Part 1. Veterinary Medicine

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ETIOLOGICAL STRUCTURE OF LEPTOSPIROSIS AMONG THE WILD BOARS AND DOMESTIC PIGS IN THE TERRITORY OF UKRAINE, ITS ANALYSIS AND CHARACTERISTICS

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Summary. Wild boars (*Sus scrofa*) are a reservoir of leptospirosis in nature and a source of infection for domestic pigs and the human, especially those at risk. Wild boars (n = 516) and domestic pigs (n = 1042) were tested for *Leptospira* spp. using microscopic agglutination test (MAT), which was conducted with 21 *Leptospira*'s serological groups. The circulation of common pathogenic leptospires among wild boars and domestic pigs in the territory of Ukraine was established. Registered positive-responding animals in the MAT among wild boars were 86.8%, among domestic pigs — 38.1%. Analysis of the etiological structure of leptospirosis among wild boars and domestic pigs showed that the dominant serological groups were *Icterohaemorrhagiae*, *Australis* (serovar *bratislava*), *Pomona*, *Canicola*, *Grippotyphosa*, *Sejroe*, *Hebdomadis*, *Tarassovi*.

Keywords: leptospirosis, etiological structure, wild boars, domestic pigs, microscopic agglutination test, Ukraine

Introduction. Leptospirosis is the natural foci-associated disease, the other name of which is 'water fever'. This zoonosis is very closely related to water, since the main route of transmission of infection is water (according to the Ukrainian Center for Monitoring and Control of Infectious Diseases of The Ministry of Healthcare of Ukraine, 164 cases of human infections in leptospirosis through the waterway of transmission throughout Ukraine, which was 50.8% of the total number of registered cases) (Adler, 2015; Mandyhra et al., 2014).

In natural foci, the source and reservoirs of pathogenic leptospires are small mammals from the genus of rodents, insectivores, predators and marsupials. The farm animals and synanthropic rodents could be the reservoirs as well in anthropological centers (Vinohrad et al., 2005; Uhovskyi, Kucheryavenko and Stepna, 2014).

In addition, recent studies have shown a significant role in the epizootology of leptospirosis in wildlife infections, in particular wild boars, which are an integral part of the fauna in many countries around the world. These animals play an important role for most viral and bacterial pathogens, including leptospirosis. It is necessary to take into account factors such as: their migration ability; ecological peculiarities of existence in the wild; omnivorous and consumption of corpses of rodents; they share pastures and rates for watering with other wildlife and livestock. Thus, infected wild boars become reservoirs and carriers of pathogenic leptospires, resulting in the creation of ideal conditions for the formation of natural foci of leptospirosis by the transfer of pathogenic leptospires from wild boars to domestic pigs and to human (Bolotskiy, 1998; Levett, 2001).

According to the literature data, wild boars (*Sus scrofa*) could be the potential reservoir for a variety of pathogenic leptospires.

For the first time, the problem of studying infectious diseases in wild boars began to be engaged in the last century. In 1986, in the USA (Texas) group of researchers examined 10 populations of this species of animals and found that they all are vectors of pathogenic leptospires (Corn et al., 1986).

Similar studies were conducted in Australia in 1998. This discovered antibodies to leptospiras in wild boars serum samples using MAT (Mason et al., 1998).

In Europe, such studies began to be conducted only in the 21st century. His first results were presented in Spain in 2002 (Vicente et al., 2002).

A year later, Italian scientists discovered specific antibodies to leptospiras and brucellas in wild boars' serum (Ebani et al., 2003). In Zagreb (Croatia), veterinarians were tested blood serums and kidney samples from wild boars and rodents and isolated three major serogroups from isolates — *Pomona*, *Australis*, *Icterohaemorrhagiae* (Cvetnic et al., 2003).

In 2006–2007, conducted a study of blood serum from wild boars caught in the suburban of Berlin (Germany), which resulted in the carriage of serogroups *Pomona* and *Australis* (serovar *bratislava*) (Jansen and Schneider, 2011).

Leptospirosis in wild boars and deer was established in Japan in 2009 (Koizumi et al., 2009). In the same year, American scientists have found that wild boars constitute a direct threat to farm animals and humans (Meng, Lindsay and Sriranganathan, 2009).

Scientists from different region in the World discovered specific leptospira antibodies in wild boars and other wildlife species during 2010–2015 (Chatfield et al., 2013; Durfee and Presidente, 1979; Espí, Prieto and Alzaga, 2010; Fornazari et al., 2009; Pappas et al., 2008; Pedersen et al., 2015; Vale-Gonçalves et al., 2015).

Multiple researchers and scholars in Russia carried out the study of wild fauna (Ananyina, 2002; Bolotskiy, 1998; Malakhov, Panin and Soboleva, 2001; and others).

In Ukraine, the study of the etiological structure of leptospirosis in wild boars was not undertaken.

Many researchers and scientists have been involved in the research of farm animals. Study of the etiological structure of leptospirosis is a very labor-intensive process, since the main hosts of the leptospirosis of one serovar can be different animals (Dovgan, Atamas and Fuchidgi, 1998).

The leading position in the etiological structure of leptospirosis among domestic pigs in Europe is occupied by serogroups *Pomona* and *Icterohaemorrhagiae* (Nardone et al., 2004; Schönberg, Staak and Arbeitsgruppe, 1987).

At the pig farms in the countries of Western Europe, North America and Asia, great attention is paid to the increasing number of positive reactions to leptospirosis with serovar *Bratislava* (serogroup *Australis*) (Meites et al., 2004; Mendoza and Prescott, 1992). For the first time in Ukraine the circulation of this serovar among the pig population was reported in 1999 (Ntahonshikira, 1999). This pathogen was registered in 80.7% of the total number of positively responsive pigs in 2004 (Ukhovskyi, 2005).

According to the results of the analysis of the data of the veterinary report of the Central State Veterinary Medicine Laboratory, the etiological structure of the pigs' leptospirosis was as follows: *Icterohaemorrhagiae* — 41.6%, *Pomona* — 14.9%, *Tarassovi* — 10.6%, *Grippotyphosa* — 2.2%, *Canicola* — 2.0%, *Hebdomadis* — 0.6%, *Sejroe* — 0.5% (Nedosekov, Ukhovskyi and Kucheryavenko, 2011).

According to the results of recent studies, the etiological structure of pigs' leptospirosis in farms of Ukraine as of the beginning of 2017 is as follows: *Icterohaemorrhagiae* — 40.2%, *Pomona* — 14.8%, *Australis* (serovar *bratislava*) — 13.4%, *Canicola* — 8.5%, *Sejroe* — 7.3%, *Hebdomadis* — 7.2%, *Tarassovi* — 4.7%, *Grippotyphosa* — 3.9% (Kulykova et al., 2016).

An analysis of literary sources about the role of wild boars in the spread of leptospirosis among wild and farm animals and human infection was the basis for research on the spread of leptospirosis among this species of animals on the territory of Ukraine.

The aim of the study was to investigate the large number of blood sera samples from wild boars and domestic pigs, to determine the etiological structure of leptospirosis, and to analyze it and to characterize the connections of leptospirosis infection in these species, taking into account the genetic affinity between wild boars and domestic pigs.

Materials and methods. All researches were performed during 2014–2016 on the basis of the Leptospirosis Laboratory of Farm Animals with the Museum of Microorganisms, on the basis of which the Scientific Research Reference Center for the study and prevention of leptospirosis in the territory of Ukraine.

Leptospira strains: Twenty-one pathogenic *Leptospira* spp. strains were genotyped. These strains were part of the bacterial collection of the Leptospirosis Laboratory of Farm Animals with the Museum of Microorganisms of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine.

Study sites: Blood sera samples from 516 wild boars were obtained by shooting hunters on the territory in hunting grounds from 375 administrative districts of all oblasts of Ukraine and the Autonomous Republic of Crimea from the State Program 'On the control of the number of wild boars in the territory of Ukraine', and kindly provided by Dr. M. P. Sytiuk.

Blood sera samples from 1,042 domestic pigs came to the laboratory from dysfunctional leptospirosis farms of Ukraine.

Serological test (MAT): The research was carried out by microscopic agglutination test (MAT) using antigens of 21 *Leptospira* serogroups recommended for research in state laboratories of veterinary medicine of Ukraine in dilutions 1:50, 1:100, 1;500, and 1:2,500. The study of blood serum from wild boars was carried out by MAT using antigens of 21 *Leptospira* serogroups (large diagnostic series), and domestic pigs — 8 *Leptospira* serogroups (small diagnostic series), which listed in Table 1.

Table 1 — List of strains used for research

No.	Serogroup	Serovar	Strain
1	*Javanica* [L]	*javanica*	Veldrat Bataviae 46
2	*Bataviae* [L]	*djatzi*	HS 26
3	*Mini* [L]	*szwajizak*	Szwajizak
4	*Sejroe* [L,S]	*polonica*	493 Poland
5	*Hebdomadis* [L,S]	*kabura*	Kabura
6	*Tarassovi* [L,S]	*tarassovi*	Perepelicyni
7	*Pomona* [L,S]	*pomona*	Pomona
8	*Grippo-typhosa* [L,S]	*grippotyphosa*	Moskva V
9	*Canicola* [L,S]	*canicola*	Hond Utrecht IV
10	*Icterohaemor-rhagiae* [L,S]	*copenhageni*	M 20

Table 1 — continuation

No.	Serogroup	Serovar	Strain
11	*Louisiana*[L]	*louisiana*	LSU
12	*Shermani*[L]	*shermani*	LT 821
13	*Panama*[L]	*panama*	CZ 214 K
14	*Semaranga*[L]	*patoc*	Patoc 1
15	*Celledoni*[L]	*whitcombi*	Whitcomb
16	*Australis*[L]	*erinacei-europaei*	Jez 1
17	*Autumnalis*[L]	*autumnalis*	Akiyami A
18	*Cynopteri*[L]	*cynopteri*	Vleermuis 3868
19	*Pyrogenes*[L]	*pyrogenes*	Saline
20	*Ballum*[L]	*ballum*	Mus 127
21	*Australis*[S]	*bratislava*	Jez-bratislava

Notes: [L] — large diagnostic series, [S] — small diagnostic series.

Results. We tested 1,558 samples of blood serum in the MAT, namely: 516 — from wild boars, 1,042 — from domestic pigs, and analyzed the results.

Research blood sera samples on leptospirosis of wild boars. In order to study the etiological structure of leptospirosis among population wild boars, we conducted a study of blood sera from all regions of Ukraine that arrived at the Laboratory of leptospirosis of IVM NAAS. The results of the research are shown in Table 2.

Table 2 — Results of studies of blood sera of wild boars on leptospirosis in MAT

Indexes	Years			Total
	2014	2015	2016	
Tested blood sera samples	109	170	237	**516**
Positive results	95	158	195	**448**
Percentage of positively blood sera samples	87.2	92.9	82.3	**86.8**
Positive reactions	288	536	553	**1,377**

In order to study leptospirosis and detect the spectrum of the etiological structure of the disease among wild boars population in Ukraine, we conducted a study of serum blood in the MAT. The results of studies in the area of regions are shown in Table 3.

Analysis of the results of Table 2 shows that in the vast majority of Ukraine (13 regions), the percentage of positive reactions ranges from 80–89%, which indicates a significant infection of wild pigs with pathogenic serogroups leptospires. In eight regions, this percentage is between 90% and 100%. Only in three regions the percentage of infection remained at the level of 70–79%.

According to numerous publications of scientists from different countries of the world, the seroprevalence of leptospirosis infection among wild boars population varies from 3% to 95% (Fig. 1). Taking into account the results of its own research, Ukraine occupies the second position in this list and is unfriendly in relation to the leptospirosis of wild boars.

Table 3 — Results of serological examination of blood serum wild boars in the territory of Ukraine

No.	Region	Studied samples of blood sera	Positive results	
			Total	%
1	AR Crimea	23	21	91.3
2	Vinnytsia	13	13	100.0
3	Volyn	15	13	86.7
4	Dnipropetrovsk	18	15	83.3
5	Donetsk	18	15	83.3
6	Zhytomyr	18	16	88.9
7	Zakarpattia	17	14	82.4
8	Zaporizhia	16	13	81.3
9	Ivano-Frankivsk	18	17	94.4
10	Kyiv	19	16	84.2
11	Kirovohrad	19	15	78.9
12	Luhansk	22	19	86.4
13	Lviv	17	17	100.0
14	Mykolaiv	20	17	85.0
15	Odesa	22	20	90.9
16	Poltava	34	31	91.2
17	Rivne	20	15	75.0
18	Sumy	25	20	80.0
19	Ternopil	22	19	86.4
20	Kharkiv	22	20	90.9
21	Kherson	19	16	84.2
22	Khmelnitskyi	22	21	95.5
23	Cherkasy	32	29	90.6
24	Chernivtsi	22	18	81.8
25	Chernihiv	23	18	78.3
	Total:	**516**	**448**	**86.8**

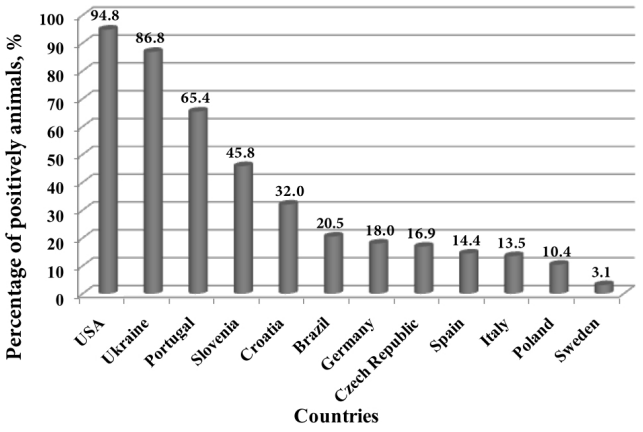

Figure 1. Seroprevalence level of leptospirosis among wild boars population in countries (2014–2016)

As a result of the serological study, 448 animals reacted positively, representing 86.8% of the total number of

investigated ones. As shown in Table 3, the highest level of infection was in 2016, and the lowest — in 2014.

The general etiological structure of the leptospirosis of wild pigs is presented in Fig. 2.

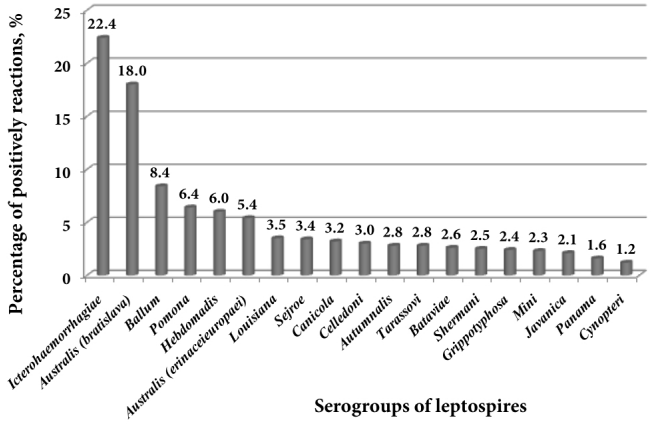

Figure 2. General etiological structure of leptospirosis of wild boars (n = 516)

Analyzing the overall etiological structure of the leptospirosis of wild pigs, shown in Fig. 2, among all positively reactive animals, the antibodies to the serotypes *Icterohaemorrhagiae* (22.4%), *Australis* (serovar *bratislava*) (18%) and *Ballum* (8.4%) were most frequently detected. *Pomona* (6.4%) and *Hebdomadis* (6%) were slightly less registered. Other serological groups ranged from 2.1% to 5.4%. The smallest etiological role was played by serogroups *Cynopteri* (1.2%) and *Panama* (1.6%).

Testing of blood sera samples on leptospirosis of domestic pigs. In total, we examined and analyzed 1,042 blood sera samples domestic pigs in MAT from 26 farms in different regions of Ukraine. The results of the research are shown in Table 4.

Table 4 — Results of studies of blood sera samples domestic pigs on leptospirosis in MAT

Indexes	Years			Total
	2014	2015	2016	
Tested blood sera samples	565	149	328	**1,042**
Positive results	237	60	100	**397**
Percentage of positively blood sera samples	41.9	40.3	30.5	**38.1**
Positive reactions	333	81	143	**557**

Positive reactions were diagnosed in 397 samples, representing 38.1% of the total number of examined animals. As shown in Table 4, with during 2015–2016 leptospirosis of domestic pigs was recorded in a relatively equal number of animals, respectively, 41.9% and 40.3%; in 2016 there was a decrease in the level to 30.5%.

The leading role in etiology of leptospirosis infection of domestic pigs was played by the serological group *Icterohaemorrhagiae*. Antibodies to it were diagnosed in 262 animals, which is 47.0%. Serogroup *Australis* was found in sick animals less frequently (16.2%). *Pomona* and *Canicola* were recorded at almost the same level (respectively 10.8% and 10.4%). The smallest etiological role was played by the serological group *Grippotyphosa*, which was noted only in 2.3% of cases (Fig. 3).

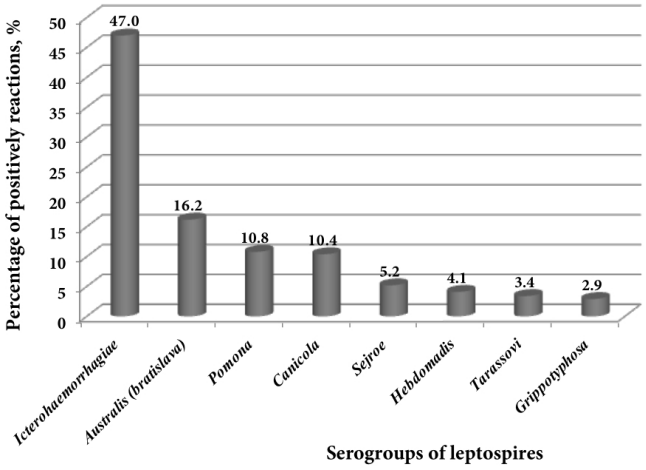

Figure 3. General etiological structure of leptospirosis of domestic pigs (n = 1,042)

Having analyzed the obtained data, common serogroups were established between positive reactions in wild boars and domestic pigs whose blood serum was investigated in the Laboratory of Leptospirosis with the Museum of Microorganisms of IVM NAAS during 2014–2016. The systematized results are shown in Table 5.

Table 5 — Etiological structure of leptospirosis of wild boars and domestic pigs on the territory of Ukraine (2014–2016)

Serogroup	Wild boars, %	Domestic pigs, %
Icterohaemorrhagiae	22.4	47.0
Australis (serovar *bratislava*)	18.0	16.2
Pomona	6.4	10.8
Canicola	3.2	10.4
Sejroe	3.4	5.2
Hebdomadis	6.0	4.1
Tarassovi	2.8	3.4
Grippotyphosa	2.4	2.9

As can be seen from Table 5, the main etiological role of wild boars and domestic pigs is played by the serological groups *Icterohaemorrhagiae* and *Australis* (serovar *bratislava*). Antibodies to the following serogroups: *Pomona*, *Hebdomadis*, *Canicola*, and others were registered less rarely. The difference in the percentage of positive reactions to serogroups due to the variability of the etiological structure, which is characteristic of leptospirosis.

Discussion and conclusions. An analysis of the publications of scientists from other countries regarding leptospirosis among wild boars populations indicates their significant level of infectiousness around the world. In particular, they can be infected by eating rodents. In order to study leptospirosis among populations of this species of animals on the territory of Ukraine, we conducted serological monitoring. Blood sera were obtained as a result of shooting at the territory of hunting grounds from 375 administrative districts of all regions and the Autonomous Republic of Crimea within the framework of the State Program 'On the control of the number of wild pigs in the territory of Ukraine'.

According to the results of our research, it has been established that the entire territory of Ukraine is unsuccessful in relation to the leptospirosis of the specified species of animals. In particular, it was investigated in MAT and analyzed 516 samples of blood sera. Of these, 448 responded positively to leptospirosis, which is 86.8%.

Based on the analysis of the results obtained, we found that in serotypes positive for wild boars, antibodies to serogroups were *Icterohaemorrhagiae* (22.4%), *Australis* (serovar *bratislava*) (18.0%), *Ballum* (8.4%), *Pomona* (6.4%), and *Hebdomadis* (6.0%) were slightly less registered. Other serological groups ranged from 2.1% to 5.4%. The smallest etiological role was played by serogroups *Cynopteri* (1.2%) and *Panama* (1.6%) (Stepna, Ukhovskyi and Sytiuk, 2015).

According to German researchers, among wild pigs, the serogroups *Pomona* and *Australis* (serovar *bratislava*) are predominant (Jansen et al., 2007).

As a result of our studies, the leptospirosis of these serogroups was also detected: *Pomona* in 6.4%, *Australis* (serovar *bratislava*) — 18.0%. Having analyzed the obtained data, there were established common serogroups between positive reactions in wild boars and domestic pigs whose blood serum was investigated in the Laboratory of Leptospirosis with the Museum of Microorganisms of IVM NAAS during 2014–2016.

The main etiological role of wild boars and domestic pigs is played by the serological groups *Icterohaemorrhagiae* and *Australis* (serovar *bratislava*). Antibodies to the following serogroups: *Pomona*, *Hebdomadis*, *Canicola*, and others were registered less rarely. The difference in the percentage of positive reactions to serogroups is due to the variability of the etiological structure, which is characteristic of leptospirosis (Ukhovskyi, 2005).

Among the domestic pig's populations, during the research period, the highest percentage of positive reactions was caused by the serological groups *Icterohaemorrhagiae* (47.0%), *Australis* (16.2%) and *Pomona* (10.8%). Together, they recorded 73.6% of the total number of positive reactions to leptospirosis. Antibodies to the serotype *Icterohaemorrhagiae* were diagnosed in 262 animals out of 365 positive-responsive, representing 66.0%. Serogroups *Australis* and *Pomona* were found in sick animals less frequently (respectively, 22.7% and 15.1%).

The obtained results confirm the data of Ukhovskyi (2005), Zon et al. (2001), Atamas, Maslennikova and Dovgan (2003), Ivanchenko and Gontar' (2010), etc., who reported the leading role of the serogroups *Icterohaemorrhagiae* and *Pomona* in the etiological structure of leptospirosis infection among domestic pigs in Ukraine. Ntahonshikira (1999), and later Ukhovskyi (2005), the significant role of the serologic group *Australis* (serovar *bratislava*) in the disease of these animals has been proven. Particular attention to the causative agent is on the part of foreign researchers. As of 2008, antibodies to it were found in 7.98% of the positively responding to the leptospirosis of the pig population in Poland, in the Netherlands — 28.67%, in the Czech Republic — 13.37%, in Denmark — about 70.0% (Ukhovskyi, 2005).

A lower percentage of positive reactions were recorded with serogroups *Canicola* (9.7%), *Sejroe* (6.2%), *Hebdomadis* (6%), and *Tarassovi* (3.4%). Antibodies to the serogroup *Grippotyphosa* have been diagnosed in only six reactions out of 535, representing 1.1% (Kulykova et al., 2016). The obtained results logically agree with the data of official reporting and foreign scientists with minor fluctuations in the percentage of certain serogroups (Atamas, Maslennikova and Dovgan, 2003; Zon et al., 2001; Ivanchenko and Gontar', 2010; Ukhovskyi, 2005; Nardone et al., 2004; Schönberg, Staak and Arbeitsgruppe, 1987).

Having systematized and analyzed the results of the research, it can be argued that the etiological structure of leptospirosis among wild boars and domestic pigs is common and has the same serological group's leptospires.

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