2007 – 11.4% (BCI, 10.56–12.29%). The least number cases were detected in 2001, 2005, and 2019 years and amounted to 1.10% (BCI, 0.90–1.34%), 1.03% (BCI, 0.76–1.36%), and 0.75% (BCI, 0.28–1.63%), respectively. Overall, from 2001 to 2019, no significant changes in the percentage of infections were registered. These figures were roughly at the same level, with the exception of 2007. The increase in the cases of infection among pigs this year is due to locations with positive reactions of leptospirosis in the Donetska and Mykolaivska regions. In absolute terms (the number of positive reactions per year), from 2001 to 2019, there was a steady downward trend in the infection rate.

Dynamics of the serovar *grippotyphosa* seroprevalence among pigs in Ukraine

During the experimental period, 1 589 positive reactions to the serovar *grippotyphosa* were received, which is 1.86% (BCI, 1.77–1.95%) of the total number of positive responding pigs. This pathogen ranks sixth in the number of positive reactions in the aetiological structure of leptospirosis in this species (Figure 4F).

The infection rates were the highest in 2006, 2015, and 2018 – 3.33% (BCI, 2.85–3.85%), 3.20% (BCI, 2.36–4.23%), and 4.20% (BCI, 3.20–5.41%), respectively, and the lowest infection rates were in 2010,

https://doi.org/10.17221/50/2021-VETMED

2013, and 2019 – 0.62% (BCI, 0.45–0.84%), 0.72% (BCI, 0.43–1.13%), and 0.38% (BCI, 0.08–1.10%), respectively.

In general, from 2001 to 2019, the percentage of swine infections by the serovar *grippotyphosa* was approximately at the same level.

Dynamics of the serovar *polonica* seroprevalence among pigs in Ukraine

In total, 713 positive reactions to the serovar *polonica* were obtained, which is 0.84% (BCI, 0.78–0.90%) of the all positive responding pigs.

According to the results of our research, this serovar is not widespread in Ukraine among the swine population (Figure 4G). Thus, the infection rates were the highest in 2010 and 2011 – 2.14% (BCI, 1.81–2.52%) and 2.04% (BCI, 1.66–2.48%), respectively, and the lowest in 2001 and 2019 – 0.07% (BCI, 0.02–0.14%) and 0.13% (BCI, 0.00–0.70%), respectively. In general, the variability of the infection indicators of this pathogen was identified during the whole analysis period. Increases in the infections of pigs with this *Leptospira* serovar are mainly associated with isolated locations with positive reactions to the disease, which are caused by violations of segregation conditions (keeping cattle and pigs separate).

Due to this, from 2001 to 2019, there is a slight increasing tendency in the infection incidences with the serovar.

Dynamics of the serovar *kabura* seroprevalence among pigs in Ukraine

During the analysed period, 573 positive reactions to the serovar *kabura* were obtained by the state veterinary laboratories of Ukraine, which is 0.67% (BCI, 0.62–0.73%) of the total number of positive responding pigs. Such a small percentage of infection indicates that this *Leptospira* serovars is not widespread in Ukraine among the pigs (Figure 4H).

The peak of the swine leptospirosis from this pathogen was in 2018 and amounted to 3.91% (BCI, 2.94–5.08%). This increase is due to small locations with positive reactions (40 pigs seropositive to the serovar *kabura*) of leptospirosis in the Sumy region. At the same time, the least number of cases were detected in 2001 [0.11% (BCI, 0.05–0.20%)], 2005 [0.13% (BCI, 0.05–0.27%)], 2013 [0.12% (BCI, 0.02–0.35%)], and 2015 [0.07% (BCI, 0.00–0.38%)].

In general, for the period from 2001 to 2019, there was a slight increase in the infection rate by this causative agent, as evidenced by the trend line.

Territorial distribution of the main *Leptospira* serovars among the swine population in Ukraine

An ecological-geographical analysis of the swine leptospirosis locations with positive reactions to each of the eight *Leptospira* serovars was also carried out, which were used for the diagnosis of this species leptospirosis in Ukraine. On the basis of the obtained data and the systematisation of the research results "Maps of the case distribution of seropositive pigs to various *Leptospira* serovars" were created by using the software QGIS v3.16.0 (Figure 5). The maps help to visualise the density of the positive sample numbers from pigs to the main *Leptospira* serovars in the context of the Ukraine regions by using different colour intensities for each region.

In order to conduct a territorial analysis with spatial patterns of the aetiological structure, a "Map of the aetiological structure of swine leptospirosis in regions of Ukraine" was compiled (Figure 6). It shows the percentages of eight *Leptospira* serovars that were used for the MAT in Ukraine by visualisation through pie charts.

As shown on the map, the aetiological structure of the swine leptospirosis in the different regions of Ukraine is not heterogeneous. For example, in the Mykolaivska area, it is various and represented by all eight serovars: *copenhageni* – 17.98% (BCI, 17.05–18.93%); *bratislava* – 13.48% (BCI, 12.65–14.33%); *canicola* – 12.15% (BCI, 11.36–12.97%); *tarassovi* – 6.85% (BCI, 6.24–7.49%); *pomona* – 3.98% (BCI, 3.52–4.48%); *grippotyphosa* – 4.01% (BCI, 3.54–4.51%); *kabura* – 2.11% (BCI, 1.78–2.49%); *polonica* – 1.57% (BCI, 1.28–1.91%). There were also cases when antibodies to several serogroups of *Leptospira* were detected in the sera samples – 37.88% (BCI, 36.70–39.08%). Similarly, a variety of aetiological structures were registered in the following areas: Volynska, Sumska, Cherkaska, Khmelnytska, etc.

However, at the same time, areas where the aetiological structure was homogeneous were also

https://doi.org/10.17221/50/2021-VETMED

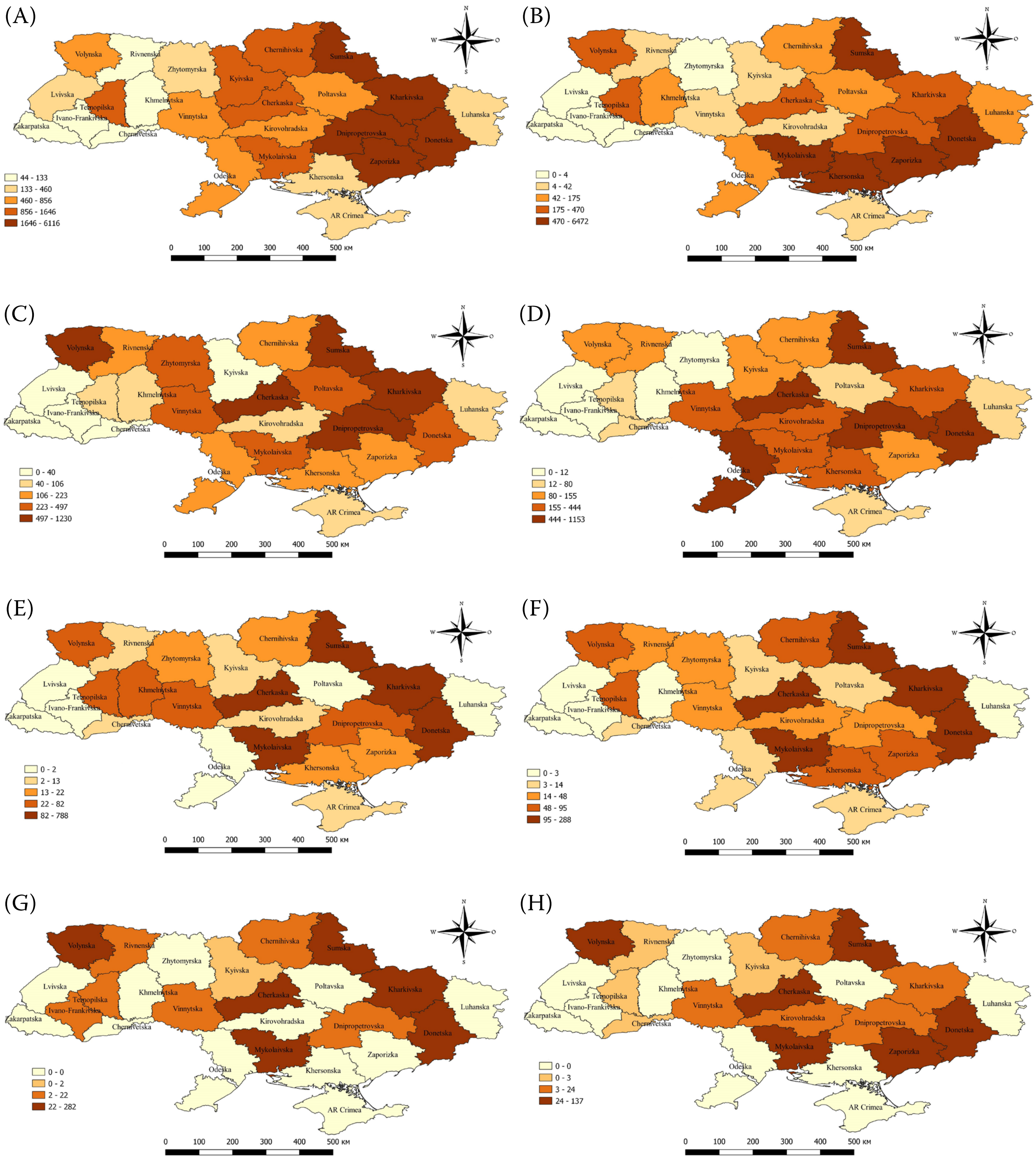


Figure 5. Maps of the case distribution of seropositive pigs to various *Leptospira* serovars (2001–2019)
(A) Sv. *copenhageni* (serogroup *Icterohaemorrhagiae*). (B) Sv. *bratislava* (serogroup *Australis*). (C) Sv. *pomona* (serogroup *Pomona*). (D) Sv. *tarassovi* (serogroup *Tarassovi*). (E) Sv. *canicola* (serogroup *Canicola*). (F) Sv. *grippotyphosa* (serogroup *Grippotyphosa*). (G) Sv. *polonica* (serogroup *Sejroe*). (H) Sv. *kabura* (serogroup *Hebdomadis*)

registered. For example, in the Zhytomyrska region, it is represented by only a few of the following serogroups of *Leptospira*: *copenhageni* – 48.00% (BCI, 44.64–51.37%); *pomona* – 48.00% (BCI, 44.64–51.37%); *canicola* – 1.83% (BCI, 1.05–2.95%); *grippotyphosa* – 1.71% (BCI, 0.96–2.81%); *tarassovi* – 0.46% (BCI, 0.12–1.17%). The Kyivska, Lvivska, Odeska, Rivnenska regions as well as the other regions are also homogeneous in terms of the aetiological structure.

https://doi.org/10.17221/50/2021-VETMED

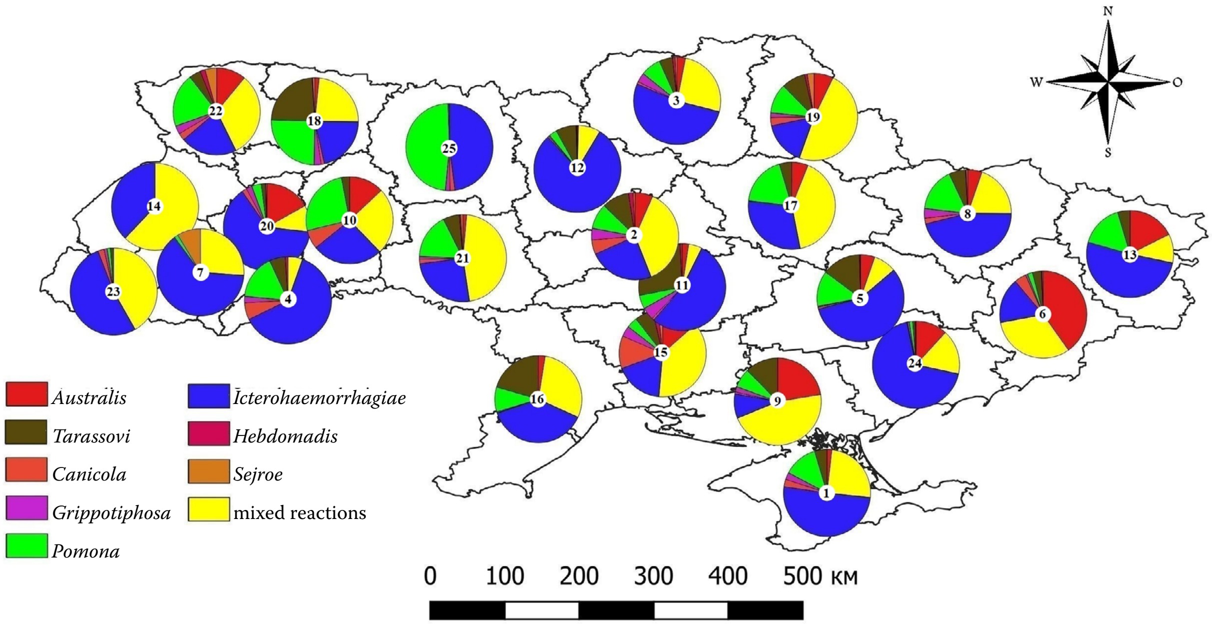


Figure 6. Map of the aetiological structure of the swine leptospirosis in the regions of Ukraine (2001–2019)
1 – AR Crimea, 2 – Cherkaska, 3 – Chernihivska, 4 – Chernivetska, 5 – Dnipropetrovska, 6 – Donetska, 7 – Ivano-Frankivska, 8 – Kharkivska, 9 – Khersonska, 10 – Khmelnytska, 11 – Kirovohradska, 12 – Kyivska, 13 – Luhanska, 14 – Lvivska, 15 – Mykolaivska, 16 – Odeska, 17 – Poltavska, 18 – Rivnenska, 19 – Sumska, 20 – Ternopilska, 21 – Vinnytska, 22 – Volynska, 23 – Zakarpatska, 24 – Zaporizka, 25 – Zhytomyrska

The spreading of leptospirosis among pigs in Ukraine

Based on the obtained results regarding the swine leptospirosis, an epidemiological ranking of the territory of Ukraine for the nineteen year period was conducted and the "Map of the cases density of swine leptospirosis in Ukraine" was created (Figure 7). Depending on the number of cases, all the regions of the country were divided into five zones of infection risk: very low, low, medium, high, and very high.

The very high risk zone includes the following regions: Donetska, Sumska, Zaporizka, Dnipropetrovska, and Cherkaska. The total number of positive cases in this zone is 59.52%. For this zone, regions with borderline indicators are the Donetska and Cherkaska regions with 18.86% and 7.72% of the cases, respectively. Areas in this zone have a very high probability of developing swine leptospirosis.

The high risk zone includes five regions: Mykolaivska, Kharkivska, Vinnytska, Volynska, and Khersonska. The total number of cases of positive responding pigs in this zone is 23.79%. Regions with borderline indicators are Mykolaivska – 7.60% and Khersonska – 3.25 % of the cases.

The medium risk zone of swine leptospirosis includes: Chernihivska, Ternopilska, Odeska, Poltavska, and Kyivska. The total number of cases of positive responding pigs in this zone is 11.10%. In this zone, the most positive and negative reactions were observed in the Chernihivska and Kyivska regions (2.69% and 1.33%), respectively.

The low risk zone of the disease includes: Kirovohradska, Lvivska, Zhytomyrska, Rivnenska, and AR Crimea. The total number of cases of positive responding pigs in this zone amounted to 4.47%. For this zone, the regions with borderline indicators are: Kirovohradska – 1.17% and AR Crimea – 0.53% of the cases.

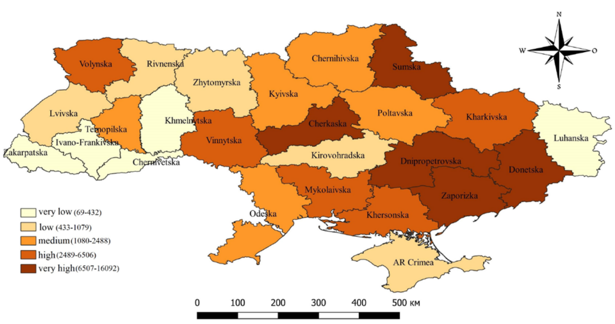


Figure 7. Map of the case densities of the swine leptospirosis in Ukraine (2001–2019)

https://doi.org/10.17221/50/2021-VETMED

In the Khmelnytska, Luhanska, Chernivetska, Zakarpatska and Ivano-Frankivska regions, the lowest number of positive reactions to leptospirosis was observed over the last nineteen years. Therefore, they are assigned to the very low risk zone of infection. During the analysed period, the total number of positive reactions in this zone was 1.12%. In the data range of this zone, the highest levels are registered in the Khmelnytska region (1.33%), and the lowest levels (in this zone, as well as in Ukraine in general) are registered in the Ivano-Frankivska region – 0.08% of the total number of all the positive responding pigs.

DISCUSSION

It is extremely important for practicing veterinarians and scientists to know which *Leptospira* serovars are common in the particular area and which of them dominate in the serological diagnosis on swine leptospirosis. This information is necessary to select the most effective vaccine, because it is highly effective only if it includes the *Leptospira* serovars, which are the causative agents in the area (Petrakovsky 2021). If it does not contain any serovar, which is the causative agent of leptospirosis in pigs or other species in this area, the vaccine will be ineffective and defective. In such cases, leptospirosis will occur despite the vaccination (Adler 2015). Also, knowledge about the prevalence of *Leptospira* serovars in the country and specific regions is necessary to align the diagnostic kit of antigens for MAT (Goarant 2016). As for Ukraine, the selection of antigens for MAT was placed on a scientific basis only in the 60s of the last century after the creation of the modern leptospires classification. The diagnostic kit of the strains recommended by scientists and experts is constantly changing. One strain of each *Leptospira* serovar with the widest antigenic spectrum from each serogroup was used in the MAT. The number and composition of the antigens is determined based on the aetiological structure of the leptospirosis for this region or country.

When selecting antigens, it should be kept in mind that the introduction into the reaction of each new antigen complicates the study and, at the same time, using a small number of strains cannot fully ensure the detection of *Leptospira*-infected animals (Koizumi 2020).

Therefore, we analysed the scope of the pigs' samples for the detection of specific antibodies to pathogenic leptospires in Ukraine for the period of 2001–2019. According to the results of our research, it is established that, since 2008, the volumes of the serological diagnostics of pigs with leptospirosis are constantly decreasing. Thus, during the period of 2008–2018, the number of tested samples decreased by 63.0%. In general, comparing the indicators of the decreasing volume of serological diagnostics with the indicators of the decreasing total number of livestock, we can say that, recently, they have significantly reduced and are not related to each other.

In the next step, we analysed the situation regarding the infection of pigs with *Leptospira* serovars during the analysed period. It was established that, from 2001 to 2019, there was a steady trend in reducing the incidence of leptospirosis among pigs. During the period of 2009–2014, there was a rapid decline in infections, which is due to two main reasons in our opinion. The first, it is an extension of the diagnostic *Leptospira* kit for MAT (the serovar *bratislava* was added in 2007) (Piotrovych et al. 2006). This, in turn, led to an increase in the number of positive reactions in 2009, and their number began to drop later. Secondly, it is the fact that, since 2009, vaccines began to be used for the prevention of leptospirosis in pigs which included the most common serovars in Ukraine. In particular, the serovar *bratislava* was added to the vaccines of Ukrainian manufacturers (Piotrovych et al. 2008).

Nowadays, according to the Ukrainian legislation, for the diagnostics of swine leptospirosis in both the regional and district laboratories of veterinary medicine, MAT is conducted with leptospires of eight serovars: *copenhageni* (serogroup *Icterohaemorrhagiae*); *bratislava* (serogroup *Australis*); *pomona* (serogroup *Pomona*); *tarassovi* (serogroup *Tarassovi*); *canicola* (serogroup *Canicola*); *grippotyphosa* (serogroup *Grippotyphosa*); *polonica* (serogroup *Sejroe*); *kabura* (serogroup *Hebdomadis*) (Ministry of Agrarian Policy and Food 1994).

According to the opinion of the leading experts in the leptospirosis field, the aetiological structure of this disease requires constant serological monitoring among farm animals and dogs, synanthropic rodents and all inhabitants of natural foci (Zhang et al. 2019; Cilia et al. 2020). Thus, we analysed the locations with positive reactions of swine leptospirosis for the period of 2001–2019 to each of the eight se-

https://doi.org/10.17221/50/2021-VETMED

rovars used in the diagnostic kit for MAT in Ukraine. It was found that there was a decrease in the number of positive reactions to *bratislava*, *pomona*, and *tarassovi* during the whole investigated period. We explain this by the fact that the polyvalent vaccine against animal leptospirosis (suis variant) which includes antigens to these pathogens (Piotrovych et al. 2008) is widely used for the prevention of swine leptospirosis in Ukraine.

At the same time, there was an increase in the number of positive reactions with the serovars *copenhageni*, *polonica*, and *kabura*. The latter two are the main aetiological factors of leptospirosis in cattle and small ruminants in Ukraine (Ukhovskyi et al. 2014). We attribute their increase to the violation of segregation conditions (keeping animals separate). It should be emphasised that although the number of positive reactions to them has increased, these pathogens are not dominant in the aetiological structure of swine leptospirosis in Ukraine. Their percentage in the aetiological structure is only 0.84% (BCI, 0.78–0.90%) for *polonica* and 0.67% (BCI, 0.62–0.73%) for *kabura*. As for the serovar *copenhageni*, for pigs, its source is rats (Boey et al. 2019). Therefore, we attribute the increase in the number of positive reactions to it with the increasing number of rats on swine farms and improper deratisation.

The percentages of swine infections by the serovars *canicola* and *grippotyphosa* were approximately at the same level.

In general, the results of our research have shown that leptospirosis is widespread among pigs in Ukraine. The average infection rate of animals for the period from 2001 to 2019 was 3.58% (BCI, 3.56–3.61%).

It was established that the serovars *copenhageni* – 33.91% (BCI, 33.59–34.23%), *bratislava* – 14.14% (BCI, 13.90–14.37%), *pomona* – 8.58% (BCI, 8.39–8.77%), and *tarassovi* – 7.12% (BCI, 6.95–7.30%) play a leading role in the aetiological structure of swine leptospirosis in Ukraine. Positive reactions with other serovars were detected less frequently: *canicola* – 3.10% (BCI, 2.98–3.22%); *grippotyphosa* – 1.86% (BCI, 1.77–1.95%); *polonica* – 0.84% (BCI, 0.78–0.90%); *kabura* – 0.67% (BCI, 0.62–0.73%) and they play a secondary role. At the same time, during the analysed period, a large number of positive reactions to several serogroups of *Leptospira* (mixed reactions) were observed, which was 29.78% (BCI, 29.47–30.09%).

To understand the territorial distribution of the main *Leptospira* serovars among the swine population in Ukraine the "Maps of the cases distribution of seropositive pigs to various *Leptospira* serovars" and the "Map of the aetiological structure of swine leptospirosis in regions of Ukraine" were created.

On the basis of the obtained data and the systematisation of the research results, we detected heterogeneity in the aetiological structure in different regions. It was found that the serovar profile of the swine leptospirosis in Ukraine has pronounced differences both in the set of serovars and in their importance for their pathology during infection.

The maps show that the highest incidence rates were identified in the eastern and central parts of Ukraine.

The epidemiological ranking of the territory of Ukraine established that the zone of very high risk of infection includes five regions: Donetska, Sumska, Zaporizka, Dnipropetrovska, and Cherkaska. Overall, 59.52% of the positive reactions were detected in this zone. In the Khmelnytska, Luhanska, Chernivetska, Zakarpatska and Ivano-Frankivska regions, the least number cases of leptospirosis were observed over the last nineteen years and, therefore, they are classified as having a very low risk of infection. According to the literature, the variability of the spectrum of pathogens and the different prevalence of swine leptospirosis in different regions is largely dependent on the natural conditions and the degree of the rodent population. However, in our opinion, the most important reasons for the widespread incidence of leptospirosis among pigs in Ukraine are violations of the raising conditions of the animals and their frequent and uncontrolled movement from one farm to another (for example, boars and sows are sometimes removed from affected *Leptospira* farms and transferred to other farms that are free from leptospirosis). At the same time, natural conditions and the rodent population play a secondary role in the occurrence of leptospirosis among this species.

In recent years, leptospirosis of farm animals in Ukraine has almost lost its landscape-stationary component. Affected farms have cropped up in different climatic and geographical areas. Currently, infections have been registered in farms in the steppe zone, where there are almost no conditions for infecting animals in nature (Kucheriavenko et al. 2005). It should be noted that until the mid-90s, leptospirosis was only registered in isolated

https://doi.org/10.17221/50/2021-VETMED

cases in this area. The reason for the current spread of this disease in the southern regions is the eradication of anthropological foci, where the pathogen is spread by domestic animals without maintaining the epizootic process by natural reservoirs and hosts.

We did not establish a relationship between the number of positive reactions to leptospirosis and the different zonings of Ukraine based on the climate, topography, etc. Therefore, we believe that the leading roles in the spread of swine leptospirosis are played by the following reasons: violation of detention conditions, insufficient deratisation, moving animals from disadvantaged farms, hypodiagnosis of leptospirosis, violation of quarantine conditions, import of new (exotic for Ukraine) pathogenic *Leptospira* serovars, violation of the vaccination timing of susceptible livestock in the foci of leptospirosis. In our opinion, natural conditions play a secondary role in the occurrence of swine leptospirosis in Ukraine.

Acknowledgement

The authors would like to acknowledge the State Service of Ukraine on Food Safety and Consumer Protection and the state laboratories of veterinary medicine in regions for presenting the official reports and for assistance.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Adler B. Vaccines against leptospirosis. Curr Top Microbiol Immunol. 2015;387:251-72.

Allan KJ, Biggs HM, Halliday JE, Kazwala RR, Maro VP, Cleaveland S, Crump JA. Epidemiology of Leptospirosis in Africa: A systematic review of a neglected zoonosis and a paradigm for 'one health' in Africa. PLoS Negl Trop Dis. 2015 Sep 14;9(9):e0003899. Erratum in: PLoS Negl Trop Dis. 2016 Mar;10(3):e0004552.

Bertasio C, Papetti A, Scaltriti E, Tagliabue S, D'Incau M, Boniotti MB. Serological survey and molecular typing reveal new Leptospira serogroup pomona strains among pigs of Northern Italy. Pathogens. 2020 Apr 29;9(5):332.

Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, Vinetz JM; Peru-United States Leptospirosis Consortium. Leptospirosis: A zoonotic disease of global importance. Lancet Infect Dis. 2003 Dec;3(12):757-71.

Boey K, Shiokawa K, Rajeev S. Leptospira infection in rats: A literature review of global prevalence and distribution. PLoS Negl Trop Dis. 2019 Aug 9;13(8):e0007499.

Cilia G, Bertelloni F, Fratini F. Leptospira infections in domestic and wild animals. Pathogens. 2020 Jul;9(7):573.

Ellis WA. Animal leptospirosis. Curr Top Microbiol Immunol. 2015;387:99-137.

Felix CR, Siedler BS, Barbosa LN, Timm GR, McFadden J, McBride AJA. An overview of human leptospirosis vaccine design and future perspectives. Expert Opin Drug Discov. 2020 Feb;15(2):179-88.

Goarant C. Leptospirosis: Risk factors and management challenges in developing countries. Res Rep Trop Med. 2016 Sep 28;7:49-62.

Guglielmini J, Bourhy P, Schiettekatte O, Zinini F, Brisse S, Picardeau M. Genus-wide Leptospira core genome multilocus sequence typing for strain taxonomy and global surveillance. PLoS Negl Trop Dis. 2019 Apr 26;13(4): e0007374. Erratum in: PLoS Negl Trop Dis. 2020 Aug 21; 14(8):e0008673.

Koizumi N. Laboratory diagnosis of leptospirosis. Methods Mol Biol. 2020;2134:277-87.

Kucheriavenko OO, Ukhovskyi VV, Aleksieieva HB. [Etiological structure of leptospirosis in pig farms in Ukraine]. J Vet Biotech. 2005 Oct;6:98-102. Ukrainian.

Ministry of Agrarian Policy and Food. [Instructions on preventive measures and healing animals from leptospirosis] [Internet]. 1994 [cited 2021 Mar 30]. Available from: http://zakon3.rada.gov.ua/laws/show/ru/z0055-94. Ukrainian.

Ngugi JN, Fevre EM, Mgode GF, Obonyo M, Mhamphi GG, Otieno CA, Cook EAJ. Seroprevalence and associated risk factors of leptospirosis in slaughter pigs; a neglected public health risk, western Kenya. BMC Vet Res. 2019 Nov 8; 15(1):403.

Petrakovsky J. Leptospirosis. In: OIE manual of standards for diagnostic tests and vaccines for terrestrial animals. Paris: Office International des Epizooties; 2021. p. 1-13.

Petri FAM, Sonalio K, de Souza Almeida HM, Mechler-Dreibi ML, Galdeano JVB, Mathias LA, de Oliveira LG. Cross-sectional study of Leptospira spp. in commercial pig farms in the state of Goias, Brazil. Trop Anim Health Prod. 2020 Nov 19;53(1):13.

Piotrovych VA, Ukhovskyi VV, Aleksieieva HB. [Substantiation of expediency of expansion of a set of diagnostic strains of Leptospira at diagnosis of leptospirosis of pigs in MAT]. J Vet Biotech. 2006 Apr;9:213-9. Ukrainian.

https://doi.org/10.17221/50/2021-VETMED

Piotrovych VA, Kucheriavenko OO, Kucheriavenko OO, Ukhovskyi VV. [Means of specific prevention of leptospirosis in animals in Ukraine]. J Vet Med Ukr. 2008 Oct;8:39-42. Ukrainian.

Poudel A, Hoque MM, Madere S, Bolds S, Price S, Barua S, Adekanmbi F, Kalalah A, Kitchens S, Brown V, Wang C, Lockaby BG. Molecular and serological prevalence of Leptospira spp. in Feral pigs (Sus scrofa) and their habitats in Alabama, USA. Pathogens. 2020 Oct 20;9(10):857.

Pyskun A, Ukhovskyi V, Pyskun O, Nedosekov V, Kovalenko V, Nychyk S, Sytiuk M, Iwaniak W. Presence of antibodies against Leptospira interrogans serovar hardjo in serum samples from cattle in Ukraine. Pol J Microbiol. 2019 Sep;68(3):295-302.

Samir A, Soliman R, El-Hariri M, Abdel-Moein K, Hatem ME. Leptospirosis in animals and human contacts in Egypt: Broad range surveillance. Rev Soc Bras Med Trop. 2015 May-Jun;48(3):272-7.

Ukhovskyi VV, Kucheryavenko OO, Kulykova VV, Aliekseieva GB, Gerilovych AP, Nedosekov VV, Potkonjak A. Prevalence and dynamics of the etiological structure of leptospirosis in cattle in Ukraine. Vet glasnik. 2014 May;68(1-2):23-30.

Vincent AT, Schiettekatte O, Goarant C, Neela VK, Bernet E, Thibeaux R, Ismail N, Mohd Khalid MKN, Amran F, Masuzawa T, Nakao R, Amara Korba A, Bourhy P, Veyrier FJ, Picardeau M. Revisiting the taxonomy and evolution of pathogenicity of the genus Leptospira through the prism of genomics. PLoS Negl Trop Dis. 2019 May 23;13(5):e0007270.

Zhang C, Xu J, Zhang T, Qiu H, Li Z, Zhang E, Li S, Chang YF, Guo X, Jiang X, Zhu Y. Genetic characteristics of pathogenic Leptospira in wild small animals and livestock in Jiangxi Province, China, 2002-2015. PLoS Negl Trop Dis. 2019 Jun 24;13(6):e0007513.

Zhang H, Zhang C, Zhu Y, Mehmood K, Liu J, McDonough SP, Tang Z, Chang YF. Leptospirosis trends in China, 2007–2018: A retrospective observational study. Transbound Emerg Dis. 2020 May;67(3):1119-28.

Received: March 30, 2021
Accepted: August 24, 2021