

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(4), 652–659
doi: 10.15421/022393

Serological prevalence of *Leptospira* spp. in horses in Ukraine

V. V. Ukhovskiy*, L. Y. Korniienko*, O. M. Chechet*, G. B. Aliekseieva*,
O. D. Polishchuk*, H. M. Mietolapova*, T. M. Tsarenko**, M. Y. Romanko*, O. O. Pyskun*

*State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

**Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine

Article info

Received 06.08.2023

Received in revised form 02.09.2023

Accepted 14.09.2023

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.: +38-068-353-63-69.
E-mail: taras.m.tsarenko@gmail.com

Ukhovskiy, V. V., Korniienko, L. Y., Chechet, O. M., Aliekseieva, G. B., Polishchuk, O. D., Mietolapova, H. M., Tsarenko, T. M., Romanko, M. Y., & Pyskun, O. O. (2023). Serological prevalence of Leptospira spp. in horses in Ukraine. Regulatory Mechanisms in Biosystems, 14(4), 652–659. doi:10.15421/022393

Leptospirosis, a zoonotic disease with constantly evolving pathogens, poses risks to both human and animal health. We investigated the prevalence of *Leptospira* spp. among horses in Ukraine from 2007 to 2021, encompassing clinical and sub-clinical cases. The study utilized data from the State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (2007–2021) to analyze leptospirosis prevalence in Ukrainian horses. Seroprevalence was calculated for each region. Equine leptospirosis outbreaks were retrospectively analyzed based on serological data from microagglutination reaction (MRA) studies using eight major serogroups. A geospatial analysis, utilizing quantile classification with five data classes, was conducted. Spanning 2007–2021, our analysis evaluated the prevalence and dynamics of equine leptospirosis in Ukraine. Serum samples tested for *Leptospira* antibodies exhibited varying dynamics over this period, with the highest and lowest rates observed in different years. Among the 125,101 horse serum samples analyzed, 10.8% tested positive for leptospirosis. The incidence rate fluctuated, peaking in certain years. The dominant serovars were Copenhageni (32.8%), Bratislava (16.1%), Grippotyphosa (15.4%), and Canicola (13.2%), while mixed reactions constituted 55.1% of positive cases. The analysis also revealed shifts in the prevalence of specific serovars over time. Geospatial analysis showcased the distribution of positive cases for each serovar across Ukraine's regions. The highest infection rates were observed in the eastern and northern regions, each characterized by distinct serovar prevalence. The etiologic composition map depicted varying serovar proportions among different regions. Furthermore, the density map delineated regions with different risk levels, with several regions falling into the "very high risk" category, while others like Odesa and Lviv were classified as low risk due to fewer positive leptospirosis cases. Detection of specific serovars like Tarassovi, Pomona, and Canicola in horses points to potential pathogen sources such as cattle, pigs, and dogs. The dominance of serovar Copenhageni (serogroup Icterohaemorrhagiae), accounting for 32.8% of seropositivity, reflects close contact with rodents, recognized reservoirs of this serogroup. These findings illuminate the evolving prevalence and distribution of leptospirosis in Ukrainian horses, offering vital insights for targeted interventions and disease management strategies. The insights garnered from this study can hold significant value for equine veterinarians, aiding the development of tailored preventive and control measures adapted to specific regions with varying risk levels. Our research offers a comprehensive examination of equine leptospirosis from both ecological and geographical perspectives, unveiling pivotal observations.

Keywords: zoonotic disease risks; equine leptospirosis dynamics; serovar prevalence shifts; geospatial analysis; pathogen sources.

Introduction

Leptospirosis is one of the most pressing zoonotic diseases. It is a global problem, as sick animals and pathogen-carrying animals pose a significant danger to humans as possible sources of leptospirosis infection. The disease has not only survived to this day, but its pathogens are evolving. Their evolution affects industrialized and developing countries (Ghazaei, 2018; Aymée et al., 2021). Animal infection is mainly carried out through direct contact with leptospore-contaminated urine or indirectly through interaction with contaminated water and/or soil (Ramos et al., 2006). Currently, based on phylogenetic analysis, *Leptospira* spp. are divided into three lineages that reflect their degree of pathogenicity: saprophytic (26), transitional (21) and pathogenic (17) (Hamond et al., 2015).

Cases of leptospirosis are often associated with poor hygiene and sanitation, so the disease is mainly reported in agricultural enterprises related to livestock farming. People working in processing plants and mining may also be at risk (Torres et al., 2016).

There is a growing number of studies that show the role of water and soil in the transmission of the pathogen to animals, and then to humans

(Vincent et al., 2019). Rains, floods, and disasters associated with water sources contribute to the spread of the pathogen (Mwachui et al., 2015). The ability of leptospores to form biofilms and cellular aggregates has been proven (Wollanke et al., 2022). Due to this, the pathogen avoids the dispersive effect of urine in large natural water sources and accumulates in quite significant quantities (Yamaguchi et al., 2018; Sato et al., 2019; Rodríguez Torrens et al., 2021).

Horses can become infected by drinking water contaminated with Leptospirae, by contact with urine or any fluids from infected animals. Leptospirae can enter the body of a susceptible animal through mucous membranes, damaged skin and transplacental routes, and subsequently colonize mainly the renal tubules (Khalili et al., 2019). Later, Leptospirae are excreted in the urine, contaminate various water sources used in agricultural production, livestock, and human consumption, and thus create conditions for human and animal infection (Pulido-Villamarin et al., 2014). The presence of *Leptospira* spp. DNA in horse semen in 50% of the studied samples confirms the possibility of sexual transmission (Hamond et al., 2015). There are studies that confirm the role of horses as sources of the pathogen for other animals and horses (Hashimoto et al.,

2007; Houwers et al., 2011). The clinical manifestations of leptospirosis vary from ultra-acute to chronic in different animal species (Båverud et al., 2009; de Vasconcelos et al., 2023). Animals also show subclinical forms of the disease, and in some individuals and species, lifelong carriage of the pathogen is observed. Horses can manifest a clinical form of the disease, including abortion, neonatal death, kidney and liver dysfunction (Hartskeerl et al., 2004; Vemulapalli et al., 2005; Dewes et al., 2020). Di Azevedo & Lilenbaum (2022) pointed out that in horses, it is necessary to speak of genital leptospirosis syndrome (EGL) (similar to the reproductive syndrome of bovine leptospirosis – BGL) (Libonati et al., 2018). The main agent causing EGL is mainly Bratislava serovar (serogroup Australis). In horses, in addition to systemic infections, eye damage and significant performance impairment have been described, especially for racehorses (Hamond et al., 2012, 2014). In 84% of cases of equine uveitis, it was found to be of leptospirosis origin (Dorrego-Keiter et al., 2016). Recurrent uveitis is an important consequence of leptospirosis infection and the main cause of blindness in horses (Verma & Stevenson, 2012).

In a study by Kulbrock et al. (2013), antibodies to *Leptospira* in horses were detected in 49% of vitreous samples from 45 vitrectomized eyes. The authors were able to isolate *Leptospira* DNA from 20 vitreous samples, and they evaluated 25 of the 45 vitrectomized eyes as positive for leptospirosis because the vitreous samples contained either *Leptospira* DNA or antibodies to the pathogen. In general, it is now generally accepted that equine leptospirosis can be associated with four syndromes: (1) ocular, characterized by equine recurrent uveitis (ERU), also called moon blindness or recurrent ophthalmia (Hartskeerl et al., 2004); (2) hepatorenal, characterized by classic jaundice symptoms (Malalana et al., 2017; Di Azevedo & Lilenbaum, 2022); (3) pulmonary, with acute respiratory failure (Pinna et al., 2013); (4) reproductive, causing reproductive disorders in mares (Velineni et al., 2016; Olmo et al., 2018). Reproductive disorders are associated with abortion and intrauterine fetal death, while the birth of infected foals is a common consequence of *Leptospira* infection (Timoney et al., 2011; Hamond et al., 2014). In addition, abortions are associated not only with local inflammation, but also with the presence of leptospires in the uterine environment (Di Azevedo & Lilenbaum, 2022). In the specialized literature, there are reports of changes in lipid metabolism due to immune activation caused by *Leptospira* (consequences of prolonged *Leptospira* carriage) (Gazi et al., 2011).

We have analyzed the serological prevalence of leptospirosis in horses in Ukraine for the period 2007–2021, identified the most epizootologically significant serovars of *Leptospira*, and provided appropriate recommendations for the control of this disease.

Materials and methods

The distribution and etiological composition of leptospirosis in horses were analyzed based on reports from the State Scientific Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise for the years 2007 to 2021. The seroprevalence for each oblast was calculated by dividing the number of positive leptospirosis samples by the total number of samples in that specific oblast.

Taking into account the requirements of the current Instruction (Meites et al., 2004), we conducted a retrospective analysis of outbreaks of equine leptospirosis caused by each of the main eight serogroups of *Leptospira* circulating in livestock farms of Ukraine among farm animals, in particular: Grippotyphosa, Canicola, Icterohaemorrhagiae, Australis, Pomona, Tarassovi, Hebdomadis, Sejroe. One strain of *Leptospira* with the broadest antigenic spectrum was used in the microagglutination reaction (MRA) from each serogroup (Table 1).

The analysis excluded data from the occupied region of the Autonomous Republic of Crimea, the city of Sevastopol, and segments of the temporarily occupied zones within the Donetsk and Luhansk regions due to unavailability. Information regarding the overall count of susceptible animals was extracted from statistical records provided by the State Statistics Service of Ukraine (<http://ukrstat.gov.ua>).

Geoinformation mapping analysis was conducted utilizing Quantum GIS 3.16.0 software, accessible for free at www.qgis.org/ru/site/forusers/download.html. Vector layers delineating the boundaries of Ukrainian regions were sourced from www.diva-gis.org/Data. Data distribution

employed quantile classification, employing 5 data classes, with each class encompassing an equal number of regions.

Table 1
List of diagnostic strains of *Leptospira*

Serogroup	Serovar	Strain
Sejroe	Polonica	493 Poland
Hebdomadis	Kabura	Kabura
Tarassovi	Tarassovi	Perepelicyni
Pomona	Pomona	Pomona
Grippotyphosa	Grippotyphosa	Moskva V
Canicola	Canicola	Hond Utrecht IV
Icterohaemorrhagiae	Copenhageni	M 20
Australis	Bratislava	Yež bratislava

Results

The amount of serological diagnostics of horses for the detection of specific antibodies to pathogenic leptospires in Ukraine for the period 2007–2021 is shown in Figure 1.

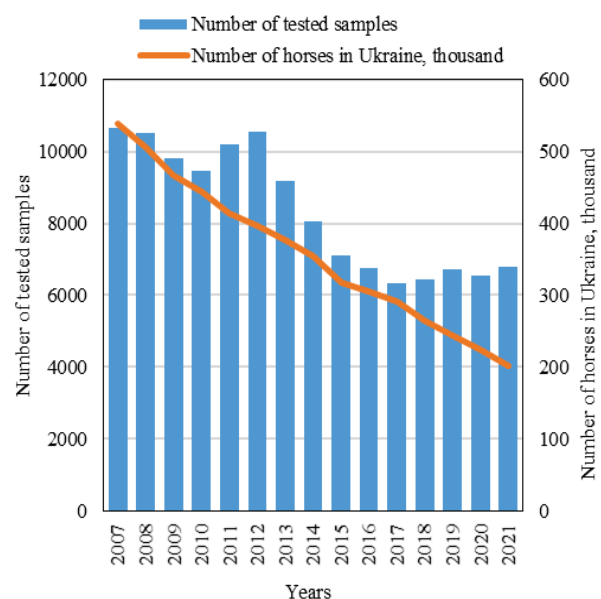


Fig. 1. Dynamics of the number of studied blood sera samples from horses on leptospirosis and total population of this species in Ukraine (2007–2021 years)

As can be seen from Figure 1, for the period 2007–2021, the largest number of blood serum samples from horses was tested in 2007, 2008 and 2012 – 10,643, 10,515 and 10,534 samples, respectively, and the smallest in 2017 and 2018 – 6,330 and 6,453 samples, respectively. It should be noted that during the period from 2012 to 2017, the volume of serological diagnostics of equine leptospirosis decreased significantly, from 10,534 samples in 2012 to 6,330 samples in 2017 (the number of samples tested during this period decreased by 39.9%). Since 2017, the number of equine serum samples tested has been approximately the same.

In order to compare the amount of research conducted and the number of horses, Figure 1 shows the dynamics of the number of livestock on the territory of Ukraine, according to the State Statistics Service of Ukraine (https://ukrstat.org/uk/druk/publicat/Arhiv_u/07/Arch_tvar_zb.htm) as of January 1 of each year. As evident from the graph, the count of horses in Ukraine has also been declining over the analyzed period. Thus, during the period of 2007–2021, the number of horses in Ukraine decreased from 538.3 to 202.0 thousand, i.e., there was a decrease in the number of horses by 62.5% during the specified period. In general, since Ukraine's independence, the number of horses has decreased by 3.5 times (from 700 thousand in 1991 to 202 thousand in 2021). At the same time, statistics show that about 95% of horses are currently used in the private sector.

During the period 2007–2021, veterinary laboratories of Ukraine examined 125,101 samples of equine blood sera, of which 13,543 were posi-

tive, which is 10.8% of the total number of samples tested. The dynamics of the incidence of leptospirosis in horses in Ukraine for the period 2007–2021 is shown in Figure 2.

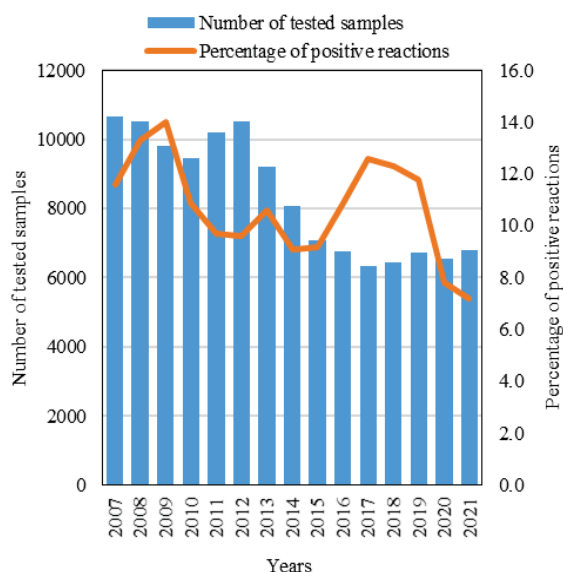


Fig. 2. Dynamics of leptospirosis infection in horses in Ukraine (2007–2021 years)

As shown in Figure 2, the leptospirosis infection rate in horses during the analyzed period was highest in 2008, 2009 and 2017 – 13.3%, 14.0% and 12.6%, respectively. The lowest infection rate was observed in recent years 2020 and 2021 – 7.8% and 7.2%, respectively. In 2014–2017, the infection rate increased slightly, and in the next four years (2018–2021), this figure began to decline again – 7.27–12.3%. In general, during the analyzed period, from 2007 to 2021, there was a steady downward trend in the number of horses seropositive for leptospirosis.

The etiological structure of equine leptospirosis in Ukraine for the period 2007–2021 is shown in Figure 3.

In the etiological structure of equine leptospirosis in Ukraine, according to the results of studies of state veterinary laboratories, the dominant serovars are: Copenhageni (serogroup Icterohaemorrhagiae) (32.8%), Bratislava (serogroup Australis) (16.1%), Grippytyphosa (serogroup Grippytyphosa) (15.4%) and Canicola (serogroup Canicola) (13.2%). Positive reactions with other *Leptospira* serovars were observed much less frequently: Tarassovi (Tarassovi serogroup) (10.7%), Pomona (Pomona serogroup) (7.8%), Polonica (Sejroe serogroup) (2.3%) and Kabura (Hebdomadis serogroup) (1.7%). It should be noted that during the analyzed period, a large number of positive reactions with different serovars of *Leptospira* (mixed reactions) were observed, which amounted to 55.1% of the total number of positive animals. We also conducted an analysis of the dynamics of the number of seropositive horses during the period 2007–2021 for each of the eight *Leptospira* serovars which are used in the diagnostics of leptospirosis in this animal in Ukraine, particularly during performance of MAT (Fig. 4).

As a result of a thorough retrospective mapping analysis of the circulation of the main diagnostic *Leptospira* serovars among the horse population in Ukraine, it was found that during the analyzed period of 2007–2021, there was a decrease in the number of positive reactions to *Leptospira* serovars Bratislava, Tarassovi, Pomona and Polonica, and an increase in the number of reactions with serovars Grippytyphosa, Canicola and Kabura. The percentage of infection of horses with the dominant serovar *Leptospira* Copenhageni was approximately the same.

Based on the results of the data analysis, we have compiled "Maps of the distribution of cases of horses seropositive to various *Leptospira* serovars" for eight *Leptospira* serogroups, which visualize the number of positive reactions of horse sera to these serogroups in the context of Ukrainian regions using different color intensities to distinguish the regions.

As illustrated in Figure 5, the highest incidence rates of leptospirosis in horses are observed in the eastern and northern parts of Ukraine. It has also been established that each serovar has its own peculiarities of territori-

al distribution in Ukraine. Thus, serovars Copenhageni, Bratislava, Grippytyphosa, Canicola and Tarassovi are most common in the eastern and northern parts of Ukraine; serovar Pomona is most common in the northern part of Ukraine; the largest number of positive reactions to serovars Polonica and Kabura were recorded in Sumy, Cherkasy, Kharkiv and Donetsk regions.

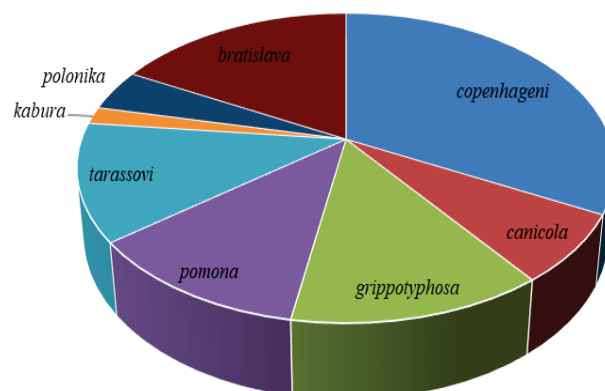


Fig. 3. Etiological structure of leptospirosis in horses in Ukraine (2007–2021 years)

Utilizing the scrutinized data, a comprehensive map illustrating the etiological composition of equine leptospirosis across all Ukrainian regions was constructed. This map visually represents the distribution of eight distinct leptospiral serovars in the period spanning from 2007 to 2021 through the utilization of pie charts, as depicted in Figure 6.

After a thorough retrospective cartographic analysis of the circulation of the main diagnostic serovars of leptospira among horses in Ukraine, it was found that the epizootic situation of leptospirosis of this animal species in different regions of Ukraine is not uniform and has its own characteristics, both in terms of the number of cases of infection of horses and the number of leptospirosis pathogens. For example, in Donetsk, Sumy and Cherkasy regions, cases of infection of horses with *Leptospirae* of all 8 *Leptospira* serovars officially included in the diagnostic range for leptospirosis testing in Ukraine are recorded, and a significant percentage of animals infected with *Leptospira* of several serogroups is noted simultaneously. Odesa, Zakarpattia, and Chemivtsi regions are characterized by a low level of leptospirosis infection in horses, so the spectrum of *Leptospira* serogroups in these regions is not diverse.

Based on the analyzed data, a map of the density of leptospirosis cases in horses in Ukraine over the past fifteen years (2007–2021) was compiled and is shown in Figure 7. On this map, all regions of the country were divided into five risk zones: very low, low, medium, high and very high.

The very high risk area includes five regions: Donetska (2,848 positive samples), Sumska (2,559), Cherkaska (1,752), Kharkivska (1,514), Chemihivska (756 positive samples). The total number of positive samples for equine leptospirosis in this zone is 9,429, which is 69.6%. The oblasts included in this zone are characterized by a very high probability of leptospirosis in the horse population.

Odeska, Khmelnytska, Chernivetska, Kyivska, Zakarpatska and Lvivska oblasts have had the lowest number of leptospirosis-positive horses over the past fifteen years, and therefore are classified as a low-risk area, with a total incidence of about 1.0% (133 positive samples were detected).

Discussion

The results of the analysis of studies of horses for leptospirosis for the period 2007–2021 showed that the seropositivity was 10.8%. Studies conducted in Brazil suggest that seropositivity among this animal species can be 28% or more (Dewes et al., 2020), 45% or more in Cuba (Rodríguez Torrens et al., 2021). Recent studies conducted in 29 US states have shown a prevalence of leptospirosis in horses of 45% (Wood et al., 2018). The detection of antibodies to the Tarassovi (10.7%), Pomona (7.8%) and Canicola (13.2%) serovars in horses indicates that cattle, pigs and dogs

may be sources of the pathogen. The latter indicates the complexity of the problem and the need for preventive measures against leptospirosis in other animal species (Whitwell et al., 2009; Shanahan & Slovis, 2011). As a rule, *Canicola* serovar is much more serious. After all, a significant

number of horses are kept in private households (95% in Ukraine today), where such animals as dogs are necessarily present. Therefore, in some studies, this "canine" serovar accounts for up to 35% or more of animals seropositive for leptospirosis (Dewes et al., 2020).

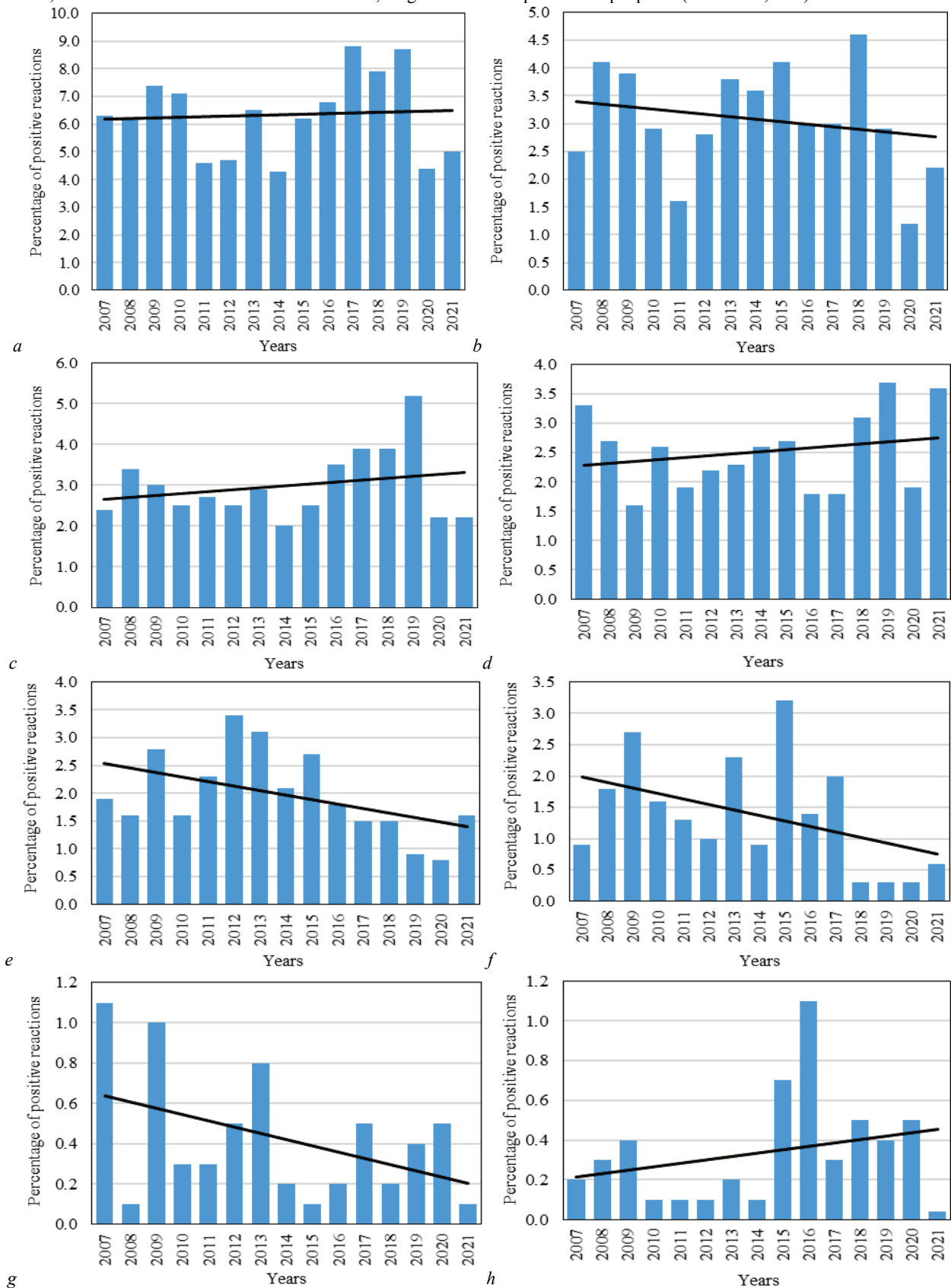


Fig. 4. Dynamics of the number of horses seropositive to various *Leptospira* serovars (2007–2021 years): *a* – sv. Copenhageni (serogroup Icterohaemorrhagiae); *b* – sv. Bratislava (serogroup Australis); *c* – sv. Grippytophosa (serogroup Grippytophosa); *d* – sv. Canicola (serogroup Canicola); *e* – sv. Tarassovi (serogroup Tarassovi); *f* – sv. Pomona (serogroup Pomona); *g* – sv. Polonica (serogroup Sejroe); *h* – sv. Kabura (serogroup Hebdomadis)

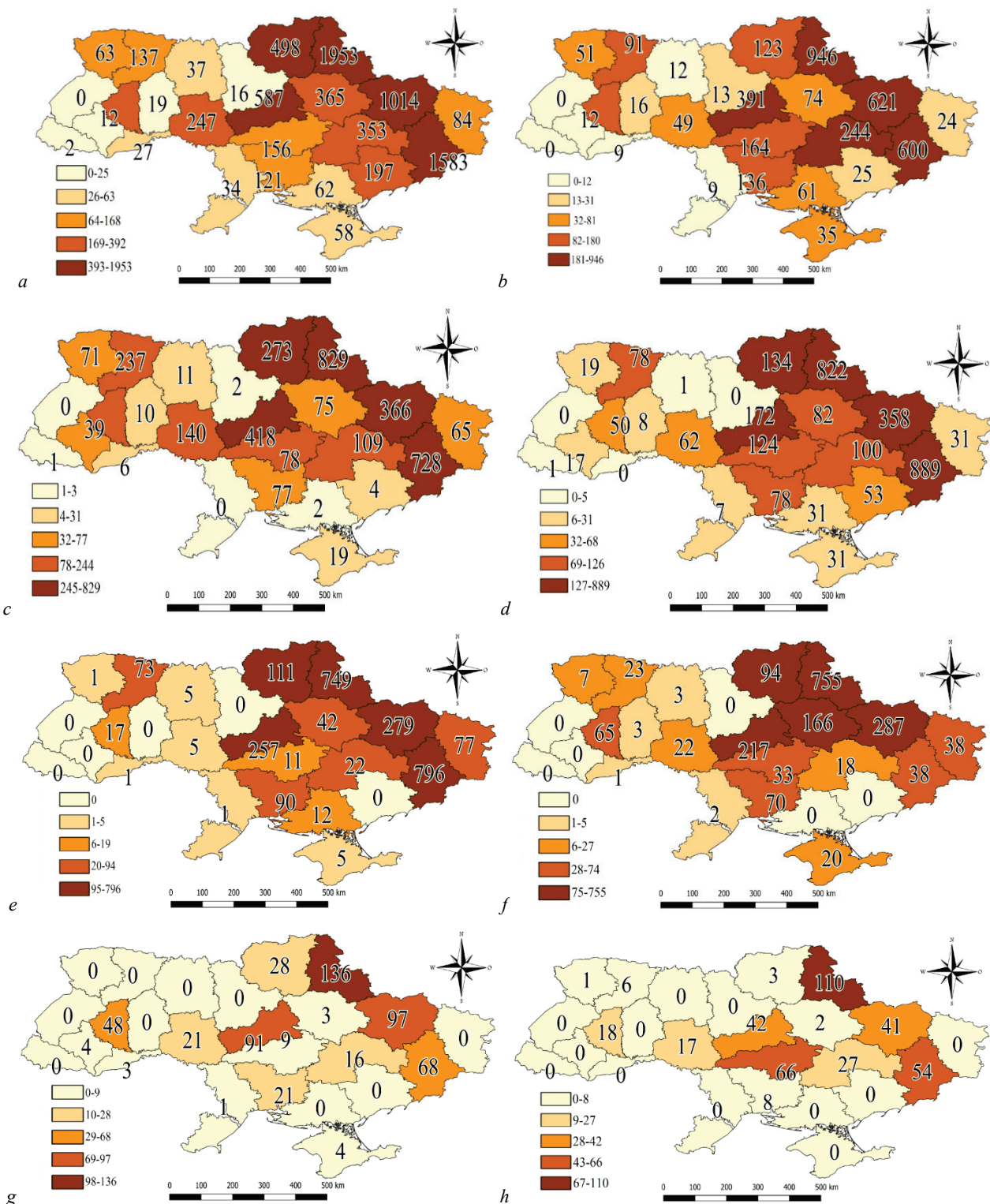


Fig. 5. Maps of the distribution of cases of horses seropositive to various *Leptospira* serovars (2007–2021 years): *a* – sv. Copenhageni (serogroup Icterohaemorrhagiae); *b* – sv. Bratislava (serogroup Australis); *c* – sv. Grippotyphosa (serogroup Grippotyphosa); *d* – sv. Canicola (serogroup Canicola); *e* – sv. Tarassovi (serogroup Tarassovi); *f* – sv. Pomona (serogroup Pomona); *g* – sv. Polonica (serogroup Sejroe); *h* – sv. Kabura (serogroup Hebdomadis)

The dominant serovar in horses in our study was Copenhageni (serogroup Icterohaemorrhagiae), with a seropositivity rate of 32.8% during the analyzed period. This serovar is associated with rodents worldwide. The latter indicates close contact of horses with rodents (mice, rats), which are the main reservoirs of this serogroup (Martins & Lilenbaum, 2013; De Souza et al., 2016). It is the Icterohaemorrhagiae serogroup that causes the highest mortality from leptospirosis in humans (Faria et al., 2008). In our studies, the second place was taken by serovar Bratislava (serogroup Australis) (16.1%), because a significant number of researchers believe that this serovar is adapted to horses (Pinna et al., 2014; Aymée et al.,

2021; de Vasconcelos et al., 2023). In the case of uveitis of leptospirosis etiology in horses, serovars Pomona, Tarassovi, Grippotyphosa, Icterohaemorrhagiae and Hardjo are mainly detected (Gilger, 2010; Dorrego-Keiter et al., 2016).

The variety of serovars detected in horses suggests that they come into contact with different species of animals (domestic, wild, companion) and thus acquire *Leptospira* spp. Domestic and wild animals are reservoirs of the pathogen in rural areas, while rats and dogs are important reservoirs in urban areas (Meites et al., 2004; Verma et al., 2013). In general, the spectrum of *Leptospira* serovars detected in Ukraine: Copenhageni (sero-

group Icterohaemorrhagiae), Bratislava (serogroup Australis), Grippotyphosa (serogroup Grippotyphosa), Canicola (serogroup Canicola), Tarassovi (serogroup Tarassovi), Pomona (serogroup Pomona), Polonica (serogroup Sejroe), Kabura (serogroup Hebdomadis) coincides with variants found in horses worldwide (Broux et al., 2012; Hamond et al., 2013; Wangdi et al., 2013; Divers et al., 2019; Muller et al., 2021).

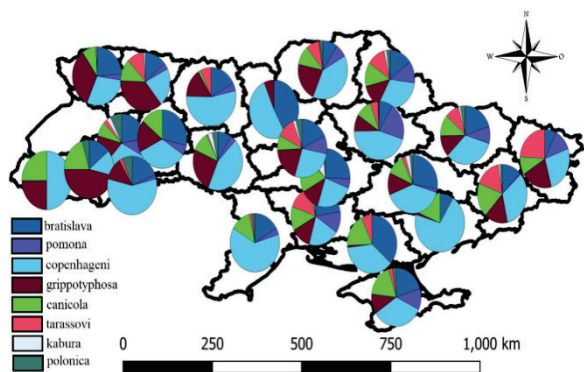


Fig. 6. Map of the etiological structure of leptospirosis of horses in Ukraine (2007–2021 years)

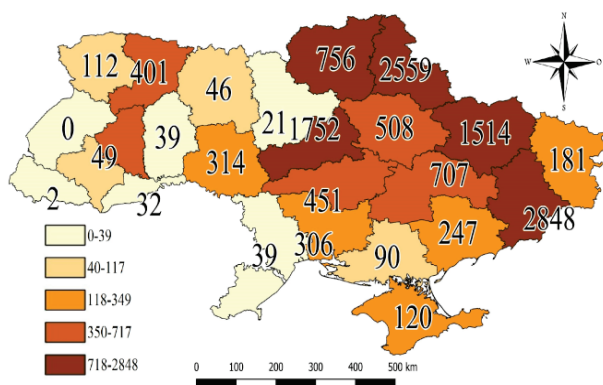


Fig. 7. Map of the density of cases of horse leptospirosis in Ukraine (2007–2021 years)

We found the most significant incidence of leptospirosis in horses in the eastern and northern regions of Ukraine. There are peculiarities of a certain territorial affiliation of serovars to different regions of Ukraine. For example, serovars such as Copenhageni, Bratislava, Grippotyphosa, Canicola and Tarassovi are most common in Eastern and Northern Ukraine. The Pomona serovar has a geographical distribution in Northern Ukraine. A significant number of seropositive horses for the Polonica and Kabura serovars were detected in Sumy, Cherkasy, Kharkiv and Donetsk regions. An analysis of the specialized literature shows that the dominant serovars in Germany and many neighboring countries are Grippotyphosa and Icterohaemorrhagiae, followed by serovar Bratislava (Yan et al., 2010; Voelter et al., 2020). In countries further to the east of Europe and in the United States of America, infections with serovars from the Pomona serogroup have been described (Divers et al., 2019). Our research and the research of these authors confirm the complexity of the leptospirosis problem. After all, it is impossible to fight only leptospirosis in horses. Control measures should be aimed at controlling leptospirosis-carrying animals, as in agricultural enterprises, where any species of animals, domestic or wild, especially rodents, can be carriers of the pathogen.

The impact of different serovars of the leptospirosis pathogen in different regions of Ukraine has regional characteristics. These include different environments where horses are kept, the presence of animals carrying the pathogen (which act as reservoirs), or its individual serovars. Consequently, significant differences in the incidence and prevalence of serovars in horses largely depend on the geographical region. Horses are often infected with Leptospirae when drinking water contaminated with the pathogen. It should be noted that the pressure of this pathogen on horses is constant, but systemic diseases are rarely recorded, the degree of severity of the infection varies, from asymptomatic to various manifestations.

In general, it is believed that older and middle-aged horses are more likely to be carriers of *Leptospira* than younger animals, and that their antibody titers are significantly higher (Båverud et al., 2009). In this case, the age of horses is almost always significantly associated with the presence of antibody titers to *Leptospira*. In general, the chances of being seropositive in this species of animal increase by about 10% with each year of life (Lees & Gale, 1994). The latter means that five-year-old horses have approximately 40% higher seroprevalence for any given serovar than yearlings. The authors confirm that similar results were obtained for almost all serovars. The latter means that as horses grow older, they are more likely to come into contact with Leptospirae and, accordingly, to form antibodies after exposure to the pathogen. Similar results were also obtained in thoroughbred horses, which at the age of 2–3 years were seropositive for *L. bratislava* in only 5% of cases, horses older than 7 years had a seropositivity of 52% (Kitson-Piggot & Prescott, 1987). This factor also affects the decrease in the number of seropositive animals among horses in Ukraine. Not only does the reduction in the number of horses by almost 3.5 times since independence (since 1991) lead to a decrease in the number of leptospirosis carriers, but in this situation the herd is significantly rejuvenated and the number of positive animals decreases.

The analysis of the incidence of leptospirosis in horses showed a heterogeneous distribution of *Leptospira* serogroups in different regions of Ukraine. Differences in natural conditions, housing conditions, and environmental factors in which the epizootic process of leptospirosis occurs affect its manifestation and intensity in different geographical areas. At present, the issue of the etiology of leptospirosis infection requires constant monitoring and analysis of the spread of *Leptospira* serovars among the horse population to determine the antigenic composition of leptospirosis pathogens in order to adjust preventive measures.

It should also be noted that serological monitoring studies are not enough to control this disease; attention should also be paid to sanitary measures, disinfection and deratization, vaccination of horses and other animal species (if necessary) to mitigate the effects of the disease in horses. The triadic approach – antibiotic therapy, vaccination and environmental management – is important in controlling the disease (Verma et al., 2013).

Therefore, our research confirms that the epizootic situation with regard to equine leptospirosis in different regions of Ukraine is not homogeneous. The information obtained can be used in the work of veterinarians working with this species of animals and taking measures to prevent and control leptospirosis. They will help in the development of specific health improvement measures in the areas (regions) of Ukraine that are unfavorable for this disease.

Conclusion

We conducted an ecological and geographical analysis of outbreaks of equine leptospirosis for each of the eight *Leptospira* serovars used in the diagnosis of leptospirosis in farm animals in Ukraine. During the period 2007–2021, 125,101 samples of equine blood sera were tested for leptospirosis, of which 13,543 were positive, which is 10.8% of the total number of samples tested. In the etiological structure of equine leptospirosis in Ukraine, the dominant serovars were: Copenhageni (serogroup Icterohaemorrhagiae) (32.8%), Bratislava (serogroup Australis) (16.1%), Grippotyphosa (serogroup Grippotyphosa) (15.4%) and Canicola (serogroup Canicola) (13.2%). Positive reactions were also obtained for the serovars Tarassovi (serogroup Tarassovi) (10.7%), Pomona (serogroup Pomona) (7.8%), Polonica (serogroup Sejroe) (2.3%) and Kabura (serogroup Hebdomadis) (1.7%). It should be noted that during the analyzed period, a significant number of positive reactions with different serovars of *Leptospira* (mixed reactions) were observed, which accounted for 55.1% of the total number of positive animals. The serovars Copenhageni, Bratislava, Grippotyphosa, Canicola and Tarassovi were most common in the eastern and northern parts of Ukraine, and the serovar Pomona was most common in the northern part of Ukraine.

The authors extend their sincere appreciation to the experts and leadership of regional establishments within the state veterinary service and veterinary laboratories for their invaluable assistance in coordinating the data collection process.

The authors declare that there is no competing of interest.

References

- Aymée, L., Gregg, W. R. R., Loureiro, A. P., Di Azevedo, M. I. N., Pedrosa, J. S., Melo, J. D. S. L., Carvalho-Costa, F. A., de Souza, G. N., & Lilenbaum, W. (2021). Bovine genital leptospirosis and reproductive disorders of live subfertile cows under field conditions. *Veterinary Microbiology*, 261, 109213.
- Båverud, V., Gunnarsson, A., Engvall, E. O., Franzén, P., & Egenvall, A. (2009). *Leptospira* seroprevalence and associations between seropositivity, clinical disease and host factors in horses. *Acta Veterinaria Scandinavica*, 51(1), 15.
- Broux, B., Torfs, S., Wegge, B., Deprez, P., & van Loon, G. (2012). Acute respiratory failure caused by *Leptospira* spp. in 5 foals. *Journal of Veterinary Internal Medicine*, 26(3), 684–687.
- de Faria, M. T., Calderwood, M. S., Athanazio, D. A., McBride, A. J., Hartskeerl, R. A., Pereira, M. M., Ko, A. I., & Reis, M. G. (2008). Carriage of *Leptospira interrogans* among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. *Acta Tropica*, 108(1), 1–5.
- De Souza, M. A., Moreira, R. Q., Bombonato, N. G., & Castro, J. (2016). Anti-*Leptospira* spp. antibodies in several animal species on the same farm. *Bio-science Journal*, 32, 202–207.
- de Vasconcelos, T. C. B., de Castro, M. P., da Silva, D. B., Barros, R. S., de Almeida, A. B., Mendes Júnior, A. A. V., Langoni, H., & Figueiredo, F. B. (2023). Serological survey of leptospirosis in horses with historical displacement through different geographic regions in Brazil. *Medicina Veterinária*, 17(1), 56–60.
- Dewes, C., Fortes, T. P., Machado, G. B., Pacheco, P. S., Silva, J. P. M., Neto, A. C. P. S., Félix, S. R., & Silva, da Éverton, F. (2020). Prevalence and risk factors associated with equine leptospirosis in an endemic urban area in Southern Brazil. *Brazilian Journal of Development*, 6(8), 58380–58390.
- Di Azevedo, M. I. N., & Lilenbaum, W. (2022). Equine genital leptospirosis: Evidence of an important silent chronic reproductive syndrome. *Theriogenology*, 192, 81–88.
- Divers, T. J., Chang, Y. F., Irby, N. L., Smith, J. L., & Carter, C. N. (2019). Leptospirosis: An important infectious disease in North American horses. *Equine Veterinary Journal*, 51(3), 287–292.
- Dorrego-Keiter, E., Tóth, J., Dikker, L., Sielhorst, J., & Schusser, G. F. (2016). Kultureller Nachweis von Leptospiren in Glaskörperflüssigkeit und Antikörpernachweis gegen Leptospiren in Glaskörperflüssigkeit und Serum von 225 Pferden mit equiner rezidivierender Uveitis (ERU) [Detection of *Leptospira* by culture of vitreous humor and detection of antibodies against *Leptospira* in vitreous humor and serum of 225 horses with equine recurrent uveitis]. *Berliner und Münchener Tierärztliche Wochenschrift*, 129(5–6), 209–215 (in German).
- Gazi, I. F., Apostolou, F. A., Liberopoulos, E. N., Filippatos, T. D., Tellis, C. C., Elisaf, M. S., & Tselepis, A. D. (2011). Leptospirosis is associated with markedly increased triglycerides and small dense low-density lipoprotein and decreased high-density lipoprotein. *Lipids*, 46(10), 953–960.
- Ghazaei, C. (2018). Pathogenic *Leptospira*: Advances in understanding the molecular pathogenesis and virulence. *Open Veterinary Journal*, 8(1), 13–24.
- Gilger, B. C. (2010). Equine recurrent uveitis: The viewpoint from the USA. *Equine Veterinary Journal*, Supplement, 37, 57–61.
- Hamond, C., Martins, G., & Lilenbaum, W. (2012). Subclinical leptospirosis may impair athletic performance in racing horses. *Tropical Animal Health and Production*, 44(8), 1927–1930.
- Hamond, C., Martins, G., Lawson-Ferreira, R., Medeiros, M. A., & Lilenbaum, W. (2013). The role of horses in the transmission of leptospirosis in an urban tropical area. *Epidemiology and Infection*, 141(1), 33–35.
- Hamond, C., Pestana, C. P., Rocha-de-Souza, C. M., Cunha, L. E., Brandão, F. Z., Medeiros, M. A., & Lilenbaum, W. (2015). Presence of leptospires on genital tract of mares with reproductive problems. *Veterinary Microbiology*, 179(3–4), 264–269.
- Hamond, C., Pinna, A., Martins, G., & Lilenbaum, W. (2014). The role of leptospirosis in reproductive disorders in horses. *Tropical Animal Health and Production*, 46(1), 1–10.
- Hartskeerl, R. A., Goris, M. G., Brem, S., Meyer, P., Kopp, H., Gerhards, H., & Wol-lanke, B. (2004). Classification of *Leptospira* from the eyes of horses suffering from recurrent uveitis. *Journal of Veterinary Medicine, Series B: Infectious Diseases and Veterinary Public Health*, 51(3), 110–115.
- Hartskeerl, R. A., Goris, M. G., Brem, S., Meyer, P., Kopp, H., Gerhards, H., & Wol-lanke, B. (2004). Classification of leptospires from the eyes of horses suffering from recurrent uveitis. *Journal of Veterinary Medicine, Series B: Infectious Diseases and Veterinary Public Health*, 51(3), 110–115.
- Hashimoto, V. Y., Gonçalves, D. D., Silva, F. G., Oliveira, R. C., Alves, L. A., Reichmann, P., Muller, E. E., & Freitas, J. C. (2007). Occurrence of antibodies against *Leptospira* spp. in horses of the urban area of Londrina, Paraná, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 49(5), 327–330.
- Houwers, D. J., Goris, M. G., Abdoel, T., Kas, J. A., Knobbe, S. S., van Dongen, A. M., Westerduin, F. E., Klein, W. R., & Hartskeerl, R. A. (2011). Agglutinating antibodies against pathogenic *Leptospira* in healthy dogs and horses indicate common exposure and regular occurrence of subclinical infections. *Veterinary Microbiology*, 148(2–4), 449–451.
- Khalili, M., Sakhaee, E., Bagheri Amiri, F., Safat, A. A., Afshar, D., & Esmaeili, S. (2020). Serological evidence of leptospirosis in Iran: A systematic review and meta-analysis. *Microbial Pathogenesis*, 138, 103833.
- Kitson-Piggot, A. W., & Prescott, J. F. (1987). Leptospirosis in horses in Ontario. *Canadian Journal of Veterinary Research*, 51(4), 448–451.
- Kulbrock, M., von Bostel, M., Rohn, K., Distl, O., & Ohnesorge, B. (2013). Studie zu Häufigkeit und Schweregrad der Equinen Rezidivierenden Uveitis bei Warmblütern [Study on frequency and severity of equine recurrent uveitis in warm-blooded horses]. *Pferdeheilkunde*, 29(1), 27–36 (in German).
- Lees, V. W., & Gale, S. P. (1994). Titers to *Leptospira* species in horses in Alberta. *The Canadian Veterinary Journal*, 35(10), 636–640.
- Libonati, H. A., Santos, G. B., Souza, G. N., Brandão, F. Z., & Lilenbaum, W. (2018). Leptospirosis is strongly associated to estrus repetition on cattle. *Tropical Animal Health and Production*, 50(7), 1625–1629.
- Malalana, F., Blundell, R. J., Pinchbeck, G. L., & Megowan, C. M. (2017). The role of *Leptospira* spp. in horses affected with recurrent uveitis in the UK. *Equine Veterinary Journal*, 49(6), 706–709.
- Martins, G., & Lilenbaum, W. (2013). The panorama of animal leptospirosis in Rio de Janeiro, Brazil, regarding the seroepidemiology of the infection in tropical regions. *BMC Veterinary Research*, 9, 237.
- Meites, E., Jay, M. T., Deresinski, S., Shieh, W. J., Zaki, S. R., Tompkins, L., & Smith, D. S. (2004). Reemerging leptospirosis, California. *Emerging Infectious Diseases*, 10(3), 406–412.
- Muller, V., Moraes, B. dos S. S., de Souza, R. P., Mousquer, M. A., Dewes, C., da Silva, É. F., & Nogueira, C. E. W. (2021). Leptospirosis seroprevalence in racehorses: A descriptive observational study. *Research, Society and Development*, 10(8), e29910817287.
- Mwachui, M. A., Crump, L., Hartskeerl, R., Zinsstag, J., & Hattendorf, J. (2015). Environmental and behavioural determinants of leptospirosis transmission: A systematic review. *PLoS Neglected Tropical Diseases*, 9(9), e0003843.
- Olmo, L., Dye, M. T., Reichel, M. P., Young, J. R., Nampanya, S., Khounsy, S., Thomson, P. C., Windsor, P. A., & Bush, R. D. (2018). Investigation of infectious reproductive pathogens of large ruminants: Are neosporosis, brucellosis, leptospirosis and BVDV of relevance in Lao PDR? *Acta Tropica*, 177, 118–126.
- Pinna, A., Martins, G., Hamond, C., Medeiros, M. A., de Souza, G. N., & Lilenbaum, W. (2014). Potential differences between *Leptospira* serovars, host-adapted (*Bratislava*) and incidental (*Copenhageni*), in determining reproductive disorders in embryo transfer recipient mares in Brazil. *The Veterinary Record*, 174(21), 531.
- Pinna, A., Martins, G., Souza, G., & Lilenbaum, W. (2013). Influence of seroreactivity to *Leptospira* and reproductive failures in recipient mares of equine embryo transfer programmes. *Reproduction in Domestic Animals = Zuchtthygiene*, 48(4), e55–e57.
- Pulido-Villamarin, A., Carreno-Beltran, G., Mercado-Reyes, M., & Ramirez-Bulia, P. (2014). Situación epidemiológica de la leptospirosis humana en Centroamérica, Suramérica y el Caribe [Epidemiological situation of human leptospirosis in Central and South America and the Caribbean]. *Universitas Scientiarum*, 19(3), 247–264 (in Spanish).
- Ramos, A. C., Souza, G. N., & Lilenbaum, W. (2006). Influence of leptospirosis on reproductive performance of sows in Brazil. *Theriogenology*, 66(4), 1021–1025.
- Rodríguez Torrens, H. de la C. Argilagos, G. B., Montes de Oca, R. V., & Garsia Casas, T. (2021). Behavior of leptospirosis according to equine and human positive reactors during a decennium previous COVID-19. *Archives of Veterinary and Animal Sciences*, 3, 1.
- Sato, Y., Mizuyama, M., Sato, M., Minamoto, T., Kimura, R., & Toma, C. (2019). Environmental DNA metabarcoding to detect pathogenic *Leptospira* and associated organisms in leptospirosis-endemic areas of Japan. *Scientific Reports*, 9(1), 6575.
- Shanahan, L. M., & Slovis, N. M. (2011). *Leptospira interrogans* associated with hydrallantois in 2 pluriparous Thoroughbred mares. *Journal of Veterinary Internal Medicine*, 25(1), 158–161.
- Timoney, J. F., Kalimuthusamy, N., Velineni, S., Donahue, J. M., Artiushin, S. C., & Fettinger, M. (2011). A unique genotype of *Leptospira interrogans* serovar *Pomona* type *kennewicki* is associated with equine abortion. *Veterinary Microbiology*, 150(3–4), 349–353.
- Torres-Castro, M., Hernández-Betancourt, S., Agudelo-Flórez, P., Arroyave-Sierra, E., Zavala-Castro, J., & Puerto, F. I. (2016). Revisión actual de la epidemiología de la leptospirosis [Current review of the epidemiology of leptospirosis]. *Revista Medica del Instituto Mexicano del Seguro Social*, 54(5), 620–625 (in Spanish).
- Velineni, S., Timoney, J. F., Artiushin, S. C., Donahue, J. M., & Steinman, M. (2016). Multiple specificities of immunoglobulin M in equine fetuses infected with *Leptospira interrogans* indicate a competent immune response. *Equine Veterinary Journal*, 48(6), 704–709.
- Vemulapalli, R., Langohr, I. M., Sanchez, A., Kiupel, M., Bolin, C. A., Wu, C. C., & Lin, T. L. (2005). Molecular detection of *Leptospira kirschneri* in tissues of a

- prematurely born foal. *Journal of Veterinary Diagnostic Investigation*, 17(1), 67–71.
- Verma, A., & Stevenson, B. (2012). Leptospiral uveitis – there is more to it than meets the eye! *Zoonoses and Public Health*, 59(2), 132–141.
- Verma, A., Stevenson, B., & Adler, B. (2013). Leptospirosis in horses. *Veterinary Microbiology*, 167(1–2), 61–66.
- Vincent, A. T., Schiettekatte, O., Goarant, C., Neela, V. K., Bemet, E., Thibeaux, R., Ismail, N., Mohd Khalid, M. K. N., Amran, F., Masuzawa, T., Nakao, R., Amara Korba, A., Bourhy, P., Veyrier, F. J., & Picardeau, M. (2019). Revisiting the taxonomy and evolution of pathogenicity of the genus *Leptospira* through the prism of genomics. *PLoS Neglected Tropical Diseases*, 13(5), e0007270.
- Voelter, K., Vial, Z., Pot, S. A., & Spiess, B. M. (2020). Leptospiral antibody prevalence and surgical treatment outcome in horses with Equine Recurrent Uveitis (ERU) in Switzerland. *Veterinary Ophthalmology*, 23(4), 648–658.
- Wangdi, C., Picard, J., Tan, R., Condon, F., Dowling, B., & Gummow, B. (2013). Equine leptospirosis in tropical Northern Queensland. *Australian Veterinary Journal*, 91(5), 190–197.
- Whitwell, K. E., Blunden, A. S., Miller, J., & Errington, J. (2009). Two cases of equine pregnancy loss associated with *Leptospira* infection in England. *The Veterinary Record*, 165(13), 377–378.
- Wollanke, B., Gerhards, H., & Ackermann, K. (2022). Infectious uveitis in horses and new insights in its leptospiral biofilm-related pathogenesis. *Microorganisms*, 10(2), 387.
- Wood, P. L., Steinman, M., Erol, E., Carter, C., Christmann, U., & Verma, A. (2018). Lipidomic analysis of immune activation in equine leptospirosis and *Leptospira*-vaccinated horses. *PLoS One*, 13(2), e0193424.
- Yamaguchi, T., Higa, N., Okura, N., Matsumoto, A., Hermawan, I., Yamashiro, T., Suzuki, T., & Toma, C. (2018). Characterizing interactions of *Leptospira interrogans* with proximal renal tubule epithelial cells. *BMC Microbiology*, 18(1), 64.
- Yan, W., Faisal, S. M., Divers, T., McDonough, S. P., Akey, B., & Chang, Y. F. (2010). Experimental *Leptospira interrogans* serovar *Kennewicki* infection of horses. *Journal of Veterinary Internal Medicine*, 24(4), 912–917.