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Monitoring the spread of leptospirosis agent as one of the reasons of low-quality milk

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ABSTRACT

On the global scale of the zoonoses problem, leptospirosis is among the five diseases that pose the greatest threat to humankind today. Leptospirosis is a worldwide zoonotic disease caused by pathogenic *Leptospira species*. In general, leptospirosis has been registered in more than 150 species of mammals. There are about 300 serovars of *Leptospira* spp. Serovar *Hardjo* is one of the most common causes of leptospirosis among cattle globally. In cows, the infection can be completely asymptomatic or cause abortions, stillbirths, infertility, and mastitis. The study's relevance is determined by the negative impact on the economy – productivity loss, and high cost of medical-preventive activities. Leptospirosis also affects humans. In this regard, the present study aimed to determine the prevalence of antibodies to *Leptospira interrogans* serovar *Hardjo* in tank milk samples from cows selected from farms in different regions of Ukraine. The method of indirect enzyme-multiplied immunoassay was used for this problem to be investigated. We have investigated 114 tank samples from 66 Ukrainian farms, of which 63.2% were positive, and 36.8% negative. It was established that antibodies to the causative agent of leptospirosis were recorded in different regions of Ukraine. It has been established that the largest number of positive samples was from Kyiv and Cherkasy regions. Our study results complement the study results of other authors and indicate the circulation of this causative agent among the cows in Ukraine, as well as being of practical value for diagnosing and controlling leptospirosis among the cattle.

Keywords: cattle, *Leptospira Hardjo*, antibodies, diagnosis, enzyme-multiplied immunoassay

INTRODUCTION

On the global scale of the zoonoses problem, according to the criteria of socio-economic rating, leptospirosis belongs to the five diseases that pose the greatest threat to humankind today. At least one million clinical disease cases of leptospirosis are annually observed among human and a crude mortality rate range from 5% to 15%. Leptospirosis affects a broad host range, including the cattle, sheep, goats and wild animals. The prevalence of serovar *Hardjo* among cattle in foreign countries is 72% – in England, 34.7% – in Ireland, 11% – in Spain, 42% – in the USA, and so on. The circulation of *L. interrogans* serovar *Hardjo* among cattle is observed in Ukraine in the range of 25.8-60.0% [1], [2].

Leptospirosis is a worldwide zoonotic disease [3] caused by infection with pathogenic *Leptospira species*. In general, leptospirosis has been registered in more than 150 species of mammals, but the infectious agent can also be detected in other classes of animals (reptiles, amphibians, etc.) [4]. There are approximately 300 serovars of *Leptospira* spp. [5] which are divided into 28 groups [6]. Leptospirosis among cattle can be caused by different serovars depending on the region and the host. Serovar *Hardjo* is one of the most common causes of leptospirosis among cattle globally.

It includes two species: *Leptospira interrogans* serovar *Hardjo* (prajinto) and *Leptospira interrogans* serovar *Hardjo* (bovis), although there are genetic and epidemiological differences between the two species; both species

are indistinguishable by serological testing [7]. Currently, cattle host this serovar, which secretes leptospire with urine [8] and secretions from the genital tract [9].

In the cattle, the infection causes significant economic losses and can be completely asymptomatic, or it can be the cause of abortions, stillborn calves, female infertility, reduced milk productivity, mastitis, birth of weak calves, embryonic mortality, as well as high cost of medical-preventive activities [10], [11], which is due to the use of antimicrobial substances, which, in turn, reduces the quality of dairy products [12].

Factors that foster the spread of the disease are many rodents, dogs and other wild animals, contaminated water and soil sources. The disease is also common in humans [13]. Symptoms of leptospirosis in humans are fever, myalgia, headache, renal failure, and pulmonary bleeding [14], [15]. Leptospire enter the organism through mucous membranes or skin failures and spread through blood [16].

Diseased animals can release the causative agent periodically or regularly for months, years, or throughout their lives. People who work in slaughterhouses, farms, meat processing plants, and veterinarians have the highest risk of disease incidence with leptospirosis [17]. As a rule, humans become infected through direct contact with infected animals that release the microorganism with their urine, or through indirect contact with contaminated water or soil. It is also reported about a possible transmission of leptospirosis through the consumption of raw milk obtained from infected cows [18], [19].

In recent years, leptospirosis in milk tank samples has yet to be studied in Ukraine. The present study aimed to investigate the prevalence of antibodies to *Leptospira interrogans* serovar *Hardjo* in tank samples of milk taken from different farms and regions of Ukraine.

In Ukraine, data on the spread of leptospirosis in tank samples of cow's milk are not systematic, and in some cases, they are absent at all, which indicates the relevance of this issue.

Scientific Hypothesis

The spread of leptospirosis in tank samples of milk in the studied farms may be significant, which will allow these farms to assess the risks of its spread and develop effective elimination measures to obtain high-quality and safe dairy products.

MATERIAL AND METHODOLOGY

Samples

Tank milk samples from cows, which were sent for the study to the serology laboratory of LLC “Veterinary Diagnostics Center” from different regions of Ukraine, were in sterile test tubes.

Chemicals

Dilution buffer Prionics Lelystard B.V (Netherlands);

Washing fluid Prionics Lelystard B.V (Netherlands);

Conjugate Prionics Lelystard B.V (Netherlands);

Chromogen (TMV) substrate Prionics Lelystard B.V (Netherlands);

Stop solution Prionics Lelystard B.V (Netherlands), (realtor UkrzooVetPostach, Kyiv, Ukraine).

Animals, Plants and Biological Materials

The animals were of different breeds (*Holstein*, *Ukrainian black* and *white*), age, duration of lactation and productivity. Information on clinical conditions, vaccination, treatment, herd size, diet, maintenance, watering, milking system, and breeding was absent. There needed to be more information on vaccination, herd size, diet, maintenance, watering, milking system, and breeding.

Instruments

Immunoenzyme analyzer Tecan Sunrise (Austria), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Tablet Microtest Plate 96 Well, F (Germany), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Dry air thermostat MicRomed (China), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Sartorius pipette dispenser (Germany), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Eppendorf pipette dispenser (Germany), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Laboratory utensils, (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Laboratory Methods

The presence of antibodies to *Leptospira interrogans* serovar *Hardjo* in tank samples of milk was determined by indirect EIA, with the use of commercial test system PrioCHECK *L. Hardjo* Ab Plate Kit (Thermo Fisher Scientific, Applied Biosystems, Lelystad, The Netherlands) [20]. EIA uses antigens to capture and quantify the amount of target antibodies present in a milk sample. The test result is the colour reaction measured by the reader in terms of optical density values. Optical densities provide a numerical-non-quantitative determination of the amount of antibodies to *L. Hardjo* in the studied sample. The final numerical result is a standardized percent positivity (PP) relative to a fixed reference sample (PP = optical density of test sample/optical density of reference sample). Recommended Prionics interpretation for PP from tank milk: PP <40% – negative for *L. Hardjo* specific antibodies, 40% PP – 60% – questionable result, and PP > 60% – positive result.

Description of the Experiment

Sample preparation: Tank milk was used for the experiment. Sampling was carried out directly on the farms, from coolers-tanks into 100 ml plastic tubes with screw caps after mixing. The samples were delivered to the laboratory at a temperature of 4-8 °C within 12 hours from their sampling. Subsequently, they were unpacked and homogenized, and 100 ml of milk was taken from each sample for the experiment.

Number of samples analyzed: 114 tank samples of milk were analyzed.

Number of repeated analyses: The retry number of each experiment for one value to be determined was 5 times.

Number of experiment replications: Each study was carried out five times, and the number of samples was three, resulting in fifteen repeated analyses.

Design of the experiment: First, we chose the farms that specialize in cattle breeding and have a dairy production direction, to select the samples from each farm individually. A team of 5 researchers conducted all studies on the investigated farms from December 2022 to September 2023. Maintaining conditions and milking procedures were assessed and documented in a standardized data collection form. When the cows were clinically examined, an anamnesis was taken, and the milk was entered into the tank; the samples were taken into sterile tubes after mixing the milk in the tank to ensure homogeneity. The samples were delivered to the laboratory at a temperature of 4-8 °C within 12 hours from selection. At the next stage, we conducted individual experiments to determine the presence of antibodies to *Leptospira interrogans* serovar *Hardjo*.

Statistical Analysis

The results were evaluated using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multifactor analysis of variance (MANOVA), LSD test. Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. Values were estimated using mean and standard deviations. The reliability of the research results was assessed according to the Student's test.

RESULTS AND DISCUSSION

According to the data of [21] the prevalence of *L. Hardjo* in tank samples of milk studied was 34.59 and 73% among unvaccinated herds in Ireland during 2018-2020, respectively. According to the data of [22] the prevalence of *L. Hardjo* in milk tank samples was 86% among unvaccinated herds in Ireland.

Studies in Brazil show that in 77 samples out of 208 animals had antibodies to leptospirosis [23]; other authors [24] indicate that the prevalence of antibodies to leptospirosis in Brazil is 52%.

For example, a study of 109 herds in Japan showed that 71 herds were positive for *Leptospira Hardjo*, and the prevalence at the herd level was 65.1% [25], which coincides with our studies. According to some sources [26], the seropositivity of animals at the herd level was 4.8% in Nepal.

These are just a few names of scientists actively working in research related to *Leptospira interrogans*. The directions of their research may differ depending on their specialization and interests.

Studies of 45 farms in India show that specific antibodies to *Leptospira Hardjo* were 27.76% [27], [28]. In the Netherlands, studies carried out during 2017-2021 indicate that *L. Hardjo* infections were detected in 120 dairy herds [29]. The authors' studies [30] in Egypt showed that 39.33% of the 236 studied animals had antibodies to leptospirosis. A total of 48 randomly selected cattle herds were studied in Algeria between 2015 and 2019. The prevalence of serovar *Leptospira interrogans Hardjo* was 31.25% [31]. The authors' study from Ethiopia [21] from 2019 to 2020 showed that out of 77 dairy farms selected for the study, 57 were marked as positive for *L. Hardjo*. In Pakistan, the prevalence of antibodies to leptospirosis among the cattle was 56.25% [32]. The studies conducted in Tanzania showed the prevalence of serovar *Leptospira Hardjo* at 13% [33]. Researchers [34] determined that the prevalence of leptospirosis in Manabí, Ecuador, at the herd level is 98.18%.

During the study period, to determine specific antibodies to *Leptospira interrogans* serovar *Hardjo*, the tank samples of milk from 16 regions of Ukraine were sent to the serology laboratory of LLC "Veterinary Diagnostics".

No samples were sent from 8 regions (Odesa, Volyn, Luhansk, Chernivtsi, Zakarpattia, Lviv, Zaporizhzhia, Rivne). The largest farms were studied from Kyiv, Cherkasy, Sumy, Khmelnytskyi, Poltava, Chernihiv, and Zhytomyr regions (Figure 1).

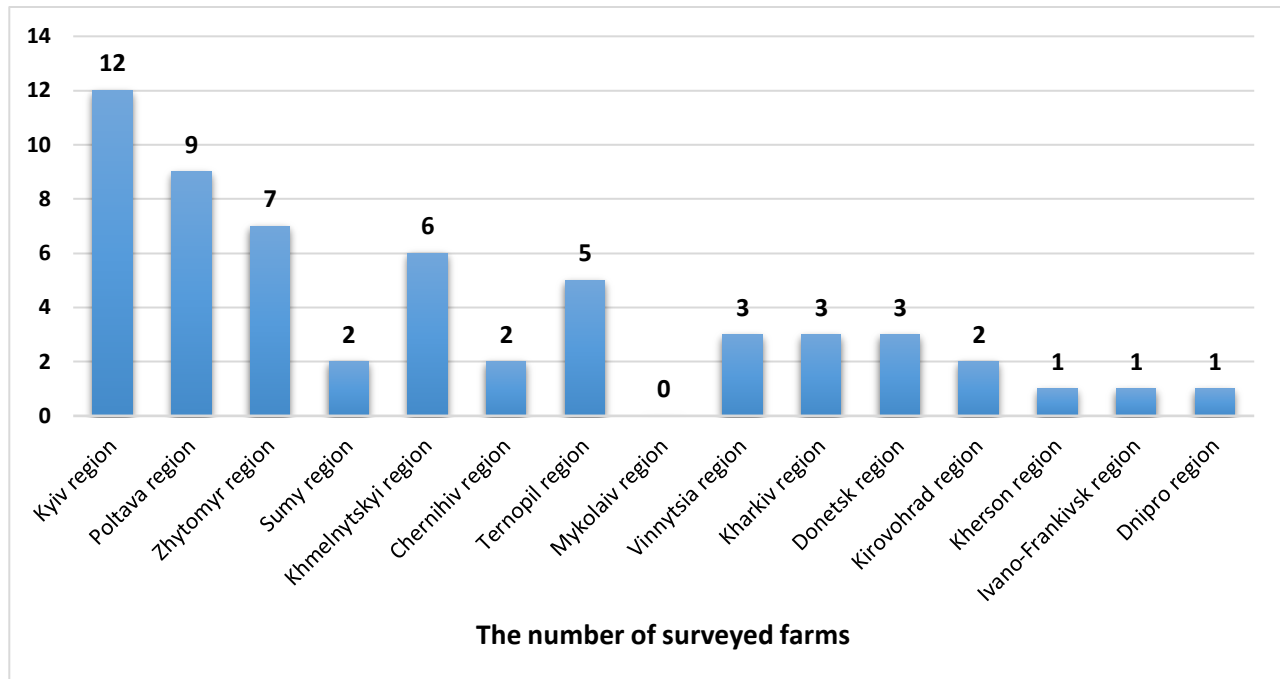


Figure 1 Number of studied farms in regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

A total of 114 tank samples of milk were studied (Figure 2).

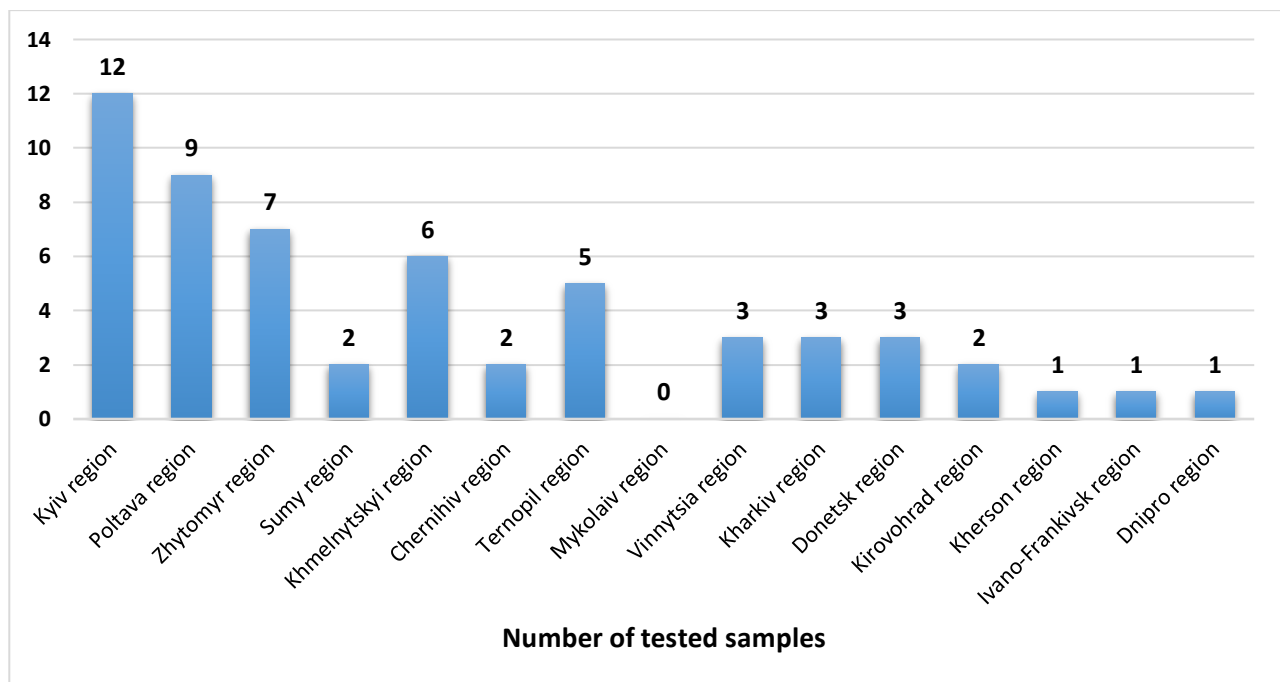


Figure 2 Number of studied milk tank samples in Regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

From 66 farms in Ukraine, 72 samples were positive (Figure 3), which was 63.2%. In turn, 42 samples were negative (Figure 4), which is 36.8%.

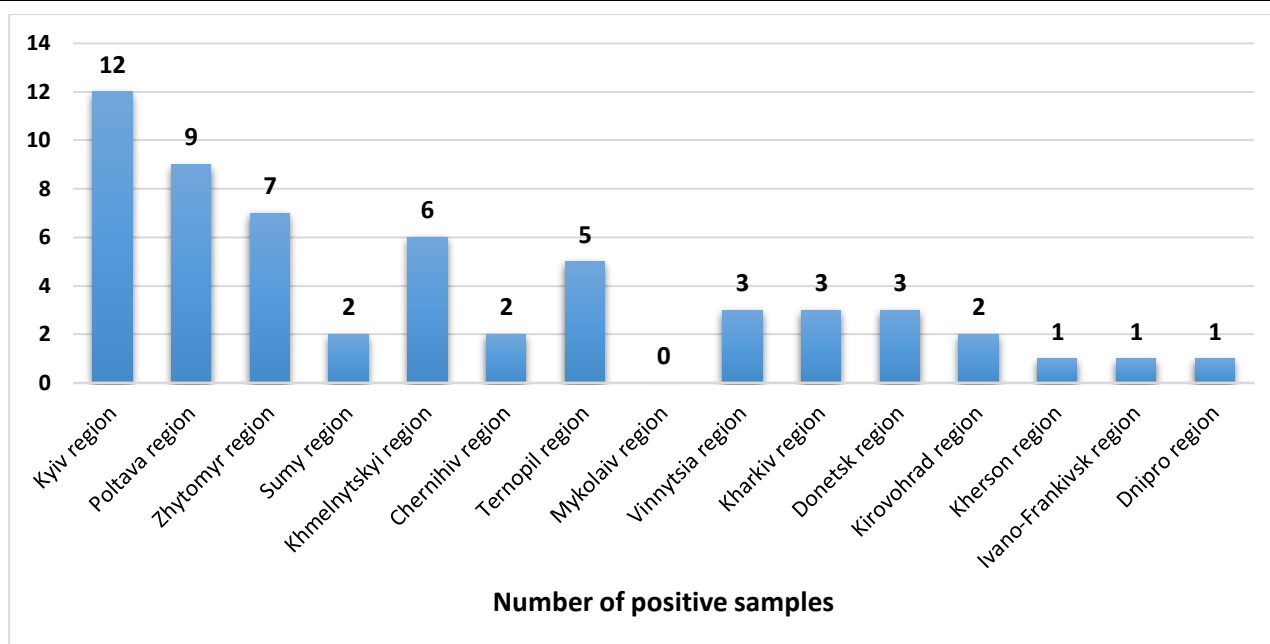


Figure 3 Number of positive milk tank samples in Regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

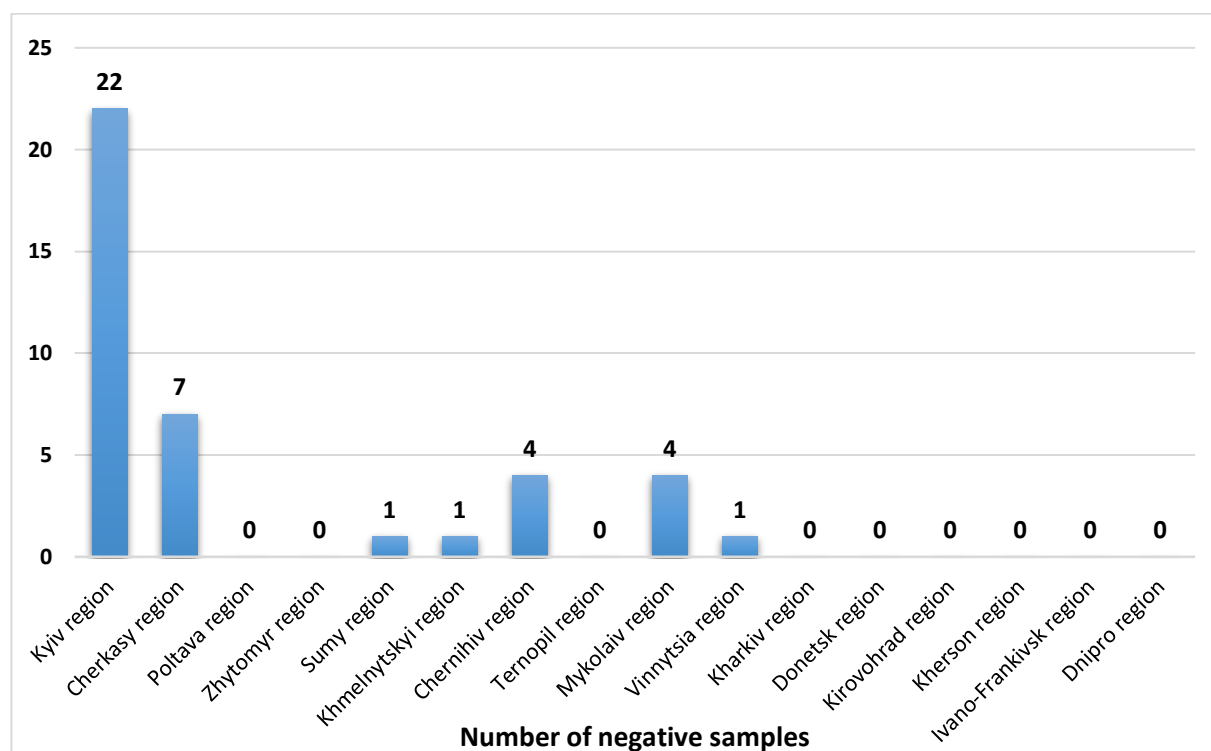


Figure 4 Number of negative tank samples of milk in Regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

A detailed analysis of the circulation data of *Leptospira interrogans serovar Hardjo* in farms and regions of Ukraine is presented in (Table 1).

Our studies showed that 12 farms (34 tank milk samples) from Kyiv region were examined from 2017 to 2020, which is 29.8%, specific antibodies to *Leptospira interrogans serovar Hardjo* were detected in 12 samples (16.6%), 22 samples (52.4%) were negative. From Cherkasy region, 11 farms (22 tank milk samples) were examined, (19.3%), positive reactions were found in 15 samples (20.8%), and negative – In 7 samples (16.7%).

From Poltava – 5 farms (9 tank milk samples) were examined (7.9%), and all 9 samples were positive (12.5%). From the Chernihiv region – 5 farms (6 tank milk samples) were examined (5.3%), 2 samples (2.8%) were positive, and 4 samples (9.5%) were negative. From the Sumy region, 5 farms (6 tank milk samples) were examined (5.3%), 2 samples (2.8%) were positive, and 4 samples (9.5%) were negative. From the Khmelnytskyi region, 5 farms (6 tank milk samples) were examined (5.3%), and all 6 samples (8.3%) turned out to be positive.

From the Vinnytsia region, 4 farms (4 tank milk samples) were examined (3.5%), 3 samples (4.2%) were positive, and 1 sample (2.4%) was negative. From the Zhytomyr region, 3 farms (7 tank milk samples) were examined (6.1%), and all 7 samples (9.7%) were positive. From Kharkiv and Donetsk regions, 3 farms (3 tank milk samples from each region) were examined from each region (2.6%), and all 3 studied samples (4.2%) were positive. From the Ternopil region, 2 farms (5 tank milk samples) were examined (4.4%), and all 5 samples (6.9%) were positive.

Table 1 Circulation of *Leptospira interrogans* serovar *Hardjo* in regions of Ukraine.

It. No.	Oblast (region)	Number of studied farms	Number of studied samples	% of the total number	Positive	%	Negative	%
1	Kyiv	12	34	29.8	12	16.6	22	52.4
2	Cherkasy	11	22	19.3	15	20.8	7	16.7
3	Mykolaiv	4	4	3.5	0	0	4	9.5
4	Vinnytsia	4	4	3.5	3	4.2	1	2.4
5	Sumy	5	6	5.3	2	2.8	4	9.5
6	Donetsk	3	3	2.6	3	4.2	0	0
7	Ternopil	2	5	4.4	5	6.9	0	0
8	Kherson	1	1	0.9	1	1.4	0	0
9	Khmelnyskyi	5	6	5.3	6	8.3	0	0
10	Ivano-Frankivsk	1	1	0.9	1	1.4	0	0
11	Dnipro	1	1	0.9	1	1.4	0	0
12	Kharkiv	3	3	2.6	3	4.2	0	0
13	Poltava	5	9	7.9	9	12.5	0	0
14	Chernihiv	5	6	5.3	2	2.8	4	9.5
15	Zhytomyr	3	7	6.1	7	9.7	0	0
16	Kirovohrad	1	2	1.7	2	2.8	0	0
Total		66	114	100	72	100	42	100

From the Kirovohrad region, 1 farm (2 tank milk samples) was examined (1.7%), and all 2 samples (2.8%) turned out to be positive. 1 farm was examined from Dnipro, Kherson, and Ivano-Frankivsk regions, 1 tank milk sample of which is (0.9%) per farm, and in all the examined samples, which is 1.4% of the total number of positive samples, it was found specific antibodies to *Leptospira interrogans* serovar *Hardjo*. During this period, 4 farms (4 tank milk samples) were examined from the Mykolaiv region (3.5%), and all 4 studied samples turned out to be negative, which is 9.5% of the total number of negative tank milk samples.

According to [31] the prevalence of *L. Hardjo* in 2009 in tank milk samples in Ireland among non-vaccinated herds studied was 34, 59 and 73%, [32] reported during 2018-2020, the prevalence of *L. Hardjo* in tank in milk samples in Ireland, the average of non-vaccinated herds was 86% – Indicating an increase in the percentage of outbreaks over a certain period.

Studies in Brazil show that in 77 samples out of 208, animals had antibodies to leptospira [33], other authors [36] indicate that the prevalence of antibodies to leptospirosis in Brazil is at the level of 52%.

For example, a study of 109 herds in Japan showed that 71 herds were positive for *Leptospira Hardjo*, and the prevalence at the herd level was 65.1% [36], which coincides with our research. Some sources [34] report that in Nepal, the seropositivity of animals at the herd level was 4.8%.

Studies of 45 farms in India show that specific antibodies to *Leptospira Hardjo* were 27.76% [35]. In the Netherlands, research conducted during 2017-2021 indicates that *L. Hardjo* infections were detected in 120 dairy herds [37]. The authors' research [38] in Egypt demonstrated that 39.33% of the 236 studied animals had antibodies against leptospirosis. A total of 48 randomly selected cattle herds were studied in Algeria between 2015 and 2019, the prevalence of serovar *Leptospira interrogans Hardjo* was 31.25% [39]. The study's results by the authors from Ethiopia [43] from 2019 to 2020 show that out of 77 dairy farms selected for the study, 57 were marked as positive for *L. Hardjo*. In Pakistan, the prevalence of antibodies against leptospirosis in cattle is 56.25% [40]. Studies conducted in Tanzania demonstrate serovar *Leptospira Hardjo's* prevalence at 13% [41]. Researchers [42] established the prevalence of leptospirosis in Manabi, Ecuador, at the herd level to be 98.18%.

CONCLUSION

According to the data of the serology laboratory of LLC “Veterinary Diagnostics Center”, specific antibodies to *Leptospira interrogans* serovar *Hardjo* were detected in 63.2% of the studied tank milk samples. The largest number of positive detection results of antibodies to *Leptospira interrogans* serovar *Hardjo* in the tank milk samples was noted in Cherkasy 15 (20.8%), Kyiv 12 (16.6%), Poltava 9 (12.5%), Zhytomyr 7 (9.7 %) and Khmelnytskyi 6 (8.3%) regions. In general, infection with *Leptospira interrogans* serovar *Hardjo* was found in almost all studied regions of Ukraine, except the Mykolaiv region. Infection with *Leptospira interrogans* serovar *Hardjo* was detected in almost all regions of Ukraine, except for the Mykolayiv region. Information about the expansion of leptospirosis of the great horned thinness is not complete, but it is possible to use Vicoristan to assess the prediction of the risks of its expansion and develop an effective program for the control of its disease. The prospects for further investigations include in-depth monitoring, which will lead to the continued availability of many milk streams directly from the dairies of Ukraine, which specialize in processing the milk of animals, as well as expanding the range of surveillance to identify new infections.

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