Open Veterinary Journal, (2024), Vol. 14(11): 2731-2744
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Review Article
DOI: *10.5455/OVJ.2024.v14.i11.2*

Submitted: 01/07/2024 Accepted: 16/10/2024 Published: 30/11/2024

A comprehensive review of paratuberculosis in animals and its implications for public health

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ABSTRACT
Paratuberculosis is an infectious disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Typically, ruminant animals including cattle, buffalo, goats, and sheep are infected with MAP. Animals get infected with MAP in a number of ways, such as by eating or drinking contaminated food or water, or by nursing from an infected mother who may have contaminated teats or directly shed the organism in milk or colostrum. Animal-derived goods like meat, dairy, and tainted surface water have the potential to spread paratuberculosis through zoonotic transmission. Reports of paratuberculosis have been received from United States, Oceania, Asia, and Africa, in addition to several European nations like Germany, Italy, and France. Paratuberculosis pathology is characterized by chronic lymphangitis, chronic enteritis, or mesenteric lymphadenopathy. In animals, wasting and watery green diarrhea are the major signs. There are two kinds of paratuberculosis diagnostic tests that are available; the goal of the first set of tests is to identify MAP while the second set consists of immunological tests. Due to similar clinical signs, some forms of the illness, such as wasting and watery green diarrhea, may be mistaken for paratuberculosis. Crohn's disease has been linked to *M. avium* subsp. *paratuberculosis* as the etiological culprit in humans. To prevent the infection from spreading to uninfected animal populations, drastic measures must be implemented. Despite the economic burden of paratuberculosis, research aimed at developing therapeutic medicines is focused on public health rather than veterinary uses. This review therefore focuses on a comprehensive detail of paratuberculosis in animals, including its public health implications and economic impact.
Keywords: Diarrhea, Intestines, MAP, Paratuberculosis, Public health.

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Introduction

The bacterium, *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is the causative agent of paratuberculosis, which is also referred to as Johne's disease in animals and is linked to Crohn's disease in humans (Garvey, 2020). The disease ("Johne's disease") named after its discoverer (Johne and Frothingham), was first identified in German cattle in 1895 and easily spread to ruminants and wild animals (Roller *et al*., 2020; Mallikarjunappa *et al*., 2021). Following a months-to-years-long preclinical phase, infected animals may exhibit signs such as diarrhea and weight loss (Idris *et al*., 2021). Because of the harm it does to animal welfare and livestock productivity, such signs have a substantial financial impact (Garcia and Shalloo, 2015).

Granulomatous inflammation of the intestinal mucosa and mesenteric lymph nodes are the disease's primary signs (Dennis *et al*., 2008). This leads to a condition known as protein-losing enteropathy, in which the intestinal mucosa becomes inflamed, making it difficult for the absorptive epithelium to absorb enough nutrients and allowing fluids and nutrients to pass through the stools (Craven and Washabau, 2019). Malabsorptive and secretory diarrhea is one of the results of this illness, and it presents clinically as loose stools in sheep and goats or projectile diarrhea in cattle (Okuni *et al*., 2020). The end result is submandibular edema at the terminal stage, decreased body weight, and decreased production of meat, milk, or wool (Idris *et al*., 2021).

Animal feces infected with paratuberculosis are the primary means of transmission (Corbett *et al*., 2019). In an uncontaminated environment, animals can contract paratuberculosis by consuming food or beverages tainted with environmental germs (Smith *et al*., 2011). Newborn animals may be exposed to high concentrations of these microorganisms through teats, polluted pasture, water, and feed, as well as through the presence of mycobacteria in colostrum and milk (Steuer *et al*., 2021). Despite the known pattern of transmission, it is challenging to stop the spread of MAP in a herd without significant management adjustments because it can live in the environment for more than a year (Tkachuk *et al*., 2013).

MAP is spread throughout the world, but infection is more common in temperate and wet climates (Steuer *et al*., 2020). A number of nations have initiated control programs aimed at suppressing or eliminating paratuberculosis in order to halt the transmission of MAP infections and establish disease-free zones (Weber *et al*., 2024). This endeavor is challenging due to the rise in animal trafficking at national and international levels (Jiménez-Martín *et al*., 2022). Furthermore, the bacterial infection causes non-specific symptoms that are only noticeable after a protracted incubation period of exposure. A protracted or variable subclinical stage of the disease in infected animals, and intermittent or continuous bacterial shedding in feces make the diagnosis of MAP infection extremely challenging (Park *et al*., 2020).

Because paratuberculosis has been reported in some places and is still spreading quickly in the livestock business, resulting in significant financial losses, the illness has caused alarm among the general people (Elmagzoub *et al*., 2020). This review paper focuses on the etiology, history, epidemiology, pathogenesis, pathology, clinical signs, diagnosis, transmission, zoonotic potential, public health importance, economic impact, treatment, vaccination, and control of paratuberculosis. This review will add to our understanding of paratuberculosis and associated hazards which could lead to better diagnosis, treatment, and control.

Etiology

MAP is a member of the *Mycobacterium avium* complex within the genus *Mycobacterium*. This is the sole genus within the *Mycobacterium* family (Garvey, 2020). Subsequent genetic research demonstrated that *M. avium* and *M. paratuberculosis* are closely related (Paustian *et al*., 2005). With a DNA homology of over 99%, these bacteria are presently regarded as belonging to a single species and are known as *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *avium* (Hodgeman *et al*., 2023). Another similarly related bacterium is known as wood pigeon mycobacteria (*M. avium* subsp. *silvaticum*), which is likewise regarded as a subspecies of *M. avium* (Tran and Han, 2014).

Mycobacterium avium is a non-photochromogenic, slow-growing bacterium. It grows on egg medium like Löwenstein-Jensen and synthetic media as acid-resistant aerobic rods (Mayahi *et al*., 2013). MAP usually cannot manufacture iron-binding siderophore; hence, it needs the growth factor mycobactin (Rathnaiah *et al*., 2017). For MAP to form visible colonies, at least 8–16 weeks are needed, and certain strains need to be cultured for 6 months (Vitense *et al*., 2021). Since MAP is largely biochemically inert, the present method of identification relies on mycobactin dependency for growth and polymerase chain reaction (PCR) detection of the IS900 insertion sequence (Sartor, 2005).

Although MAP is normally quite uniform, there are occasional phenotypic variations. Specifically, some ovine strains are referred to as "ovine types" because of their pigmentation and other cultural characteristics. Despite the fact that some strains seem to prefer their hosts, they may still produce clinical illness in cattle (Imperiale *et al*., 2017).

History

Johne and Frothingham not only described the clinical features of the illness but also noted the presence of acid-fast organisms in granulomatous lesions and conjectured that the illness might be an unusual type of bovine tuberculosis (Borham *et al*., 2022). The causal agent, known as *Mycobacterium* enteritidis chronikae pseudotuberculosis bovis Johne, was initially identified by Twort and Ingram in 1912 (*Twort* and Ingram, 1912;

Mohan *et al*., 2013). In 1913, the Scottish surgeon Dalziel initially referred to the condition as chronic regional ileitis when he coined the term Crohn's disease. At the time, its term was derived from a Crohn paper published in 1932 (Aitken *et al*., 2024). Later on, this illness was referred to as paratuberculosis or Johne's disease, and MAP was identified as the causative agent. Since its first description in North America in 1908, paratuberculosis has been reported in almost every country (Kuenstner and Kuenstner, 2021); although most case reports prior to 1908 originated in Northern and Western Europe (Sweeney *et al*., 2012).

Epidemiology

Typically, ruminants including cattle, buffalo, goats, and sheep are infected with MAP (Schrott *et al*., 2023); however, donkeys, antelopes, llamas, pigs, alpacas, horses, deer, and camels are among the other animals that might become infected (Roller *et al*., 2020). Despite the fact that paratuberculosis is a global disease, it is unknown how common it is in most nations. This is a result of the issue's low priority, which stems from ongoing worries about other diseases in many nations, as well as the challenge of precisely identifying diseased animals because of their lengthy incubation period (McAloon *et al*., 2017). However, in many nations, data are accessible based on bacteriological surveys conducted in slaughterhouses and, more recently, on the identification of antibodies in serum or milk samples kept in bulk tanks (Field *et al*., 2022). The majority of studies employ disparate methodologies, which makes it challenging to compare study findings. The majority of findings show that paratuberculosis is very prevalent in domestic livestock (Gurung *et al*., 2018).

In addition to several European nations like Germany, Italy, and France, reports of paratuberculosis, especially in sheep, goats, dairy and beef cattle, alpacas, llamas, and deer have been received from Oceania, Asia, Middle East, and Africa, (Okuni, 2013; Gautam *et al*., 2018; Acharya *et al*., 2020; Ekundayo and Okoh, 2020; Idris *et al*., 2021). Caprine paratuberculosis has been documented in the United States (Missouri), Brazil, and Canada (Dimareli-Malli *et al*., 2013). Because there are no standardized diagnostic tests or funds for research on ruminants, no studies have been done to provide a reliable estimate of the prevalence of paratuberculosis in sheep and goats in the United States (Pithua and Kollias, 2012).

Pathogenesis

The small intestinal mucosa is the entry point for ingested bacteria into the intestinal wall. It has been demonstrated that specific M cells found in the epithelium lining the dome area of Peyer's patches serve as the point of entrance in experimentally infected calves and goats (Secott *et al*., 2004). Following that, subepithelial macrophages phagocytose the bacteria. These bacteria will progressively multiply in macrophages and cause inflammatory and immunological reactions because they are resistant to intracellular destruction (Casey *et al*., 2015). Animals usually contract the infection early in life before the development of clinical illness because resistance increases with age (Romdhane *et al*., 2017). This might be because the immune system is still developing in young animals and the ileal Peyer's patches, which atrophy with age, and provide easier access to the intestinal mucosa (Koets *et al*., 2015). Animals with clinical illnesses typically have been incubated for longer than two years (Douarre *et al*., 2010).

The majority of MAP-infected animals are able to manage their illness, and only 10%–15% of infected animals experience overt paratuberculosis or Johne's disease (Rossi *et al*., 2017). However, animals that are subclinically sick produce less, which has serious financial ramifications for livestock farmers (Bakker *et al*., 2000). The MAP-infected animals may not recover from the illness and may carry the MAP pathogens for the remainder of their lives; some may periodically excrete bacteria that aid in the further spread of infection (Aitken *et al*., 2024). Although no lesions were seen following experimental infection in some investigations, it is still possible that these animals had latent infections (Vazquez *et al*., 2014). It is unknown what precise immunopathological pathways lead to the development of clinical paratuberculosis. The course of MAP infection is influenced by the animal's age, general health, genetics, and infectious dosage (Lombard, 2011).

sPathology

Paratuberculosis pathology is characterized by chronic lymphangitis, chronic enteritis, or mesenteric lymphadenopathy (Hailat *et al*., 2012). The mesenteric lymphatic ducts and the gut are the primary locations for macroscopic lesions (Marquetoux *et al*., 2018). The mesenteric and serosal lymphatic vessels enlarge and become thicker (Pierce, 2009) while the lymph nodes in the mesenterium are edematous, enlarged, and pallid (Navarro *et al*., 1998). Intestinal lesions can be diffuse or segmental, and are most often found in the ileum, and sometimes, throughout the intestinal tract (Olsen *et al*., 2002). The intestinal tract and mesenteric lymph nodes exhibit multicentric to diffuse granulomatous inflammation in histopathological lesions which are categorized as mild, moderate, and severe (Lei *et al*., 2008). Langhans large cells (inclusing the sporadic Langhans large cells) which contain a high concentration of acid-fast bacilli, particularly in macrophages in the paracortical zone of the mesenteric lymph nodes and villi are present in mild lesions (Sikandar *et al*., 2012). In moderate lesions, groups of macrophages or several Langhans giant cells are found in the mesenteric lymph nodes, intestinal mucosa, and submucosa (Marquetoux *et al*., 2018); while in severe lesions, large numbers of macrophages and Langhans giant cells that penetrate the intestinal wall's layers as well as the lymphatic channel lumen are found (Bannantine and Stabel, 2002). The intestinal crypts grow and the villi become

blunt. The paracortical zones and subcapsular sinuses of mesenteric lymph nodes have been noted to be filled with inflammatory cells (Mohammed *et al*., 2023) while intestinal mucosa, mesenteric lymph nodes, intestinal Peyer's patches, and lymphoid nodules contain inflammatory cells (Navarro *et al*., 1998). There are transverse folds in the mucosa and an edematous thickened intestinal wall (Mohammed *et al*., 2023). Lesions are occasionally visible in other organs, particularly the liver and hepatic lymph nodes (Scherrer *et al*., 2019). Additionally, the degree of pathological damage is not necessarily correlated with the intensity of the clinical symptoms. When the illness is severe, the animal may also have submandibular edema, serous effusion inside the body cavity, alopecia, and cachectic anemia (Okuni *et al*., 2020).

Histopathological results in experimental cattle infected with MAP between 1953 and 1975 have been documeted (Fecteau, 2018); the observed lesions in cattle infected with MAP are comparable to those in reported mild lesions of paratuberculosis and can be identified two to four months after infection (Scherrer *et al*., 2019).

Clinical signs

Paratuberculosis is a long-standing feverless disease (Tkachuk *et al*., 2013). In animals, wasting and watery green diarrhea are the major symptoms (Cheng *et al*., 2020). Although young animals are prone to the disease, animals older than 2 years old typically exhibit clinical symptoms (Garcia and Shalloo, 2015). Older cattle are less likely to become infected after exposure. The disease progresses in phases, with stage I (silent infection) occurring in young calves that do not exhibit any clinical symptoms and do not shed (Roller *et al*., 2020). Only interferon gamma (IFN-γ) or allergy skin tests are capable of diagnosing an allergy. Stage II (subclinical infection) is the next stage, marked by the absence of clinical symptoms and the pathogen's sporadic discharge, usually in trace amounts (Lee *et al*., 2023). To find such cases, repeat culture or PCR is typically necessary.

Stage III (clinical cases) is when clinical symptoms first appear, and the immune system and exposure level determine how severe the signs are. Signs, especially in infected animals, include decreased milk production linked to frequent, watery green diarrhea (intermittent or chronic), and progressive but persistent weight loss (Tiwari *et al*., 2006). However, the animal's appetite and vital signs remain normal. PCR, enzyme-linked immunosorbent assay (ELISA), or stool culture can be used to diagnose this illness.

Next, in stage IV (advanced clinical stage), bottle jaw (submandibular edema) and cachexia develop as a result of blood protein being depleted as a result of malabsorption and diarrhea (Peek *et al*., 2018). Infected animals were reported to persist in losing weight until death with observable pallid mucous membranes and thin carcasses (Donat *et al*., 2024). Significant alterations in the terminal ileum of deceased animals, which may encompass the entire gastrointestinal tract were discovered after autopsy in a study (Singh *et al*., 2010). The mesentery, afferent lymphatic vessels, and intestinal tract walls were noted to be thicker (Elze *et al*., 2013) while calcified or caseous white nodules in the enlarged mesenteric lymph nodes were also reported (Tiwari *et al*., 2006).

Diagnosis

There are two kinds of diagnostic tests available for paratuberculosis. The goal of the first set of tests is to identify *M. avium* subsp. *paratuberculosis*. These tests have a high specificity, and as the disease progresses, their sensitivity rises in proportion to the bacterial pathogen load that are present in the infected animal. These diagnostics include fecal smear microscopic inspection, fecal sample culture, and PCR detection of insertion sequence IS900 (Arsenault *et al*., 2019). All MAPs discovered to date have 14–18 copies of IS900, which is the sole genetic factor known to distinguish them from other *M. avium* subspecies (Grant, 2021). Both *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium avium* subsp. *avium* have another insertion sequence, IS1311, and polymorphisms in both genes enable distinction between and within subspecies (Turenne *et al*., 2008). Various techniques have been attempted for molecular subtyping in order to distinguish between MAPs. Restriction Fragment Length Polymorphism (RFLP) is one of the most widely utilized techniques nowadays, and MAP strains can result in distinct IS900 RFLP patterns (van Soolingen *et al*., 2001). Investigations are being conducted on additional techniques, including IS1311 polymorphism typing, multiplex PCR type, the study of randomly amplified polymorphic DNA, and pulsed field gel electrophoresis (Djønne *et al*., 2005).

The second group consists of immunological tests. These include the gamma-interferon test, the complement fixation test, the agargel immuno-diffusion test, the delayed-type hypersensitivity test or intradermal test, and the ELISA which uses a range of distinct antigens (Wlodarczyk *et al*., 2014).

Naturally, in more advanced stages of the disease, conventional MAP bacteriological culture of a stool sample is the most sensitive test, 100% specific, and hence regarded as the "gold standard" (Dimareli-Malli and Sarris, 2001). It is challenging to evaluate its "true" sensitivity, or the capacity to identify sick animals at a particular moment in time. The only way to accomplish this is to track animals in infected groups for an extended amount of time. An approximate estimate of the sensitivity is 35% (Ellingson *et al*., 2004).

Eliminating bacteria and other quickly growing fungi from the sample is crucial because the organism grows very slowly, making the culture evaluation process take up to 6 months to complete (Carvalho *et al*., 2012). Because mycobacteria have extremely thick, lipid-rich, and acid-resistant cell walls, the sample can be

treated with an acid, like oxalic acid, or a chemical, like hexadecylpyridinium to decontaminate it (Vilchèze, 2020). Testing becomes extremely labor-intensive, costly, time-consuming, and sample-limited as a result of these decontamination methods. However, severe bacterial or fungal development results in significant sample loss (Eshraghisamani *et al*., 2023). Furthermore, the extended culture period hinders prompt detection of positive samples, indicating that the animal has shed the bacteria and subsequently infected additional animals for a minimum of several months prior to the animal being taken out of the herd (Cunha *et al*., 2020). This restricts the test's application in certification schemes.

PCR can be a desirable substitute for culture techniques due to their speed, lack of issues with overgrowth by other microbes, and excellent specificity when using the appropriate primers (Douarre *et al*., 2010). Despite its benefits, this approach has not yet proven viable in being routinely used as a large-scale diagnostic tool. The primary drawback of this method is the laborious sample preparation process needed to get rid of PCR reaction inhibitors, which are found in feces and significantly lower test sensitivity (Hu *et al*., 2015). The methodology is currently more expensive than classic culture methods due to the significant reduction in the number of samples that can be processed.

For large-scale testing needed for certification or eradication campaigns, serological testing in ELISA format is the test of choice due to its affordability, ease of sample collection, vast capacity, and quick turnaround time in the laboratory (Moyano *et al*., 2021). However, because the antibody response develops in the latter stages of the disease, stool culture and antibody ELISA has been previously reported to be only appropriate for use as assays in older animals (Bridges and van Winden, 2021). As a result, when the animal exhibits clinical symptoms, the sensitivity of the ELISA will rise from zero in the early stages of the illness to 100% (Mason *et al*., 2024).

Thus far, crude fractions of several microorganisms have been used as antigens to detect serum antibodies to MAP (Karuppusamy *et al*., 2021). On the other hand, similar bacteria found in the environment can cause false positive results. For instance, many mycobacteria species can be grown from soil and water sources (Primm *et al*., 2004). Consequently, pre-absorbing serum samples with crude fractions of different mycobacteria increases the specificity of ELISA by removing cross-reacting antibodies (Bahmanjeh *et al*., 2021). Ever since it was made commercially available, ELISA has emerged as the most used immunoassay test for detecting paratuberculosis in cattle (Muskens *et al*., 2000).

The absorbed ELISA was advocated for a number of years to be the sole exam needed for certification programs (Field *et al*., 2022). However, the reported relative sensitivity of the absorbed ELISA rapidly dropped from 57% to 33% when different crude fractions of the bacterium was used as antigens. This implies that only one-third of the animals that were simultaneously confirmed to be positive in fecal culture would be detected by the ELISA (Mason *et al*., 2024).

Skin tests and gamma-interferon assays are examples of diagnostic procedures that target early (T cell-mediated) immune responses (Trajman *et al*., 2013). These tests could be intriguing additions to the diagnostic toolkit for identifying paratuberculosis. However, in young animals, the antigens commonly utilized in these assays elicit non-specific reactions (Köhler *et al*., 2021). Consequently, if more specific antigens for paratuberculosis are identified, the diagnosis of paratuberculosis will be very helpful (Vazquez *et al*., 2013). Even though a number of research groups have previously made claims, the antigen is now unknown.

Control and elimination of paratuberculosis in the near future will be extremely challenging due to the limited sensitivity of currently available diagnostic tests, particularly assays appropriate for detecting MAP in young animals in the early stages of illness.

Differential diagnosis

Due to similar antigenic components or cross-reactivity in immunological test, some forms of the illness can be mistaken for paratuberculosis. Marked emaciation in animals suffering from paratuberculosis can be confused with bovine tuberculosis, although bovine tuberculosis is not accompanied by chronic diarrhea (Carvalho *et al*., 2012). Symptoms of chronic diarrhea in cases of paratuberculosis can be confused with bovine viral diarrhea, but it should be noted that the causative agent in both is clearly different (Okuni *et al*., 2020). Apart from that, paratuberculosis can be confused with salmonellosis, coccidiosis, and helminthiasis, where these three diseases are also accompanied by diarrhea; but usually, these three diseases occur acutely and the causative agents are different (Chigerwe and Heller, 2018).

Transmission

Animals can come into contact with this organism in several ways, such as by eating or drinking contaminated food or water, or by nursing from an infected mother who may have contaminated teats or directly shed the organism in milk or colostrum (Biemans *et al*., 2021). One of the most common sources of infection in animals that are vulnerable is contaminated milk (Knific *et al*., 2022). In cattle that are both clinically and subclinically affected, intra-uterine transmission is also a possibility (Field *et al*., 2022). There is a higher chance of transplacental transmission to offspring in pregnant animals with advanced infection (Judge *et al*., 2006); however, other animal species can also experience in utero transmission. The probability of transfer by artificial or natural insemination to the mother animal or fetus has not been examined, despite the fact that MAP has been isolated from semen (Fechner *et al*., 2017).

Zoonotic potential

Animal-derived products like meat, dairy, and tainted surface water have the potential to spread paratuberculosis through zoonotic transmission (Shabana and Aljohani, 2019). The crippling chronic inflammatory bowel disease in humans known as Crohn's disease may be connected to MAP (McNees *et al*., 2015). Recently, a Crohn's disease susceptibility gene (Nod2 on chromosome 16) which connects the innate immune response, bacterial components, and the onset of the disease was identified (Ashton *et al*., 2023). The monocyte response to bacterial lipopolysaccharide (a component of many gastrointestinal bacterial species) is dysfunctional due to gene defects (Scanu *et al*., 2007). In a previous investigation, PCR-based assays were used to compare the detection rates of MAP DNA in patient and control tissues with Crohn's disease (Ellingson *et al*., 2003). The PCR results were supported by the results of previous serologic investigations; however, MAP was not reported to be isolated from Crohn's disease patients (Mendoza *et al*., 2009).

Public health importance

Crohn's disease, a chronic granulomatous infection of the bowel, has been linked to *M. avium* subsp. *paratuberculosis* as the etiological culprit in humans (Rosenfeld and Bressler, 2010). When Crohn first characterized the illness in 1932, the early theory of a mycobacterial genesis was based on the disease's resemblance to intestinal tuberculosis (Chiodini *et al*., 2012). Mycobacteria-like spheroblasts were recovered from Crohn's disease patients in 1984, and these cells were subsequently identified as MAP (Zhang *et al*., 2017). These discoveries sparked a renewed interest in mycobacteria and Crohn's disease. Several organizations have tried to employ PCR techniques to validate the presence of MAP DNA in the intestines of infected patients since the unique IS900 sequence was discovered (Albuquerque *et al*., 2017). While it appears that MAP is occasionally detected in the intestines of Crohn's disease patients, it is unclear if this is a result of the bacteria being involved in the disease's etiology or if the discovery is merely coincidental (Mintz and Lukin, 2023). Confounding results from immunological studies of the connection between mycobacteria and Crohn's disease are comparable to those from PCR-based investigations (Mendoza *et al*., 2009). It is not unexpected that assays measuring the immune response are unable to definitively diagnose paratuberculosis, considering how challenging it is to use these tests for the diagnosis of Crohn's disease. Assuming a mycobacterial etiology, the Crohn's disease patients included in a previous research study were probably at different phases of the illness and would have triggered diverse immune responses (Tsianos *et al*., 2011). Additionally, there are instances where using immunosuppressive medications have been reported to make matters worse. It has also been noted that more precise and well-defined antigens are needed because the antigens utilized in different experiments are typically crude whole cell extracts or pure protein derivatives (PPDs) (Li *et al*., 2023).

Economic impact

Several countries have eradicated paratuberculosis as a result of the implementation of control measures (Weber *et al*., 2024). Significant financial losses are incurred due to this disease, both directly and indirectly. These losses include early culling, decreased body weight and milk production, decreased herd immunity, and higher expenses associated with diagnosis, treatment, and limiting the trade in live animals and their products (Smith and van Winden, 2019). The US loses over $200 million in milk production each year as a result of paratuberculosis (Rasmussen *et al*., 2021). A similar effect occurred in Italy, where MAP infection in a dairy herd caused profit efficiency to drop from 84% to 64% (Sardaro *et al*., 2017). A paratuberculosis management program's cost-efficiency connection can be observed by contrasting the annual cost of disease control (about $30) with the average annual loss of paratuberculosis per cow ($79) (Groenendaal *et al*., 2015).

Treatment

Prolonged studies have been conducted to treat paratuberculosis (Johne's disease) with antibiotics. Unfortunately, there is currently no cure for Johne's disease in animals, like there is for other mycobacterial infections (Sweeney *et al*., 2012). Despite the economic burden of paratuberculosis, research aimed at developing therapeutic medicines is focused on public health rather than veterinary uses (Juste, 2012). Antibiotics such as rifabutin, clarithromycin, metronidazole, clofazimine, and ciprofloxacin are effective in treating MAP infections linked to Crohn's disease in humans (Iaquinto *et al*., 2024).

Due to the connection between autoimmune disorders and Johne's disease, treatment for autoimmune diseases (such as immunosuppressants or immunomodulators) improves the disease's clinical symptoms in people without curing the underlying cause of the illness (Kim *et al*., 2023).

The connection between human Crohn's disease, ruminant Johne's disease, and gut microbiota may lead to the creation of novel treatment strategies for these conditions in the future. Treating intestinal dysbiosis using fecal microbiota transplantation or probiotics (such as *E. coli* Nissle1917, *Clostridium butyricum* MIYAIRI 588, and *Bifidobacterium longum*/Synergy 1) appears to be a potential approach for treating Crohn's disease (Yoshimatsu *et al*., 2021).

Probiotics can help to restore microbial balance by increasing the number of *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and Roseburia species; decreasing the number of pathogenic *E. coli*, *Salmonella*, *Listeria*, MAP, *Yersinia*, *Clostridium difficile*, *Desulfovibrio*, and *Bilophila wadsworthia* in the intestine**,** and removing pro-inflammatory

Actinobacteria and Proteobacteria (Aldars-García *et al*., 2021). Similarly, people with Crohn's disease can fully recover after receiving pluripotent stem cell therapy. Stem cells can simultaneously correct immunological abnormalities, repair intestinal ulcers, and restore normal intestinal function (Manieri and Stappenbeck, 2011). The combination of stem cell therapy and microbial dysbiosis correction represents future potential in the optimal therapeutic strategy for patients with Johne's disease (Mohammadi *et al*., 2023).

Advances in nanotechnology have launched a revolution in the medical field and improved the diagnosis, treatment, and prevention of Johne's disease. This approach can eradicate organisms that are intracellular or that have a high profile of antibiotic resistance, including mycobacterial infections (Hunt *et al*., 2019). Specialized gallium nanoparticles have recently been created to suppress the growth of mycobacteria and control the production of cytokines by host macrophages (Choi *et al*., 2017).

Vaccination

The vaccination against paratuberculosis is typically administered to ruminants in order to minimize clinical signs since it lessens the severity of clinical cases and the amount of MAP released by diseased animals (Links *et al*., 2021). Vaccination is a cost-effective strategy compared to other control strategies. However, because vaccination tampers with skin tests used to diagnose tuberculosis, it is not regarded as the optimal option as a control measure and is even banned in some nations (Buddle *et al*., 2018). On the other hand, vaccination campaigns have been implemented in an effort to control paratuberculosis in a number of nations, including Australia, New Zealand, Spain, India, and the Netherlands (Bastida and Juste, 2011). Fortunately, an effective treatment strategy from a previous study has been implemented to address this disease, which involves using a combination of proteins and peptides in skin tests rather than conventional test reagents (Harris and Barletta, 2001).

Vaccinating ruminants against paratuberculosis at an early age is advised in order to avoid interfering with tuberculosis diagnosis (Garrido *et al*., 2013). Australian sheep vaccination trials showed that the age at which a vaccination becomes effective is 8 months (Windsor, 2006). There have been reports of antibodies lasting up to 42 months following vaccination; however, environmental infections may have a long-lasting booster impact that contributes to these long-lasting antibodies (Boretti, 2024). Infected dairy goats in the Netherlands often receive one vaccination in their first few months of life (Idris *et al*., 2021).

Presently, live (uninactivated and attenuated) and inactivated whole cell vaccines (such as Gudair®, Mycopar®, and Silirum®), along with subunit vaccinations that have been utilized in certain situations with lesser levels of protection, are used to prevent paratuberculosis (Hanafy *et al*., 2023). There is no difference in the effectiveness of attenuated and uninactivated vaccines. However, many nations do not favor live vaccinations because they may only partially protect against certain diseases by lowering clinical cases as opposed to completely eliminating infections, which frequently weaken the immunity of vaccinated animals when they are sold to other groups. Additionally, there may be concerns about public health if humans are infected (Pollard and Bijker, 2021).

Control

There is increasing interest on paratuberculosis due to the potential link between MAP and Crohn's disease, as well as growing knowledge of zoonotic illnesses and food safety concerns. Furthermore, several nations have successfully eliminated bovine tuberculosis, and increased attention is also being paid to the monetary damages brought on by paratuberculosis (Garcia and Shalloo, 2015). To restrict the spread of illnesses and create areas and cattle free of disease, several nations have started disease control or eradication initiatives (Weber *et al*., 2024). This endeavor is challenging due of the rise in animal trafficking on a national and international level (Dow and Alvarez, 2022). The protracted incubation time and the high number of animals with subclinical infections, which are challenging to distinguish using current techniques, are the primary challenges (Park *et al*., 2017).

To prevent the spreading of MAP to uninfected animal populations, drastic measures must be implemented. The inclusion of additional animals to the herd is the primary problematic factor (Cashman *et al*., 2008). The paratuberculosis diagnostic tests now in use are frequently inadequate to determine an animal's actual infection status; as a result, it is critical to understand the animal's origins and the transmission status of the entire herd (Donat *et al*., 2024). This can only be accomplished by repeatedly testing the entire herd. Various testing methods should be used in different age groups due to the type of infection (Holstad *et al*., 2005). It is not appropriate to permit animals from groups with lower or unknown paratuberculosis status to interact or enter with groups that are paratuberculosis-free (Sweeney *et al*., 2012). Pasturing on pastures contaminated by bacterial pathogens is another method of transmission (Garvey, 2020). Since MAP can persist in the environment for extended periods of time, it is best to avoid grazing on pastures where animals get sick (Eppleston *et al*., 2014).

When paratuberculosis is identified in herds, it is critical to work toward limiting the disease's spread. Suspected diarheaogenic or sick animals with signs of MAP infections should be removed from the herd as they may contribute in diseases spread (Mortier *et al*., 2014). Since young animals are more susceptible to infection, it is crucial to handle neonates in an ideal environment and according to a well-established routine (Harris and Barletta, 2001). After birth, calves

should be kept apart from adult animals and should only receive colostrum from their own mothers or animals that have been shown to be paratuberculosis-free (Steuer *et al*., 2020).

Conclusion

Paratuberculosis is an infectious disease that needs public attention because of its zoonotic potential and its impact on economic decline. Early diagnosis needs to be done, considering that exposure to MAP infection might have been going on for a long time. Treatment and control need to be carried out to suppress the spread of this disease.

Acknowledgments

The authors thank Universitas Airlangga and Badan Riset dan Inovasi Nasional.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

The authors thank Universitas Airlangga for managerial support, Salma Firdausya Qurrotunnada Noor, Eunice Wong Hui Wen, Joo Jia Yin, Rahma Novhira for technical support. This research funded by the Directorate of Research and Community Service, Deputy for Strengthening Research and Technology, Ministry of Research and Technology/National Research and Innovation Agency for the 2022 fiscal year, Chancellor's Decree number: 770/UN3.14/PT/2022.

Author's contributions

DKM, ARK, IBM, and MKJK drafted the manuscript. WW, IM, AOA, and SMY revised and edited the manuscript. NS, RIM, SWP, and KAF took part in preparing and critical checking of the manuscript. RR, IF, SW, and SRA edited the references. All authors read and approved the final manuscript.

Data availability

All references are open access, so data can be obtained from the online web.

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