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ANALYSIS OF THE RESULTS OF SEROLOGY STUDIES FOR PARATUBERCULOSIS AMONG CATTLE IN UKRAINE OVER THE FIVE YEARS

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Abstract. *Paratuberculosis, also known as paratuberculosis enteritis, is a chronic infectious disease caused by the acid-resistant bacterium Mycobacterium avium subsp. paratuberculosis (MAP), belonging to the Mycobacteriaceae family. The causative agent affects a wide range of animals, but the most sensitive to it are domestic ruminants (cattle, cattle, etc.), which are recognized as the main reservoirs of infection for various types of animals, as well as humans. However, the zoonotic potential of this pathogen is speculative and requires the further study. In Ukraine, according to the current regulatory documentation, the main methods of paratuberculosis diagnosing are serological and bacteriological techniques. The authors analyzed the results of serological research on paratuberculosis among cattle in Ukraine during 2019–2023 by systematizing the data of their own research and official reports of the regional state laboratories of veterinary medicine. The research results are presented in the article without taking into account the temporarily occupied territories, as well as the part of the territory where military operations are being conducted. In total, over a period of five years, 41679 samples were serologically tested in the complement fixation test (CFT). During the work, specific antibodies to the pathogen were detected in 36 samples (prevalence about 0.1%; BCI, 0.06–0.12%) from ten regions. The analysis of the received serological studies shows that this disease has a sporadic character and a significant tendency to decrease the number of positive cases. Thus, in 2019 and 2020, 17 positive samples were detected, and in 2023 – only two. Visualization of the obtained data indicates that antibodies were detected mainly in samples among animals from the eastern, northern, central and northwestern regions of the country.*

Keywords: mapping, MAP, paratuberculosis enteritis, serological studies, Johne's disease.

Paratuberculosis, also known as paratuberculosis enteritis and Johne's disease, is a chronic, contagious disease of the gastrointestinal tract caused by *Mycobacterium avium subsp. paratuberculosis* (MAP) that mainly affects various species of ruminants (*Ruminantia*). The signs in affected cattle and sheep are mainly characterized by slow weight loss and progressive diarrhea, which, in turn, leads to a loss of milk and meat productivity (Fecteau, 2018).

The etiological factor of paratuberculosis is the *bacterium Mycobacterium avium subsp. paratuberculosis* (MAP). It is an acid-fast rod in the *M. avium* complex of organisms belonging to the *Mycobacteriaceae* family, order *Actinomycetales*. It is an obligate pathogen of animals. The only place where these pathogens can multiply is in macrophage cells. When MAPs are excreted into the environment with feces, colostrum or milk, they can survive for a long time (up to a year) in soil and water. The main route of transmission is alimentary (especially fecal-

oral). A susceptible animal ingests MAP, from where it penetrates the intestinal wall through the epithelium. *Mycobacteria* then induce disease by stimulating a dysregulated inflammatory response, which is maintained by a non-adaptive immune response. There, MAPs are taken up by intestinal macrophages and exist in the form of spheroplasts (without a cell wall) in a latent or non-pathogenic state for years. Once inside the macrophage, the mycobacterium causes the production of a number of inflammatory mediators, including cytokines (TNF-a, interleukin (IL)-1, etc.). They, together with T-lymphocytes, cause damage of intestinal tissues, which causes progressive diarrhea (Stevenson, 2015).

Based on the ability to infect different susceptible hosts, strains of the pathogen are divided into two types. Thus, type II strains (also known as type C) were initially identified in cattle, but now have a wide range of carriers, including sheep, goats, camels, and non-ruminant wildlife (Fernández et al., 2014). Type I strains (type S) are found mainly in ruminants, but are increasingly being recorded in other species, including deer, camels, alpacas, and cattle in direct contact with ruminants (Li et al., 2016). Type III strains were a separate “intermediate” group, but are now considered a subtype of type I (Dimareli-Malli et al., 2013). In addition, according to molecular genetic studies, there are two different exotic strains of “bison type (B)” - one in North America and the other in Asia. The latter has been isolated from cattle, buffalo, sheep, goats, deer, bison, rabbits, wild boars and is the predominant strain in the Asian region (Aiello, 2016).

Paratuberculosis (PTB) is most commonly reported among domestic ruminants (cattle, buffalo, camels, buffalo, etc.), which act as the main reservoirs of infection. This disease is considered one of the most relevant for cattle worldwide. However, the causative agent *Mycobacterium avium subsp. paratuberculosis* has also been isolated from a significant number of wild animals, including moose, reindeer (*Rangifer tarandus*), bison (*Bison bison*), bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), and various antelope species (Aiello, 2016).

Paratuberculosis (PTB) is most commonly reported in domestic ruminants (cattle, buffalo, camels, buffaloes, etc.), which are the main reservoirs of infection. This disease is considered one of the most relevant for cattle worldwide. However, the causative agent *Mycobacterium avium subsp. paratuberculosis* has also been isolated from a significant number of wild animals, namely: elk, reindeer (*Rangifer tarandus*), bison *(Bison bison*), bighorn sheep (*Ovis canadensis*), snow goats (*Oreamnos americanus*) and various species of antelopes (Aiello, 2016). In addition, according to WHO data, many species of non-ruminant mammals (rabbits, horses, hares, pigs, dogs, etc.) and even some species of birds (jackdaws, gulls, crows, starlings, sparrows) are susceptible to MAP (Aiello, 2016). These “atypical” hosts are infected through eating infected prey or through contact with sick animals and, according to current ideas, are considered a “dead end” of infection (they do not shed MAP in feces or milk to pose a risk of transmission) (Beard et al., 2001).

Humans are also susceptible to MAP, but the zoonotic potential of the disease requires further study. For example, *M. avium ssp. paratuberculosis* is sometimes found in the intestinal tract of some healthy people (Dow & Alvarez, 2022). This microorganism is also believed to play a role in causing Crohn's disease (a chronic gastrointestinal disease of unknown etiology). There is evidence both for and against this hypothesis, but a causal role has not yet been proven (McNees et al., 2015). There are also a number of scientific articles on the possibility of paratuberculosis playing a certain role in the onset or progression of a number of other diseases, such as: sarcoidosis, diabetes mellitus types 1 and 2, multiple sclerosis, HIV, Hashimoto's thyroiditis (Elmagzoub et al., 2022; Ozana et al., 2022; Estevinho et al., 2023). The most consistent evidence of a possible link has been described for Crohn's disease and type 1 diabetes.

Although paratuberculosis was first described over 100 years ago, the disease remains widespread in Europe, North America, South America, Asia, Australia and Africa and causes

significant economic losses (Garcia & Shalloo, 2015). This is facilitated by the fact that a significant part of the livestock after infection becomes asymptomatic chronic carriers of the pathogen, and clinical signs of the disease may appear only after several years. Therefore, in the absence of monitoring laboratory tests, MAP can circulate unnoticed in the herd for years. In addition, it is necessary to take into account the differential diagnosis from bovine tuberculosis (bTB) and the lack of specific treatment measures.

Intravital and postmortem methods are used to diagnose paratuberculosis. Intravital methods include allergic, microscopic, serological and molecular genetic (examination of washings from mucous membranes and biological fluids and secretions). Postmortem methods include histological, bacteriological and molecular genetic (examination of pathological material) methods. The effectiveness of these methods depends on the stage of the disease.

Serological methods can be useful for screening diagnostics and for the diagnosis confirming in animals with clinical signs. Thus, enzyme-linked immunosorbent assay (ELISA), which are more sensitive and allow to detect of subclinically infected cattle and small cattle more effective are commercially available. At the same time, the complement fixation test (CFT) and agar gel immunodiffusion test (AGID) are quite effective for identifying clinical cases of the disease (Jain et al., 2021).

Considering all of the above, the relevance of measures for the diagnosis and prevention of paratuberculosis among ruminants remains at a fairly high level for Ukraine. This disease is included in the list of diseases of state monitoring by serological and bacteriological diagnostic methods. In addition, paratuberculosis is included in the list of diseases subject to mandatory reporting to the World Organization for Animal Health (WHO), which is specified in the "Terrestrial Animal Health Code".

The aim of study was to analyze the results of serological studies on bovine paratuberculosis in Ukraine for the period 2019–2023.

Materials and methods of research. On the basis of the scientific and research department of immunological research of the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary Sanitary Expertise (SSRILDVSE, Kyiv), the results of our own serological studies were systematized, and reports of regional laboratories of the State Service for Food and Consumer Protection (RDL) were analyzed in accordance with the reporting form No. 2-Vet. over the past 5 years.

In accordance with the current regulatory documentation, the studies were conducted in the complement fixation test (CFT).

The results of the studies are presented in the article without taking into account the temporarily occupied territory of the Autonomous Republic of Crimea, the city of Sevastopol and part of the temporarily occupied territories of the Donetsk and Luhansk regions, as well as part of the territory where military operations are / were conducted.

Information on the total number of susceptible cattle was obtained from the website of the State Statistics Service of Ukraine (http://ukrstat.gov.ua/). Binomial confidence intervals (BCI) were calculated to estimate seroprevalence using the “Clopper-Pearsor exact” method with a confidence level of 0.95 using the “R epitools” software package, available at https://epitools.ausvet.com.au/.

Mapping of data on the positive reactions was performed in the freely available Quantum GIS software version 3.16.0 (https://surl.li/qmznox), taking into account geographical coordinates (latitude and longitude) using the “Dot density layer” plugin. Vector layers for the borders of the regions of Ukraine were downloaded from https://www.diva-gis.org/Data.

Results. In total, during 2019–2023, 41,679 blood serum samples from cattle were examined on the basis of the National Institute of Veterinary and Animal Health and Animal Health. At the same time, as shown in Table 1, during the analyzed five years, 36 positive samples were detected, which is about 0.1% of the total number of those examined (BCI, 0.06–0.12%).

The absolute majority of positive reactions (94.4%) were recorded during 2019–2020 – 17 samples each (prevalence of 0.18% and 0.14%, respectively). Two more samples reacting positively to the paratuberculosis pathogen were identified in 2023 (BCI, 0.04–0.13%). Regarding the number of studies conducted by year, we see that their number decreased by 1.5 times starting in 2022 and remained at the same level in 2023.

Table 1

Systematized results of serological diagnostics of bovine paratuberculosis in Ukraine during 2019–2023

Years	Research results in the CFT		Prevalence, %	Binomial confidence intervals (BCI)	
	Number of samples tested	Number of positive samples		Lower 95 % CL	Above 95 % CL
2019	9365	17	0.18	0.11	0.29
2020	11838	17	0.14	0.08	0.23
2021	9339	–	–	–	–
2022	5702	–	–	–	–
2023	5435	2	0.04	0.04	0.13
Total	41679	36	0.09	0.06	0.12

Systematized data by region indicate that during the analyzed period, the largest number of samples was serologically tested from animals from Ternopil region (6,113 blood serum samples). Slightly fewer samples (within 2,129–2,931) were tested from cattle from Kyiv, Odessa, Donetsk, Kharkiv, Dnipropetrovsk and Zhytomyr regions. The smallest number of samples was tested from animals from Lviv and Kherson regions – 695 and 629 blood serum samples, respectively. At the same time, positive reactions were recorded in ten regions in the east, north, north-west, and also in the center of the country (Fig. 1).

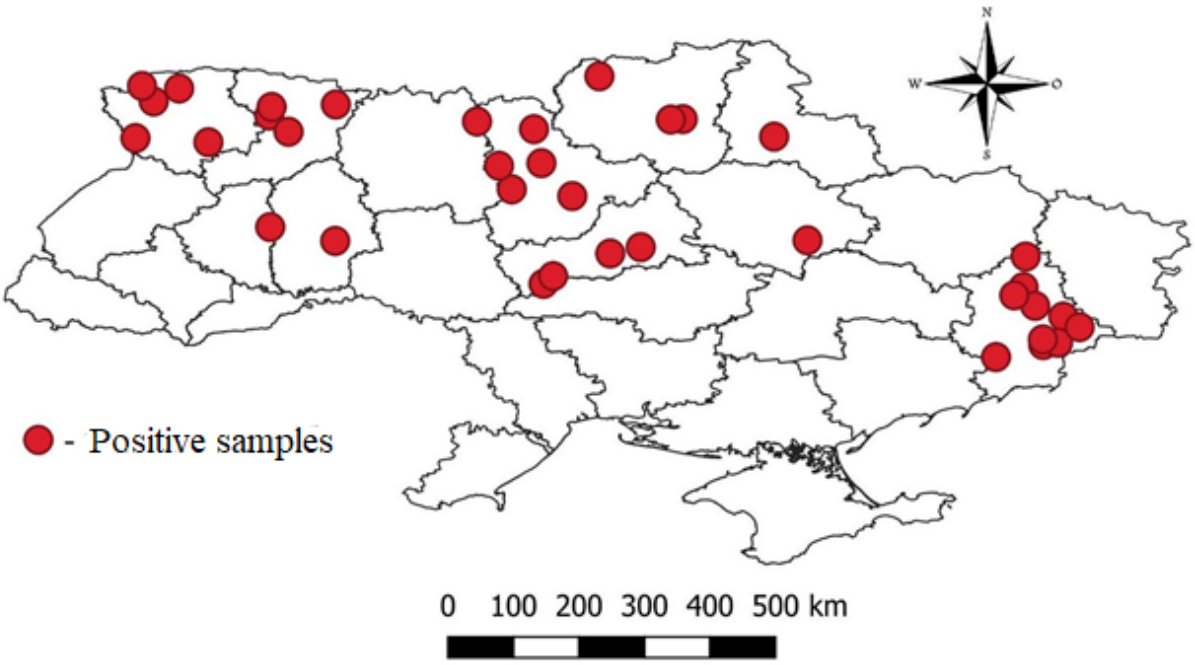

Figure 1. Map-scheme of the density of positive reactions among cattle in Ukraine during 2019–2023

The largest number of positive reactions was recorded in Donetsk region – ten (prevalence in this region 0.44%; BCI, 0.21–0.8%), which is 27.8% of the total number of positive samples detected throughout Ukraine. Five positive animals were found in Volyn and Kyiv regions (prevalence 0.25%; BCI, 0.08–0.59% and 0.17%; BCI, 0.06–0.4%, respectively); four in Rivne and Cherkasy (0.22%; BCI, 0.06–0.57% and 0.26%; BCI, 0.07–0.65%, respectively); three in Chernihiv (0.33%; BCI, 0.06–0.95%); two in Khmelnytskyi (0.22%; BCI, 0.03–0.81%) and one each in Poltava, Zhytomyr and Sumy regions (0.07%, 0.05% and 0.08%, respectively, with BCI less than 0.01%).

Analysis of indicators in dynamics indicates that, starting from 2022, the number of serological studies conducted has significantly decreased in the eastern and southern regions, in particular, by almost four times in Zaporizhia and Sumy regions, by more than two times in Dnipropetrovsk and Kharkiv regions, by one and a half times in Odessa, etc. At the same time, samples from animals from Donetsk and Luhansk regions were not studied at all in 2023.

Discussion. According to WOAH, paratuberculosis is widespread throughout the world. Only Sweden and some states of Australia have currently confirmed the absence of cases of paratuberculosis in their territories (Aiello, 2016). As for Europe, as of 2022, a large-scale analysis of official reporting on diagnostic and preventive work over the past 24 years was conducted and it was found that MAP was most often registered in the countries of southern and western Europe. PTB has been declared a notifiable disease in 65% of countries, but only 19% of them have regular active surveillance for the disease (Fanelli et al., 2022). However, by 2017, 57.4% of European countries reporting paratuberculosis were classified as enzootic for it (Fanelli et al., 2020). The disease usually presents as sporadic clinical cases in most species, including cattle and sheep, often affecting even young animals (Fecteau, 2018).

Regarding the epizootic situation in Eastern European countries, the literature data is very limited and the disease is neglected. Sporadic cases of paratuberculosis among cattle have been described in our country. Thus, in 2019, Zavgorodniy and co-authors examined 1,098 samples from cattle from 19 farms in 9 regions of Ukraine and 17 of them were positive. At the same time, no specific antibodies were detected in 24 samples from cattle (Zavgorodniy et al., 2019). Large-scale studies on the circulation of MAP in Ukraine have not been conducted.

The diagnosis of paratuberculosis is complex and includes the diagnosis of clinical cases of the disease and the detection of subclinical infection. The latter is necessary for disease control both in an individual farm and at the national or international levels (Windsor, 2015). Currently, no diagnostic method has been developed that would have 100% sensitivity and specificity in both subclinical and clinical cases. Registration of subclinical infection mainly depends on the detection of specific antibodies by serological methods, indication of the MAP pathogen from feces, milk or tissues collected during autopsy, detection of DNA by PCR or demonstration of delayed-type hypersensitivity (DTH). The choice of test depends on the purpose and degree of sensitivity and specificity required at the level of the study of an individual animal or the herd as a whole (Arteche-Villasol et al., 2021).

Given the neglect of the epizootic situation regarding paratuberculosis in Eastern European countries, the authors decided to systematize and analyze the results of serological studies on the circulation of specific antibodies to MAP pathogens in different regions of our country. The results obtained indicate that the epizootic situation regarding bovine paratuberculosis in farms of various forms of ownership is fully controlled. Thus, although over 2019–2020 17 positive samples were registered in the CFT, the prevalence of positive reactions remained quite low and amounted to less than 0.2% of the total number of samples tested. In recent years, the frequency of detection of positively reacting animals has significantly decreased (in 2023, only 2 samples from animals from Rivne and Sumy regions were detected). During the analyzed period, the highest prevalence was recorded in animals

from Donetsk region (0.44%; BCI, 0.21–0.8%), somewhat lower – in cattle from Chernihiv (0.33%; BCI, 0.06–0.95%), Cherkasy (0.26%; BCI, 0.07–0.65%), Volyn (0.25%; BCI, 0.08–0.59%), Rivne and Khmelnytskyi (0.22%; BCI, 0.06–0.57% and 0.22%; BCI, 0.03–0.81%, respectively).

Conclusions. Analysis of serological studies conducted on paratuberculosis among cattle in Ukraine demonstrates that this disease is sporadic in nature and there is a significant tendency to reduce the number of positive cases. In addition to the meticulous work of the veterinary medicine service, in our opinion, a significant impact on improving the epizootic situation is also due to the decrease in the number of studies conducted, which after 2022 decreased almost twice due to military operations.

In addition, we definitely do not have information on the circulation of MAP among representatives of wild fauna, which significantly hinders the study of the epizootic process in our country and the implementation of effective measures to combat the pathogen. In addition, it is necessary to introduce the use of molecular genetic diagnostic methods (PCR) into laboratory practice and gradually switch from studies in the complement fixation reaction to the enzyme-linked immunosorbent assay method.

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АНАЛІЗ РЕЗУЛЬТАТІВ СЕРОЛОГІЧНИХ ДОСЛІДЖЕНЬ НА ПАРАТУБЕРКУЛЬОЗ СЕРЕД ВРХ В УКРАЇНІ ЗА П'ЯТЬ РОКІВ

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Резюме. *Паратуберкульоз, також відомий як паратуберкульозний ентерит є хронічним інфекційним захворюванням, що спричиняється кислотостійкою бактерією Mycobacterium avium subsp. paratuberculosis (MAP), що належить до родини Mycobacteriaceae. Збудник вражає широкий спектр тварин, проте найбільш чутливими до нього є свійські жуйні (ВРХ, ДРХ тощо), які визнані головними*

резервуарами інфекції для різних видів тварин, а також і людини. Проте, зоонозний потенціал збудника має спекулятивний характер та потребує подальшого вивчення. В Україні, відповідно до чинної нормативної документації основними методами діагностики паратуберкульозу є серологічні та бактеріологічні техніки. Авторами проведено аналіз результатів серологічних досліджень на паратуберкульоз серед ВРХ в Україні впродовж 2019–2023 рр. шляхом систематизації даних власних досліджень та офіційних звітів регіональних державних лабораторій ветеринарної медицини. Результати досліджень представлені в статті без урахування тимчасово окупованих територій, а також частини території, де ведуться / велися військові дії. Загалом, за період п'яти років було серологічно досліджено 41 679 зразків в реакції зв'язування комплементу (РЗК). У ході роботи було виявлено специфічні антитіла до збудника у 36 зразках (превалентність близько 0.1%; ВСІ, 0.06–0.12%) із десяти областей. Аналіз одержаних серологічних досліджень показує, що це захворювання має спорадичний характер та значну тенденцію до зменшення кількості позитивних випадків. Так, у 2019 та 2020 рр. виявлено по 17 позитивних зразків, а у 2023 – лише два. Візуалізація одержаних даних вказує на те, що антитіла більшою мірою виявлялися у тварин із східних, північних, центральних та північно-західних регіонів країни.

Ключові слова: картографування, МАР, паратуберкульозний ентерит, серологічні дослідження, хвороба Йоне

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