Porcine Research

Presence of antibodies against *Brucella* spp. in serum samples from wild boars in Ukraine

Anton Pyskun, Olesia Polishchuk, Iryna Piankivska, Olena Pyskun, Olexandr Moroz, Olexandr Pishchanskyi, Halyna Aliekseieva

State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine. Corresponding author: A. Pyskun, anton.pyskun1989@gmail.com

Abstract. The article presents data of research that were conducted during 2015–2018 in the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv). Authors investigated the possible circulation of *Brucella* spp. in wild boars from forestries in 14 regions of Ukraine. Serological studies were performed by complement fixation test (CFT), Rose Bengal Plate Agglutination test (RBT) and enzyme-linked immunosorbent assay (ELISA). In addition, positive samples were tested by ELISA to *Yersinia enterocolitica*. In general, 1,344 samples of blood sera were investigated and analyzed. In 152 (11.3%) of them were detected antibodies to the *Brucella* spp. by CFT and RBT, and in 140 (10.4%) – by ELISA. Considering cross-reactivity of serological tests to brucellosis and yersiniosis, the seroprevalence of antibodies to *Brucella* spp. exposure in wild boars in investigated regions was calculated to 5.0%.
Key Words: brucellosis, *Brucella suis* biovar 2, yersiniosis, serological studies, feral pigs.

Introduction. Brucellosis (undulant fever, Mediterranean fever or Malta fever) – is a zoonotic disease of bacterial etiology, that is characterized by abortions, orchitis, epididymitis, arthritis, lesions of the central nervous system, etc. with global distribution (Corbel 2006). The infection often leads to long-term reproductive failure, causing significant economic losses in farming worldwide (Olsen et al 2012; OIE 2018).

Due to prevention measures the epizootic situation of this zoonosis improved in recent years and, for example, despite sporadic outbreaks, the European Union countries are considered officially free of porcine brucellosis (Algers et al 2009). According to the data of World Organization for Animal Health (OIE), only a few countries in Northern and Central Europe, as well as Canada, Japan, Australia and New Zealand, are considered free from the animal brucellosis (OIE 2018). The official reporting from State Laboratories of Veterinary Medicine in Ukraine show that the last confirmed case of brucellosis was registered in 1992 in cattle and in 2008 – in pigs (Chemych 2017).

This zoonotic disease caused by gram-negative *bacilli* of the genus *Brucella* that are presented by 12 species of microorganisms (*B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. ceti*, *B. inopinata*, *B. microti*, *B. papionis*, *B. pinnipedialis* and *B. vulpis*). It affects all species of farm animals and a large number of wild mammals, but susceptibility to them in different species of animals is different (Corbel 2006; OIE 2018). The key farm species which play main role in the spreading of *Brucella* spp. are the major food-producing animals: cattle (*B. abortus* and *B. melitensis*), sheep (*B. ovis*), goats (*B. melitensis*) and pigs (*B. suis*). Others, including bison, camels, dogs, buffalo, reindeer, horses, and yaks are less important, but they can be very significant local sources of infection in some countries and regions (Corbel 2006).

Wild mammals, especially wild boars, which are the main reservoir of infection, play the significant role in the spreading of brucellosis among farm animals (Lama & Bachoon 2018). In addition, feral pigs (*Sus scrofa*) are an environmentally destructive

invasive species that act as a reservoir for viral, bacterial and parasitic pathogens (African and classical swine fevers, leptospirosis, escherichiosis, trichinellosis etc.) which are transmissible to humans and other animals through direct interaction with wild boars, contaminated food or indirectly through contaminated environment (Artois et al 2002; Rossi et al 2005; Ruiz-Fons et al 2008; Faber et al 2015; Żmudzki et al 2016; Lama & Bachoon 2018). In science literature are described cases when wild boars represented a source of infection for cattle (bovine tuberculosis) (Naranjo et al 2008), as well as for domestic pets and other wildlife (Gortazar et al 2007; Martin et al 2011). For example, hunting dogs have infected and died of Aujeszky's disease after contact with wild boar in Italy (Moreno et al 2015).

The Eurasian wild boar (*S. scrofa*) is widespread in most countries of Europe. Over the past 50 years have revealed a massive increase in the population with a current annual continental hunting bag of over 2.2 million wild boar (Meier & Ryser-Degiorgis 2018). Generating milder winters with less snow and climate changes in general, may affect their spread into northern regions (Fredriksson-Ahomaa 2019).

Brucella suis biovar 2 (BSB2) is an important pathogen in wild boars with a broad geographical distribution (Spain, Portugal, Poland, France, Germany, Italy etc.) (Muñoza et al 2019). At the same time, the porcine brucellosis can be caused by three biovars (1, 2, and 3) of the five *B. suis* biovars (Algers et al 2009; Olsen et al 2012; OIE 2018; Muñoza et al 2019). Despite the significant amount of data in the foreign scientific literature, brucellosis in wild fauna of Ukraine is still insufficiently studied.

According to the recommendations of World Organization for Animal Health (OIE), the confirmation of suspect or clinical cases of brucellosis are carried out by identifying the causative agent by bacteriological methods (staining methods or culture) or by identifying the DNA fragments (PCR). However, a lot of important aspects in the diagnosis of this zoonosis are based on serological methods (RBT, CFT, and ELISA). These methods allow determine population freedom from infection, individual animal freedom from infection, contribute to eradication policies, and herd/flock prevalence of infection (surveillance) (OIE 2018).

There is no information in literature about the registration of *Brucella* spp. in wild fauna in Ukraine, so the aim of the study was to determine the possible presence of antibodies to these pathogens in blood serum samples from wild boars in Ukraine.

Material and Method

Wild boars sera. Samples of blood sera were collected from wild boars killed by hunters from 2015 to 2018 (n = 1,344) conducted within the National surveillance programs aimed on African swine fever virus.

Samples were collected randomly from forestries in 14 oblasts of Ukraine: Poltava, Mikolayiv, Khmelnytskyi, Kirovohrad, Kharkiv, Chernivtsi, Chernihiv, Zakarpattya, Zaporizhzhya, Vinnytsia, Cherkasy, Odesa, Zhytomyr and Dnipropetrovsk. These oblasts present central, northern, southern, western and eastern regions of Ukraine.

Sera samples were transported to the laboratory refrigerated at 4°C in 2 mL tubes, centrifuged and stored frozen at -20°C. Subsequently, sera was thawed and analyzed for the presence of *Brucella* spp. antibodies.

Serological studies. Studies were conducted at the immunology department of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv) during 2015–2018.

Sera were tested by the Rose Bengal test (RBT) with an antigen produced by the Kherson Biofactory (Kherson, Ukraine), a complement fixation test (CFT) and indirect enzyme-linked immunosorbent assay (ELISA) (IDvet, Grabels, France) according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2018).

For the CFT, the following compounds were used: *Brucella* antigens, rabbit hemolytic serum, and guinea pig complement (the commercial kit of the State Company «Veterinary Medicine», Kharkiv, Ukraine), sheep blood in Alsevers (blood of sheep from

an experimental holding), calcium–magnesium veronal buffer (Virion/Serion, Würzburg, Germany).

Samples were recorded as seropositive if the CFT, ELISA and/or RBT were positive, and these were further screened for presence of *Yersinia enterocolitica* antibodies by an indirect ELISA (Pigtype Yopscreen, Labor Diagnostik Leipzig, Leipzig, Germany).

Results and Discussion. Brucellosis is an important zoonotic bacterial infection of virtually all examined mammalian species including farm animals that may cause significant economic losses (Corbel 2006).

Different species of animals are hosts for various *Brucella* spp., but wild boars and hares are considered the main reservoirs of infection in wild fauna (Gortazar et al 2007; Martin et al 2011; Lama & Bachoon 2018). Thus, these species are maintenance hosts for *B. suis* biovar 2 (BSB2) (Corbel 2006; Szulowski et al 2013; Muñoza et al 2019). In the scientific literature from different countries described cases of infection by this pathogen in other species too (cattle, pigs.) (Szulowski et al 2013; Muñoza et al 2019).

It is considered that this variant has a low pathogenicity for humans then biovars 1 and 3 (Corbel 2006), but in literature were published two confirmed cases in humans (Teyssou et al 1989; Paton et al 2001; Mailles et al 2017).

Before our study, there was no information in Ukrainian and foreign literature sources about circulation of *Brucella* spp. in wild boars in Ukraine, so we conducted serological studies to determine the possible circulation of antibodies to these pathogens in blood serum of these animals.

Over the whole period, 1,344 samples of wild boars sera were investigated, and 152 positive reactions by CFT have been detected, which constitutes 11.3%. The obtained data of serological research by CFT are presented in Table 1.

Table 1

Results of serological research of samples from wild boars by CFT during 2015–2018

Years	*Regions*	*Number of tested samples*	*Positive reactions by CFT*	
			Number of positive samples	Percentage of positive samples, %
2015	Poltava, Mikolayiv, Khmelnytskyi, Kirovohrad, Kharkiv,and Chernihiv	286	24	8.4
2016	Poltava, Zakarpattya, Zaporizhzhya, Chernivtsi, Khmelnytskyi, and Vinnytsia	304	32	10.5
2017	Cherkasy, Vinnytsia, Mikolayiv, Odesa, Zaporizhzhya, Kirovohrad, Chernihiv, Poltava, Zhytomyr, Khmelnytskyi, Dnipropetrovsk, and Chernivtsi	391	58	14.8
2018	Zakarpattya, Odesa, Kirovohrad, Vinnytsia, Khmelnytskyi, Zhytomyr, Mikolayiv, Poltava, and Cherkasy	363	38	10.5
	Total	1344	152	11.3

As shown in Table 1, during 2015–2018 were tested 286, 304, 391 and 363 samples respectively. Antibodies of *Brucella* spp. were found within the 8.4–14.8% range of animals.

Comparative studies were performed by RBT and indirect ELISA. The obtained data showed that the number of positive reactions by RBT in different regions was the same as for CFT and accounted for 11.3% (antibodies were detected in the same serum samples).

At the same time, the number of animals reacting positively by ELISA was slightly lower (10.4%). This is due to the fact that positive samples in the low titers by CFT and/or RBT (two and three pluses in titer 1:5) were identified as negative by ELISA.

The different number of positive samples to *Brucella* spp. by CFT or RBT and ELISA was registered in Kharkiv, Khmelnytskyi, Kirovohrad, Odesa, Poltava, Vinnytsia, Zaporizhzhya, and Zhytomyr oblasts (Table 2).

Table 2

Results of serological research of samples from wild boars by CFT, RBT and ELISA from different regions of Ukraine during 2015–2018

Regions	*Number of tested samples*	*Positive reactions by CFT*		*Positive reactions by RBT*		*Positive reactions by ELISA*	
		Number of positive samples	Percentage of positive samples, %	Number of positive samples	Percentage of positive samples, %	Number of positive samples	Percentage of positive samples, %
Cherkasy	74	6	8.1	6	8.1	6	8.1
Chernihiv	58	7	12.1	7	12.1	7	12.1
Chernivtsi	101	15	14.9	15	14.9	15	14.9
Dnipropetrovsk	71	7	9.9	7	9.9	7	9.9
Kharkiv	85	7	8.2	7	8.2	5	5.9
Khmelnytskyi	99	8	8.1	8	8.1	7	7.1
Kirovohrad	91	10	11.0	10	11.0	8	8.8
Mikolayiv	110	6	5.5	6	5.5	6	5.5
Odesa	78	8	10.3	8	10.3	7	9.0
Poltava	243	28	11.5	28	11.5	26	10.7
Vinnytsia	146	29	19.9	29	19.9	27	18.5
Zakarpattya	66	5	7.6	5	7.6	5	7.6
Zaporizhzhya	54	7	13.0	7	13.0	6	11.1
Zhytomyr	68	9	13.2	9	13.2	8	11.8
Total	1344	152	11.3	152	11.3	140	10.4

Systematized results show that antibodies to *Brucella* spp. were detected in samples from all investigated regions of Ukraine by all three serological reactions. Analyzing the results in different regions of Ukraine, least frequently antibodies were detected by CFT and/or RBT within the 5.5–8.1% of the total number of investigated samples from Mikolayiv, Zakarpattya, Cherkasy, Khmelnytskyi, and Kharkiv oblasts. In the wild boars from Dnipropetrovsk, Odesa, Kirovohrad, Poltava, Chernihiv, Zaporizhzhya, Zhytomyr, and Chernivtsi regions, antibodies were found within the 9.9–14.9% range of animals. The highest levels of positive reactions were detected in Vinnytsia region reaching 19.9%.

Figure 1 illustrates the intensity of detection of antibodies to *Brucella* spp. among wild boars sera samples from different regions of Ukraine during 2015–2018.

In general, as shown on map (Figure 1), the highest levels of positive reactions on brucellosis were registered in central part of the country (8.1–19.9%), less – in west (7.6–14.9%), north (12.1–13.2%) and east (8.2–13.0%) regions. The lower incidence of positive samples in those collected in the south part of the country (5.5–10.3%).

According to the current edition of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2018), *Y. enterocolitica* O:9, may cause antibody responses and false positive serological reactions in brucellosis tests may occur, impeding accurate serological diagnosis (OIE 2018). So, at the last stage of the studies, differential diagnosis of positive reactions from yersiniosis was performed by ELISA. As a result, from the 152 positive samples on brucellosis 85 were positive on yersiniosis.

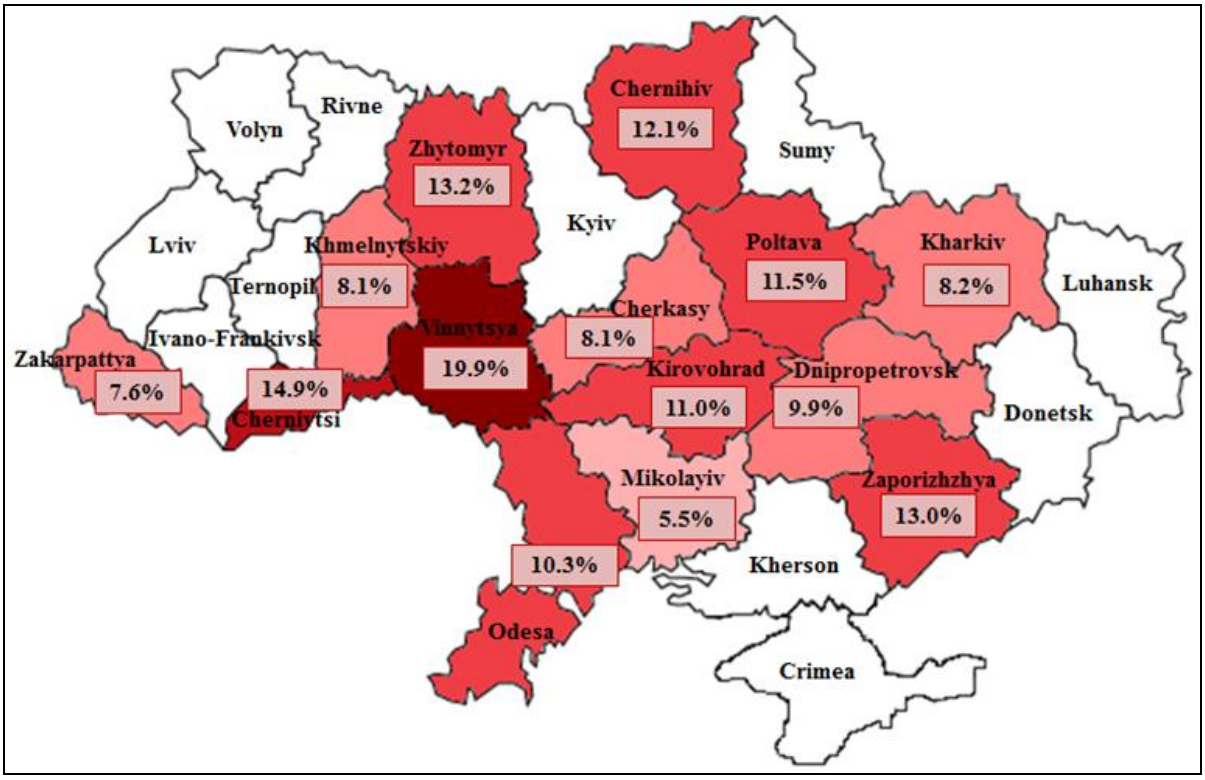

Figure 1. The percentage of antibodies among sera samples from the total number of investigated on brucellosis wild boars in different regions during 2015–2018 (N=1,344).

Considering cross-reactivity of serological tests to brucellosis and yersiniosis, the seroprevalence of antibodies to *Brucella* spp. exposure in wild boars in investigated regions was calculated to 5.0% (67 from 1,344 investigated samples).

Obtained data are relatively low in comparison to certain other European countries. For example, in 2018 were published data by Latvian scientists that seroprevalence of antibodies to *Brucella* spp. in wild boars in their country was 14.0% (Grantina-Ievina et al 2018).

In our opinion, this is related to the depopulation (shooting) of wild boars in Ukraine due to the outbreaks of African swine fever (from 2012 to date, 500 cases of this disease have been recorded). According to the official reports of the State Laboratories of Veterinary Medicine, the number of wild boar populations in Ukraine decreased from 61,549 individuals to 31,786 during 2015–2018 (almost half). Thus, in 2018, hunters killed an average of 125 heads per week.

In the neighboring countries of Eastern Europe, the infections caused by BSB2 were reported in Poland, Romania and Hungary (Szulowski et al 2013; Muñoza et al 2019). Scientific data on the prevalence of *Brucella* spp. in wild boars in Russia and Belorussia have not been published, but a few cases of domestic pig brucellosis have been recorded in Estonia and Latvia (Grantina-Ievina et al 2018). At the same time in Russia have been described cases of this zoonosis among humans and other species of animals (Sannikova et al 2015; Sotnikov et al 2015).

In our opinion, further research is needed to make a more meaningful analysis of the epizootic situation with this pathogen in different regions. However, the data obtained show circulation of antibodies to *Brucella* spp. among wild boars in Ukraine. Perhaps these pathogens were circulated on the territory of Ukraine constantly, because wild boars are migrating animals that constantly move between regions and borders of states.

Conclusions. For the first time, we investigated the possible circulation of *Brucella* spp. in wild boars in Ukraine. At the same time, in the Europe, the highest risk of brucellosis infection is associated with wild fauna (wild boars and hares). The results of our research

in our opinion indicate the circulation of *Brucella* spp. among wild boars in Ukraine. In general, positive reactions were detected in all regions, from which samples of sera were received. Lower or higher incidence of positive samples can be linked with fact that samples were randomly collected.

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Authors:
Anton Pyskun, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Immunology department, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: anton.pyskun1989@gmail.com
Olesia Polishchuk, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Immunology department, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: polishy@bigmir.net
Iryna Piankivska, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Immunology department, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: iryna.piankivska@gmail.com
Olena Pyskun, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Research department of organization of scientific and international work, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: stepnahelen@gmail.com
Olexandr Moroz, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Epizootology department, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: moroz-vet@ukr.net
Olexandr Pishchanskyi, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Administrative department, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: dndildvse@vetlabresearch.gov.ua
Halyna Aliekseieva, State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Immunology department, Ukraine, Kyiv, Donetska St. 30, 03151, e-mail: serolog@i.ua

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