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The epizootic situation of bovine paratuberculosis in Ukraine for the period 2019–2023

A. V. Pyskun, O. D. Polishchuk, O. L. Kravtsova, L. Y. Korniienko,
V. V. Ukhovskiy, H. M. Mietolapova, O. V. Pishchanskyi, G. B. Aliekseieva

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

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State Scientific and Research
Institute of Laboratory
Diagnostics and Veterinary
and Sanitary Expertise,
Donetska st., 30,
Kyiv, 03151, Ukraine.
Tel.: +38-099-415-80-96.
E-mail:
anton.pyskun1989@gmail.com

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Paratuberculosis (Johne's disease) is a contagious infectious disease, the etiological agent of which affects various species of mammals, mainly ruminants. The causative agent is the acid-resistant bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which belongs to the family Mycobacteriaceae. The most sensitive to it are domestic ruminants (cattle, sheep, goats etc.), which are the main reservoirs of infection for the various species of animals and humans (the zoonotic potential of the pathogen requires further study). In Ukraine, the main methods for diagnosing paratuberculosis are serological and bacteriological techniques. The authors analyzed the epizootic situation of paratuberculosis among cattle in Ukraine during 2019–2023 by systematizing the data of their own research and official reports of state veterinary laboratories in regions. The results of the investigation are presented without taking into account the temporarily occupied territories of Luhansk and Donetsk oblasts and the Autonomous Republic of Crimea, as well as part of the territory where military operations were conducted. In total, over the five-year period, 41 679 samples were examined serologically in the complement fixation test and 13 405 – bacteriologically, by microscopy and cultivation of biological materials on the nutrient media. During the investigation, specific antibodies to the pathogen were detected in 36 samples (prevalence, about 0.1%; BCI, 0.06–0.12%) from the ten regions. The analysis of the serological studies shows that the disease is sporadic and has a significant downward trend in the number of positive cases (17 positive samples were detected in 2019 and 2020, and only two in 2023). As for bacteriological examination, no positive cases have been registered during the 5-year period. In addition to the scrupulous work of the veterinary medicine service, both the persistent decrease in the cattle population and the ongoing hostilities in Ukraine have a considerable impact on improving the epizootic situation, as the number of serological tests conducted after 2022 has almost halved (especially in the eastern and southern regions), and bacteriological tests have decreased fivefold. The visualization of the data shows that antibodies were mostly detected in animals from the central, eastern, northern, and northwestern regions of the country.

Keywords: Johne's disease; MAP; epizootic process; serological studies; bacteriological studies; mapping.

Introduction

Paratuberculosis (PTB), commonly known as Johne's disease and paratuberculosis enteritis, is a long-term infectious disease that targets the gastrointestinal tract. It is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which mainly affects various species of ruminants. Affected cattle and small ruminants mainly show slow weight loss and progressive diarrhea, which in turn leads to loss of milk and meat production (Juste & Perez, 2011; Fecteau, 2018).

The etiologic factor of paratuberculosis is the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP). This is an acid-resistant bacillus in the *M. avium* complex of organisms belonging to the family Mycobacteriaceae, order Actinomycetales. The previous names of this microorganism were *Mycobacterium paratuberculosis* and *M. johnei* (Aiello & Moses, 2016). MAP is an obligate pathogen of animals. The only place where these pathogens can multiply is in the 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 the soil and water. The main route of transmission is alimentary (especially fecal-oral), but vertical transmission has also been described. A susceptible animal consumes MAP, allowing it to penetrate the intestinal wall through the epithelial layer. Disease is induced by mycobacteria through the stimulation of an unregulated inflammatory response, which is enhanced by a maladaptive immune reaction. In the intestines, MAP are absorbed by macrophages and remain as spheroplasts (lacking a cell wall) in a dormant or non-patho-

genic state for years. After entering the macrophage, the mycobacterium triggers the production of various inflammatory mediators, such as cytokines (TNF- α , interleukin (IL)-1, etc.). They, together with T-lymphocytes, cause damage to intestinal tissue, which leads to progressive diarrhea (Rosenfeld & Bressler, 2010; Stevenson, 2015).

According to the ability to infect different susceptible hosts, the pathogen strains 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 hosts, including sheep, goats, camels, and non-ruminant wildlife (Fernández et al., 2014). Type I (type S) strains are found primarily in small ruminants, but are increasingly reported in other species, including deer, camels, alpacas, and cattle in direct contact with sheep and goats (Li et al., 2016). Type III strains were a separate "intermediate" group, but are now considered a subtype of type I (Dohmann et al., 2003; Dimareli-Malli et al., 2013). In addition, according to molecular genetic studies, there are two distinct 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 & Moses, 2016).

Most often, paratuberculosis (PTB) is registered among domestic ruminants (cattle, sheep, goats, camels, buffaloes, etc.), which are the main reservoirs of infection. It is considered one of the most important diseases affecting cattle on a global scale. However, the pathogen *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*), mountain goats (*Oreamnos americanus*) and various species of antelope (Aiello & Moses, 2016; de Silva, 2021). In addition, according to WOAHA data, many non-ruminant mammalian species (rabbits, horses, hares, pigs, dogs, etc.) and even some bird species (eiders, gulls, crows, starlings, sparrows) are susceptible to MAP (Aiello & Moses, 2016; Alonso & Martínez, 2021). These “atypical” hosts become infected by eating infected prey or through contact with infected animals and are considered to be a “dead-end” of the infection according to the current thinking (they do not shed MAP in their feces or milk to pose a risk of transmission) (Beard et al., 2001).

Humans are also susceptible to this pathogen, but the zoonotic potential of the disease requires further study. For example, *M. avium* ssp. *paratuberculosis* is sometimes detected in the intestinal tract of some healthy people (Waddell et al., 2015; Dow & Alvarez, 2022). It is also believed to play a role in causing Crohn’s disease (a chronic gastrointestinal disease of unknown etiology). There is evidence both in favor of and against this hypothesis, and 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 role in the onset or progression of a number of other diseases, such as sarcoidosis, types 1 and 2 diabetes, 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 more than 100 years ago, the disease is still widespread in Europe, North America, South America, Asia, Australia and Africa and causes significant economic losses (Garcia & Shalloo, 2015; McAloon et al., 2016; Alonso & Martínez, 2021). This is facilitated by the fact that a significant proportion of the livestock after infection become 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 undetected in the herd for years. In addition, the differential diagnosis from bovine tuberculosis (bTB) and the lack of specific treatment measures must be taken into account.

For the diagnosis of paratuberculosis, both lifetime and postmortem methods are used. The lifetime methods include allergic, microscopic, serological and molecular genetic (examination of washings from the mucosal membranes and biological fluids and secretions). Postmortem methods include histological, bacteriological, and molecular genetic (examination of the pathologic material) methods. The effectiveness of these methods depends on the stage of the disease. Ziehl-Nielsen staining can be used for diagnosis in clinical cases. Accumulations of small, highly acid-resistant microorganisms in the feces indicate that symptoms may be caused by MAP. The microorganisms can also be found in swabs from the intestinal mucosa or lymph node incision surfaces. In samples from some infected animals, mycobacteria may be rare or absent. Histopathology can also help in the diagnosis. Immunostaining techniques can detect the pathogen in the tissue samples, but antibodies can cross-react with antigens of the other mycobacteria (Alonso & Martínez, 2021).

MAP or its nucleic acids can be detected in the feces, intestinal tract, and associated lymph nodes during autopsy. Typically, recommended specimens for culture include samples of ileum, mesenteric and ileocecal lymph nodes, and liver, although the microorganism can also be found in other tissues. Milk and environmental samples can also be tested to detect unhealthy herds (Arteche-Villasol et al., 2021). *M. avium* subsp. *paratuberculosis* can grow on a range of specialized media, but some strains, particularly those belonging to type I (S), are more difficult to isolate. A number of type I and type II strains have been found to grow on Middlebrook 7H10 and 7H11 media with mycobactin J, although some do not grow on other commonly used media. Therefore, it is suggested to cultivate these bacteria on several media. *M. avium* subsp. *paratuberculosis* is slow-growing, and depending on the strain and culture medium, results can be obtained within weeks or months (Carvalho et al., 2012).

Molecular genetic testing methods, especially PCR and Loop mediated isothermal amplification (LAMP), have proven to be quite effective (Vary et al., 1990). Specialized genetic techniques, such as pulsed-field electrophoresis, are useful for epidemiological studies and allow one to distinguish between type I and type II strains (Carvalho et al., 2012; Bein-

hauerova et al., 2021). An intradermal tuberculin test based on purified proteins from *M. avium* subsp. *paratuberculosis* or *M. avium* can detect the delayed-type hypersensitivity reaction, but it is not sensitive enough and nonspecific reactions are common (Alonso & Martínez, 2021). Serological tests can be useful for the screening diagnosis and for confirming the diagnosis in animals with clinical signs. For example, enzyme-linked immunosorbent assays (ELISA) are available that are more sensitive and allow for more efficient detection of subclinically infected cattle and small ruminants. At the same time, the complement fixation test (CFT) and the agar gel immunodiffusion reaction (AGID) are quite effective for the identification of clinical cases (Gilardoni et al., 2012; Jain et al., 2021; Jurado-Martos et al., 2023).

Given all of the above, the relevance of measures to diagnose and prevent paratuberculosis among ruminants remains at a fairly high level for Ukraine. The PTB is on the list of diseases that are subject to state monitoring using serological and bacteriological diagnostic techniques. Furthermore, it is listed among the diseases that require mandatory reporting to the World Organization for Animal Health (WOAH), according to the Terrestrial Animal Health Code.

Materials and methods

We analyzed the epizootic situation of bovine paratuberculosis in Ukraine within the period 2019–2023. For this aim, the results from serological studies of 30 728 cattle samples were conducted and systematized at the Immunological Department of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv). In addition, reports from the regional laboratories of the State Service of Ukraine for Food Safety and Consumer Protection, following reporting form 2-Vet, were reviewed, covering serological (10 951 samples) and bacteriological (13 405 samples) studies conducted over the last 5 years.

In compliance with the current “Instruction on measures for the prevention and elimination of paratuberculosis enteritis (paratuberculosis) in cattle” (approved in 1975), serological studies were conducted in the complement fixation test (CFT), and bacteriological – by microscopic examination of feces and sowing samples on selective nutrient media (Dubos-Smith, Herold with mycobactin, Levenstein-Jensen, Petraniani). The studies were conducted according to the methods recommended by the WOAHA and presented in the Terrestrial Animal Health Code (Alonso & Martínez, 2021).

Research results are presented in the article, excluding the temporarily occupied areas of the Autonomous Republic of Crimea, the city of Sevastopol, and portions of the temporarily occupied Luhansk and Donetsk regions, as well as part of the territory where military operations were conducted. Data on the total number of susceptible cattle was sourced from the State Statistics Service of Ukraine’s website (<http://ukrstat.gov.ua>). Binomial confidence intervals (BCI) were calculated to assess a seroprevalence using the Clopper-Pearson exact method with a confidence level of 0.95 using the R epitools software package (Ausvet, version 2020, Australia) (<http://epitools.ausvet.com.au>).

Mapping of data on serological and bacteriological studies by region was performed using the freely available Quantum GIS software version 3.16.0 (International Quantum GIS Project, Germany, www.qgis.org/ru/site/forusers/download.html). The data on positive reactions were mapped in the same program taking into account geographic coordinates (latitude and longitude) using the “Dot density layer” and “Standard Deviation Ellipse” plugins. Vector layers representing the borders of Ukrainian regions were downloaded from www.diva-gis.org/Data. A quantile with 6 data classes was selected for classification.

Results

In total, 41 679 blood serum samples (serological investigation) and 13 405 cattle fecal samples (bacteriological research) were tested in Ukraine during 2019–2023. Regarding the serological tests, as shown in Table 1, 36 positive samples were detected during the analyzed five years, representing approximately 0.1% of all samples tested (BCI, 0.06–0.12%).

Table 1

Systematized results on the serological diagnosis of bovine paratuberculosis in Ukraine during 2019–2023 according to the reports of SSRILDVSE and laboratories in regions

Years	Research results in the CFT		Prevalence, %	Binomial confidence intervals (BCI)	
	number of samples tested	number of positive samples		lower 95% CL	upper 95% CL
2019*	9 365	17	0.18	0.11	0.29
2020*	11 838	17	0.14	0.08	0.23
2021*	9 339	–	–	–	–
2022*	5 702	–	–	–	–
2023*	5 435	2	0.04	0.04	0.13
Total	41 679	36	0.09	0.06	0.12

Note: * – research results are presented in the article, excluding the temporarily occupied areas of the Autonomous Republic of Crimea, the city of Sevastopol, and portions of the temporarily occupied Luhansk and Donetsk regions, as well as part of the territory where military operations were conducted.

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

The systematized data obtained by region indicate that during the analyzed period, the largest number of samples was serologically examined from animals in Ternopil'ska region (6 113 blood serum samples). Somewhat fewer samples (in the range of 2 129–2 931) were from cattle in Kyiv'ska, Odeska, Donetsk, Kharkiv'ska, Dnipropetrovska and Zhytomyr'ska regions. The smallest number of samples was tested from animals in Lviv'ska and Kherson'ska regions – 695 and 629 blood serum samples, respectively (Fig. 1a). At the same time, positive reactions were recorded in ten regions in the center, east, north and northwest of the country (Fig. 1b, 1c). 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-reactive animals were detected in Volyn'ska and Kyiv'ska regions (prevalence 0.25%; BCI, 0.08–0.59% and 0.17%; BCI, 0.06–0.4%, respectively); four in Rivnenska and Cherkaska regions (0.22%; BCI, 0.06–0.57% and 0.26%; BCI, 0.07–0.65%, respectively); three in Chernihiv'ska (0.33%; BCI, 0.06–0.95%); two in Khmelnytska (0.22%; BCI, 0.03–0.81%) and one each in Poltav'ska, Zhytomyr'ska and Sumska regions (0.07%, 0.05% and 0.08%, respectively, with BCI less than 0.01%).

The analysis of indicators in the dynamics shows that, since 2022, the number of serological tests has significantly decreased in the eastern and southern regions, in particular by almost four times in Zaporizka and Sumska regions, by more than two times in Dnipropetrovska and Kharkiv'ska regions, by one and a half times in Odeska region, etc. At the same time, samples from animals in Donetsk and Luhansk regions were no longer tested at all in 2023 (Table 2).

As for the results of bacteriological studies, their dynamics did not change significantly during the analyzed period by oblasts, but the total number decreased almost fivefold. In total, biological material (fecal samples) was tested in eight regions of Ukraine (Fig. 2).

The largest number of samples were tested from animals in Vinnytska, Volyn'ska and Dnipropetrovska regions (4 214, 3 537 and 2 854 samples, respectively). The fewest of these studies were carried out in the laboratories of Odeska and Rivnenska regions (four and one samples, respectively). No positive results were obtained during microscopy and culture on nutrient media.

Discussion

According to the WOAHI, paratuberculosis is widespread worldwide. Only Sweden and some states in Australia have confirmed the absence of paratuberculosis cases in their territories (Aiello & Moses, 2016; Alonso & Martinez, 2021). As for Europe, as of 2022, a large-scale analysis of official reporting on diagnostic and prevention work over the past 24 years was conducted and found that MAP was most often registered in southern and western Europe. In 65% of countries, PTB is declared a reportable disease, but only 19% of them regularly implement active monitoring of this disease (Fanelli et al., 2022). At the same time, by 2017, 57.4% of

European countries where paratuberculosis was reported were classified as enzootic for it (Fanelli et al., 2020). This disease usually manifests itself in sporadic clinical cases in most species, including cattle, sheep and goats, often affecting even young animals (Carvalho et al., 2012; Fecteau, 2018).

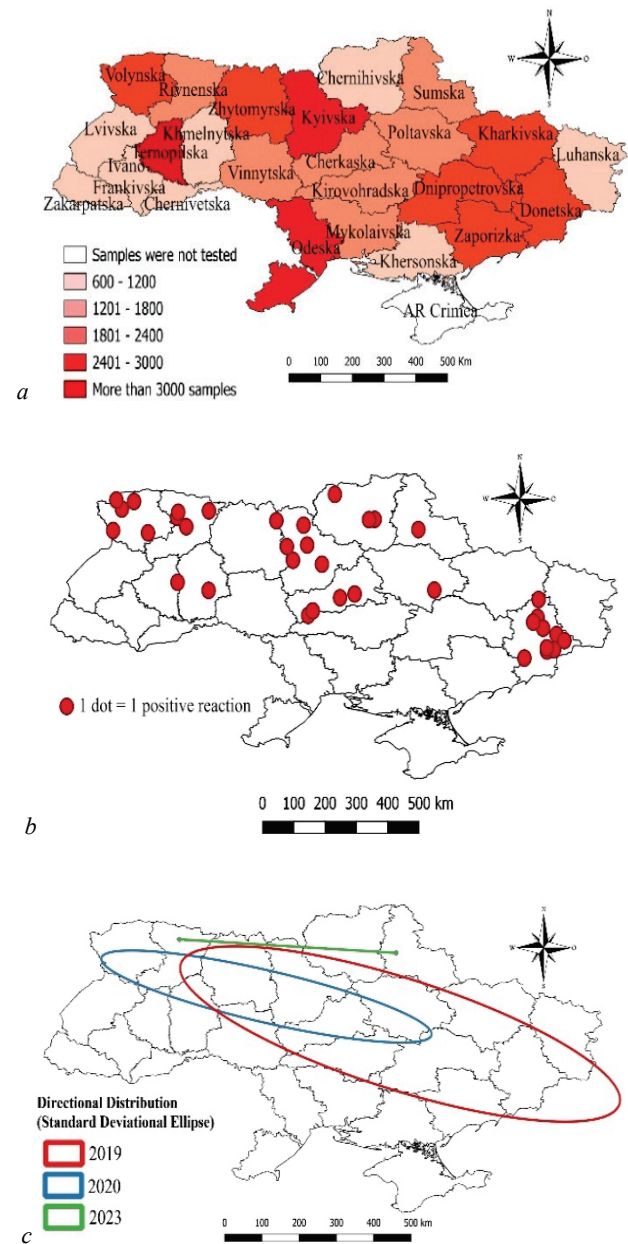


Fig. 1. Map-scheme of the results of serological studies among cattle in Ukraine during 2019–2023: *a* – density of studies conducted; *b* – density of positive reactions; *c* – directional distribution of positive cases by years

Table 2

Dynamics of conducted serological studies on bovine paratuberculosis in Ukraine during 2019–2023 according to the reports of regional laboratories and SSRILDVSE

Oblast	Number of tested samples in CFT by year / number of positive samples				
	2019	2020	2021	2022	2023
Vinnyska	289	394	267	203	198
Volynska	503 / 1	599 / 4	436	228	216
Dnipropetrovska	567	620	520	211	214
Donetska	803 / 9	735 / 1	653	106	–
Zhytomyrska	407	647 / 1	412	288	375
Zakarpatska	244	197	237	210	215
Zaporizka	521	673	502	143	127
Ivano-Frankivska	289	266	277	151	166
Kyivska	648	760 / 5	671	358	494
Kirovohradska	303	423	289	144	152
Luhanska	323	332	302	83	–
Lvivska	222	179	36	125	133
Mykolaivska	340	489	195	219	155
Odeska	556	731	599	311	341
Poltavska	231	330 / 1	462	133	184
Rivnenska	377 / 1	471 / 2	448	259	230 / 1
Sumska	317	388	327	118	89 / 1
Ternopilska	686	1523	1228	1490	1186
Kharkivska	602	587	529	267	247
Khersonska	118	181	97	112	121
Khmelnyska	278 / 2	305	212	36	59
Cherkaska	359 / 1	489 / 3	311	189	214
Chernivetska	178	264	163	175	166
Chernihivska	204 / 3	255	166	143	153
Total	9 365 / 17	11 838 / 17	9 339	5 702	5 435 / 2

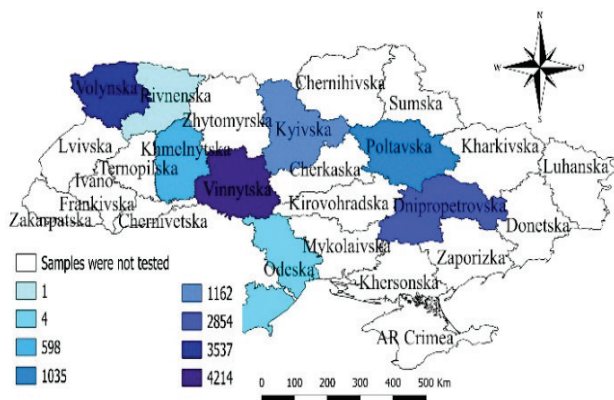


Fig. 2. Map-scheme of the bacteriological studies conducted among cattle in Ukraine in 2019–2023

As for the epizootic situation in Eastern Europe, the literature is very limited and the disease is neglected. In our country, sporadic cases of paratuberculosis among cattle have been described. Thus, Zavgorodniy with co-authors in 2019 examined 1 098 samples from cattle from 19 farms in 9 regions of Ukraine and found 17 positive samples. At the same time, no specific antibodies were detected in 24 samples from small ruminants (Zavgorodniy et al., 2019). No larger-scale studies on the circulation of MAP in Ukraine have been conducted.

The diagnosis on paratuberculosis is complex and includes the diagnosis of clinical cases and the detection of subclinical infection. The latter is necessary for controlling the disease both on an individual farm and at the national or international level (Windsor, 2015). To date, no diagnostic method has been developed that has 100% sensitivity and specificity in both subclinical and clinical cases (Alonso & Martínez, 2021). The registration of subclinical infection mainly depends on the detection of specific antibodies by serological methods, the indication of the MAP pathogen from feces, milk or tissues collected during autopsy, the detection of DNA by PCR or the demonstration of cell-mediated reactions (CMR).

The choice of a test depends on the purpose and the degree of sensitivity and specificity required at the level of the individual animal or herd (Arteche-Villasol et al., 2021; Beinhauerova et al., 2021).

Taking into account the neglect of the epizootic situation on paratuberculosis in Eastern Europe, we decided to systematize and analyze the results of the serological and bacteriological studies on the circulation of MAP pathogens and specific antibodies to them in different regions of our country. The results obtained suggest that bovine paratuberculosis on farms of various ownership types is completely under control. Thus, although in 2019–2020, 17 positive samples were recorded 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 positive animals has decreased significantly (in 2023, only two samples from animals in Rivnenska and Sumska regions were detected). During the analyzed period, the highest prevalence was recorded in animals from Donetska region (0.44%; BCI, 0.21–0.8%), slightly less in cattle from Chernihivska region (0.33%; BCI, 0.06–0.95%), Cherkaska (0.26%; BCI, 0.07–0.65%), Volynska (0.25%; BCI, 0.08–0.59%), Rivnenska and Khmelnytska (0.22%; BCI, 0.06–0.57% and 0.22%; BCI, 0.03–0.81%, respectively).

In general, the obtained data indicate the sporadic nature of outbreaks of paratuberculosis among cattle in Ukraine and are logically consistent with the results of other Ukrainian researchers (Zavgorodniy et al., 2019). The progress in controlling PTB in Ukraine is attributed to the continuous and meticulous implementation of preventive measures by the veterinary service and farm administration (animal owners). Our research has shown that over the past 5 years, no bacteriologically confirmed cases have been registered in any of the regions and no pathogen culture has been detected in animals during sufficiently extensive studies (13 405 samples of biological material were analyzed). Besides the diligent efforts of the veterinary medicine service, we believe that the consistent decrease in cattle numbers in Ukraine has significantly contributed to the improvement of the epizootic situation. Evidence for this comes from an analysis showing the correlation between the reduction in cattle numbers and the improvement in bovine paratuberculosis recovery rates. Therefore, the cattle population on Ukrainian farms of different ownership types decreased by 1 million during the period of the presented research (from 3.3 million in 2019 to 2.3 million in 2023). Similar trends were also observed during the analysis of the epizootic situation for other infectious diseases (in particular, tuberculosis) (Komiienko et al., 2021). In addition, due to the hostilities, the number of serological tests conducted after 2022 decreased by almost half (especially in the eastern and southern regions), and bacteriological tests – by five times.

However, despite the controlled epidemiological situation with regard to paratuberculosis, no studies have been conducted on the possible circulation of MAP among wildlife in Ukraine. This could greatly hinder the complete eradication of the disease in our country, as literature suggests the presence of natural infection foci in other countries (Waddell et al., 2016; Arrazuria et al., 2017). Thus, the European rabbits should be distinguished from natural carriers, which, according to the literature, may play a leading role in the spread of paratuberculosis among domestic ruminants and act as a leading source of the pathogen in certain countries (in particular, in Scotland) (Beard et al., 2001; Dohmann et al., 2003).

Conclusions

The analysis of serological studies on paratuberculosis among cattle in Ukraine shows that this disease is sporadic and shows a marked tendency to decrease the incidence of positive cases. Thus, in 2023, specific antibodies to MAP were detected in only two samples, while in 2019 and 2020, 17 samples were positive. Alongside the meticulous efforts of the veterinary service, the continual decrease in cattle numbers and ongoing hostilities in Ukraine, in our opinion, play a substantial role in enhancing the epizootic situation, as the number of serological tests conducted after 2022 has almost halved.

Regarding the bacteriological studies, during the 5-year period, the pathogen was not registered either by microscopy or cultivated from cultures of biological material on nutrient media. However, we do not have accurate information on the circulation of MAP among wildlife, which

significantly hinders the study of the epizootic process in our country and the implementation of the effective control measures. In addition, it is necessary to introduce the use of molecular genetic diagnostic methods (PCR) into laboratory practice and gradually move from complement fixation test studies to enzyme-linked immunosorbent assay.

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References

- Aiello, S. E., & Moses, M. A. (2016). Paratuberculosis. In: The Merck veterinary manual, 11th edition. Merck and Co, Kenilworth. Pp. 762–764.
- Alonso, B., & Martínez, P. (Ed.). (2021). Chapter 3.1.16: Paratuberculosis (Johne's disease). Terrestrial Animal Health Code, 12th Edition. Agroatimentaria, Buenos Aires. Pp. 1–16.
- Arrazuria, R., Juste, R. A., & Elguezal, N. (2017). Mycobacterial infections in rabbits: From the wild to the laboratory. *Transboundary and Emerging Diseases*, 64(4), 1045–1058.
- Arteche-Villasol, N., Gutiérrez-Expósito, D., Vallejo, R., Espinosa, J., Elguezal, N., Ladero-Auñón, I., Royo, M., Del Carmen Ferreras, M., Benavides, J., & Pérez, V. (2021). Early response of monocyte-derived macrophages from vaccinated and non-vaccinated goats against *in vitro* infection with *Mycobacterium avium* subsp. *paratuberculosis*. *Veterinary Research*, 52(1), 69.
- Beard, P. M., Daniels, M. J., Henderson, D., Pirie, A., Rudge, K., Buxton, D., Rhind, S., Greig, A., Hutchings, M. R., McKendrick, I., Stevenson, K., & Sharp, J. M. (2001). Paratuberculosis infection of nonruminant wildlife in Scotland. *Journal of Clinical Microbiology*, 39(4), 1517–1521.
- Beinhauerova, M., Beinhauerova, M., McCallum, S., Sellal, E., Ricchi, M., O'Brien, R., Blanchard, B., Slana, I., Babak, V., & Kralik, P. (2021). Development of a reference standard for the detection and quantification of *Mycobacterium avium* subsp. *paratuberculosis* by quantitative PCR. *Scientific Reports*, 11(1), 11622.
- Carvalho, I. A., Campos, V. E., Souza, I. M., Zanardo, L. G., Filho, J. D., Gomes, M. J., & Moreira, M. A. (2012). Diagnosis of paratuberculosis in cattle: Microbiological culture, serology and PCR. *Brazilian Journal of Microbiology*, 43(2), 581–585.
- de Silva, K. (2021). Developing smarter vaccines for paratuberculosis: From early biomarkers to vaccine design. *Immunological Reviews*, 301(1), 145–156.
- Dimareli-Malli, Z., Mazaraki, K., Stevenson, K., Tsakos, P., Zdragas, A., Giantzi, V., Petridou, E., Heron, I., & Vafeas, G. (2013). Culture phenotypes and molecular characterization of *Mycobacterium avium* subsp. *paratuberculosis* isolates from small ruminants. *Research in Veterinary Science*, 95(1), 49–53.
- Dohmann, K., Strommenger, B., Stevenson, K., de Juan, L., Stratmann, J., Kapur, V., Bull, T. J., & Gerlach, G. F. (2003). Characterization of genetic differences between *Mycobacterium avium* subsp. *paratuberculosis* type I and type II isolates. *Journal of Clinical Microbiology*, 41(11), 5215–5223.
- Dow, C. T., & Alvarez, B. L. (2022). *Mycobacterium paratuberculosis* zoonosis is a One Health emergency. *EcoHealth*, 19(2), 164–174.
- Elmagzoub, W. A., Idris, S. M., Isameldin, M., Arabi, N., Abdo, A., Ibrahim, M., Khan, M. A., Tanneberger, F., Bakhiet, S. M., Okuni, J. B., Ojok, L., Gameel, A. A., Abd El Wahed, A., Bekaert, M., Mukhtar, M. E., Amanzada, A., Eltom, K. H., & Eltayeb, E. (2022). *Mycobacterium avium* subsp. *paratuberculosis* and microbiome profile of patients in a referral gastrointestinal diseases centre in the Sudan. *PLoS one*, 17(4), e0266533.
- Estevinho, M. M., Cabeda, J., Santiago, M., Machado, E., Silva, R., Duro, M., Pita, I., Morais, R., Macedo, G., Bull, T. J., Magro, F., & Sarmento, A. (2023). Viable *Mycobacterium avium* subsp. *paratuberculosis* colonizes peripheral blood of inflammatory Bowel disease patients. *Microorganisms*, 11(6), 1520.
- Fanelli, A., Buonavoglia, D., Pleite, C. M. C., & Tizzani, P. (2020). Paratuberculosis at European scale: An overview from 2010 to 2017. *Veterinaria Italiana*, 56(1), 13–21.
- Fanelli, A., Galgano, M., Sposato, A., & Buonavoglia, D. (2022). Assessment of Paratuberculosis International Official Reporting in Europe using the information supplied to the WOAHP by National Veterinary Services. *Veterinaria Italiana*, 58(2), 215–224.
- Fecteau, M. E. (2018). Paratuberculosis in cattle. *The Veterinary Clinics of North America. Food Animal Practice*, 34(1), 209–222.
- Fernández, M., Benavides, J., Sevilla, I. A., Fuertes, M., Castaño, P., Delgado, L., García Marín, J. F., Garrido, J. M., Ferreras, M. C., & Pérez, V. (2014). Experimental infection of lambs with C and S-type strains of *Mycobacterium avium* subspecies *paratuberculosis*: Immunological and pathological findings. *Veterinary Research*, 45(1), 5.
- García, A. B., & Shalloo, L. (2015). Invited review: The economic impact and control of paratuberculosis in cattle. *Journal of Dairy Science*, 98(8), 5019–5039.
- Gilardoni, L. R., Paolicchi, F. A., & Mundo, S. L. (2012). Bovine paratuberculosis: A review of the advantages and disadvantages of different diagnostic tests. *Revista Argentina de Microbiología*, 44(3), 201–215.
- Jain, M., Kumar, A., Polavarapu, R., Gupta, S., Aseri, G. K., Sharma, D., & Sohal, J. S. (2021). Development of rELISA using novel markers for the diagnosis of paratuberculosis. *Journal of Immunological Methods*, 497, 113105.
- Jurado-Martos, F., Cardoso-Toset, F., Tarradas, C., Galán-Relaño, Á., Sánchez-Carvajal, J. M., Ruedas-Torres, I., Vera-Salmoral, E., Larenas-Muñoz, F., Carrasco, L., Gómez-Laguna, J., Lorenzo, B. H., & Luque, I. (2023). Evaluation of the diagnostic accuracy of the serological test for paratuberculosis in cattle according to tuberculosis status. *The Veterinary Record*, 193(9), e3313.
- Juste, R. A., & Perez, V. (2011). Control of paratuberculosis in sheep and goats. *The Veterinary Clinics of North America. Food Animal Practice*, 27(1), 127–138.
- Korniienko, L. Y., Pyskun, A. V., Ukhovskiy, V. V., Karpulenko, M. S., Moroz, O. A., Pyskun, O. O., Tsarenko, T. M., & Alikseieva, G. B. (2021). Retrospective analysis of the control and prevention of tuberculosis among cattle in Ukraine in the period 1994–2020. *Regulatory Mechanisms in Biosystems*, 12(2), 301–306.
- Li, L., Katani, R., Schilling, M., & Kapur, V. (2016). Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* on dairy farms. *Annual Review of Animal Biosciences*, 4, 155–176.
- McAloon, C. G., Whyte, P., More, S. J., Green, M. J., O'Grady, L., Garcia, A., & Doherty, M. L. (2016). The effect of paratuberculosis on milk yield – A systematic review and meta-analysis. *Journal of Dairy Science*, 99(2), 1449–1460.
- McNees, A. L., Markesich, D., Zayyani, N. R., & Graham, D. Y. (2015). *Mycobacterium paratuberculosis* as a cause of Crohn's disease. *Expert Review of Gastroenterology and Hepatology*, 9(12), 1523–1534.
- Ozana, V., Hruska, K., & Sechi, L. A. (2022). Neglected facts on *Mycobacterium avium* subspecies *paratuberculosis* and type 1 diabetes. *International Journal of Molecular Sciences*, 23(7), 3657.
- Rosenfeld, G., & Bressler, B. (2010). *Mycobacterium avium paratuberculosis* and the etiology of Crohn's disease: A review of the controversy from the clinician's perspective. *Canadian Journal of Gastroenterology*, 24(10), 619–624.
- Stevenson, K. (2015). Genetic diversity of *Mycobacterium avium* subspecies *paratuberculosis* and the influence of strain type on infection and pathogenesis: A review. *Veterinary Research*, 46(1), 64.
- Vary, P. H., Andersen, P. R., Green, E., Hermon-Taylor, J., & McFadden, J. J. (1990). Use of highly specific DNA probes and the polymerase chain reaction to detect *Mycobacterium paratuberculosis* in Johne's disease. *Journal of Clinical Microbiology*, 28(5), 933–937.
- Waddell, L. A., Rajić, A., Stärk, K. D., & McEwen, S. A. (2015). The zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: A systematic review and meta-analyses of the evidence. *Epidemiology and Infection*, 143(15), 3135–3157.
- Waddell, L., Rajić, A., Stärk, K., & McEwen, S. A. (2016). *Mycobacterium avium* ssp. *paratuberculosis* detection in animals, food, water and other sources or vehicles of human exposure: A scoping review of the existing evidence. *Preventive Veterinary Medicine*, 132, 32–48.
- Windsor, P. A. (2015). Paratuberculosis in sheep and goats. *Veterinary Microbiology*, 181(1–2), 161–169.
- Zavgorodniy, A. I., Pozmogova, S. A., Goncharova, N. V., Kalashnyk, M. V., & Bilushko, V. V. (2019). The study of epizootic sera obtained from ruminant animals in complement fixation test (CFT) with the use of paratuberculosis antigen. *Veterinary Medicine*, 105, 41–45.