The study consisted of 14,468 samples of bovine serum. Anti-MAP antibodies were identified in 349 cattle sera.

**Material and methods.** The materials for this study consisted of 14,468 samples of bovine serum across Poland. Determination of anti-MAP antibody levels was performed using an immunoenzymatic Paratuberculosis Screening Ab Test p/n P07130-5® (IDEXX, Westbrook, Maine 04092, United States).

**Results.** The study consisted of 14,468 samples of bovine serum. Anti-MAP antibodies were identified in 349 cattle sera.

**Conclusions.** Successful paratuberculosis screening programs can lead to a reduction of MAP in animal products. As the role of MAP in Crohn’s disease is not fully understood, future research should be conducted to address this knowledge gap and avoid a potential zoonotic public health problem.

**Keywords:** Mycobacterium avium spp. paratuberculosis, paratuberculosis, Crohn’s disease, cattle, public health

**Streszczenie**

**Wprowadzenie.** Mycobacterium avium spp. paratuberculosis (MAP) zwany także prątkiem Johne’a jest czynnikiem etiologicznym paratuberkulozy, wyniszczającej choroby występującej u zwierząt. Jednym z czynników niezbędnych do rozwoju choroby Crohna u ludzi jest obecność MAP, stąd też obecność w środowisku tych drobnoustrojów, a przede wszystkim ocena ich występowania u zwierząt gospodarskich w odniesieniu do choroby Crohna, stanowiły obecne badania, nieopisane do tej pory w Polsce.

**Materiał i metody.** Materiał do badań stanowiło 14,468 bydlęcych surowic pochodzących z terenu całej Polski. Oznaczenie poziomu anty-MAP wykonano przy użyciu immunoenzymatycznego testu Paratuberculosis Screening Ab Test p/n P07130-5® (IDEXX, Westbrook, Maine 04092, United States).

** Wyniki.** W badanej grupie 14468 bydła zdrowego pochodzenia z terenu całej Polski, w 349 sztukach waszych zgłoszono obecność MAP.

**Wnioski.** Otrzymane wyniki sugerują konieczność badania bydła w Polsce na obecność MAP, aby uniknąć potencjalnie niepokojącego problemu zdrowia publicznego.

**Słowa kluczowe:** Mycobacterium avium spp. paratuberculosis, paratuberkuloza, choroba Leśniowskiego-Crohna, zdrowie publiczne
Introduction

According to current taxonomy, mycobacteria belong to the family *Mycobacteriaceae* and the genus *Mycobacterium*. There are 2 main groups of mycobacteria, tuberculous mycobacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM). The MTBC consists of 11 different species, whereas the NTM group has over 200 different species and is constantly expanding as new species are discovered [1-3]. NTM is the most widely distributed group of mycobacteria, with the most common pathogens being those included in the *Mycobacterium avium* complex (MAC) [4]. Currently, MAC includes more than 200 mycobacterial species, of which at least 30 are pathogenic to humans and animals [5,6]. Of these, the most common pathogens affecting humans are *M. avium*, *M. intracellulare*, and *M. chimaera*. Most laboratories are unable to distinguish between numerous species and subspecies because they lack the required molecular testing methods [4]. Accurate identification of the species is important as recent studies have shown there are differences in the source of environmental exposure involved [7], degree of MAC pathogenicity [8], and in the outcome of antimycobacterial therapy [9,10].

*Mycobacterium avium* spp. *paratuberculosis* (MAP) is a Gram-positive, acid-fast bacterium. They stain blue during Gram staining and red in Ziehl-Neelsen staining, which are characteristics of acid-fast mycobacteria. A common name for the disease caused by this microorganism is Johne's disease, which is common in veterinary practice [11]. MAP is classified as a member of the MAC and is the etiological agent of paratuberculosis (Johne's disease), a severe chronic gastrointestinal disease that affects domesticated and wild cattle worldwide [12-14]. Johne's disease is characterized by debilitating and profuse diarrhea that ultimately leads to the death of the infected animal [15]. The disease is a burden for the dairy industry by endangering the health of cattle which can have a severe economic impact on the infected animals [13]. Not long after the isolation of paratuberculosis in cattle, medical doctors noted some similarities with inflammatory bowel disease in humans. The disease was first described in 1904 by the Polish physician, Antoni Leśniowski, hence the name Leśniowski-Crohn's disease which is often used in Polish literature. A similar intestinal disease was described by a Scottish physician, Thomas Kennedy Dalziel, in 1913. However, it wasn’t until 1932 that a detailed description of the disease was described by an American gastroenterologist, Burrill Bernard Crohn, and his colleagues Leon Ginsburg and Gordon Oppenheimer, hence the current name, Crohn’s disease. An increasing number of researchers have suggested that one of the factors necessary for the development of Crohn’s disease is an infection by MAP [16]. Although MAP DNA has been found in biopsied tissue from patients with Crohn’s disease, the zoonotic potential of Johne's disease is not fully understood but is an important topic of research [16].

The aim of the paper is the evaluation of the prevalence of anti-MAP antibodies in bovine serum in Poland from 2011 to 2020.

Material and methods

The materials for the study consisted of 14,468 samples of bovine serum from various regions of Poland. The study was performed as part of the Multiannual Program implemented by the National Veterinary Institute – National Research Institute in Puławy. The cattle surveyed in this study were primarily destined for export beyond Poland’s eastern border. About 10% of the cattle tested had clinical signs of diarrhea.

Determination of anti-MAP antibody levels was performed using an immunoenzymatic Paratuberculosis Screening Ab Test p/n P07130-5® (IDEXX, Westbrook, Maine 04092, United States). This is a qualitative serological testing method in accordance with the Instruction No. 18/99 of the Chief Veterinary Surgeon dated November 2, 1999, and the requirements of the Polish Accreditation Center. This test is highly sensitive and results can be obtained the same day.

Results

The study consisted of 14,468 samples, anti-MAP antibodies were confirmed in 349 (2.41%). Details of the confirmed cases by year and place of origin are shown in Table 1.
Table 1. Number of cattle tested for paratuberculosis by year, number of seropositive animals, and place of origin

| Year | Number of cattle tested | Number of animals in which anti-MAP was detected | Place of origin of seropositive animals (provinces) |
|------|-------------------------|-----------------------------------------------|--------------------------------------------------|
| 2011 | 1,394                   | 42                                            | Opole, Warmian-Masurian, Pomeranian, Podlaskie, Subcarpathian, Lublin, West Pomeranian |
| 2012 | 2,583                   | 42                                            | Kuyavian-Pomeranian, Silesian, Greater Poland, Podlaskie, Warmian-Masurian, Świętokrzyskie, Subcarpathian, Lublin |
| 2013 | 2,189                   | 91                                            | Lesser Poland, Kuyavian-Pomeranian, Lower Silesian, Masovian, Greater Poland, Podlaskie, Łódź, Lublin, Pomeranian, West Pomeranian, Warmian-Masurian, Opole |
| 2014 | 1,110                   | 17                                            | Lubuskie, Opole, Lublin, Greater Poland, Masovian |
| 2015 | 1,039                   | 23                                            | Lublin, Warmian-Masurian, Pomeranian, Greater Poland, Łódź, Masovian |
| 2016 | 887                     | 6                                             | Greater Poland, Kuyavian-Pomeranian, Lublin, Opole |
| 2017 | 960                     | 30                                            | Greater Poland, Opole, Masovian, Kuyavian-Pomeranian |
| 2018 | 1,232                   | 36                                            | Kuyavian-Pomeranian, Greater Poland, Świętokrzyskie, Lublin, Łódź |
| 2019 | 1,636                   | 42                                            | Greater Poland, Pomeranian, Opole, Podlaskie, Masovian, Kuyavian-Pomeranian |
| 2020 | 1,438                   | 20                                            | Kuyavian-Pomeranian, Greater Poland, Opole, Podlaskie |

Discussion

Crohn's disease is a chronic inflammatory bowel disease. In some patients, partial resection of the intestine is the only effective treatment option. The age of onset Crohn's disease typically occurs is between the ages of 20-40. The number of patients diagnosed with Crohn's disease continues to increase worldwide [17]. The incidence and prevalence of Crohn's disease are higher in developed countries compared to developing countries. It is also more frequent in patients from urban areas than in rural areas. The prevalence of Crohn's disease is highest in Europe (322 per 100,000 population), Canada (319 per 100,000), and the USA (214 per 100,000) [18]. In Asia, where some countries are undergoing rapid urbanization, an increase in the annual incidence of Crohn's disease has been reported (0.54 per 100,000 population) [19]. As more and more scientific reports confirm the involvement of MAP in Crohn's disease, it may be reasonable to begin screening programs to evaluate herds of cattle for paratuberculosis [16,20,21].

Paratuberculosis is included on the list of diseases requiring mandatory registration according to Annex No. 3 of the Law of March 11, 2004. "On protection of animal health and control of infectious animal diseases", the costs of diagnostic tests and elimination of the sick animal are borne by the owners [22,23]. In Poland, no programs exist to eradicate paratuberculosis in cattle, therefore the number of sick animals is often underestimated. The results published in this manuscript indicate that in Poland, the percentage of seropositive animals using the Elisa test was 2.41% relative to the number of tested samples. Considering that the cattle population in Poland is 6.27 million, the authors of this text estimate the percentage of seropositive animals to be much higher.

The main symptoms of paratuberculosis are persistent diarrhea and gradual weight loss leading to cachexia in the final stage of the disease [20,22,23]. The infected animals subsequently have difficulty absorbing the necessary nutrients. The damaged intestinal mucosa results in the loss of proteins leading to chronic hypoproteinemia, trace element deficiencies, and micronutrient deficiencies essential for normal function [11]. Cachexia is preceded by prolonged, untreatable diarrhea, sometimes lasting many months. The more intense it is, the quicker the animal's death. In the course of paratuberculosis, the intensity of diarrhea may vary and even periodically disappear. In sheep and goats, because of their body's high ability to reabsorb water in the intestines, diarrhea usually does not occur but the stools are mushy and deformed. Appetite and feed intake are usually not impaired [23].

With anti-MAP antibodies having been confirmed in the serum of cattle in Poland, a high risk of spreading the disease within the environment exists. According to the data presented, Johne's disease was confirmed in almost every province. The acid-fast mycobacteria that cause paratuberculosis are resistant to many therapeutic agents. Given the relatively unambiguous symptoms (diarrhea), the following entities should be considered in
the differential diagnosis: bovine viral diarrhea (BVD), bovine viral diarrhea and mucosal disease (BVD/MD), acidosis due to poor nutrition, hepatic steatosis, fascioliasis, and chronic salmonellosis [22]. By the time the disease entity is properly diagnosed, the infection has likely already spread between animals and the bacteria has already seeded the environment. Interesting work by Waddell et al. was published in 2015 and 2016, summarizing the scientific evidence from 255 research papers on the occurrence of MAPs in food, water, and other media highlighting the high probability of human exposure [20,21].

Conclusions

Successful Johne’s disease prevention and control programs can lead to a reduction in MAP in animal products [15,24]. As the role of MAP in Crohn’s disease is not fully understood, future research should be conducted to address this knowledge gap and avoid a potential zoonotic public health problem.

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