REVIEW ON BOVINE PARATUBERCULOSIS

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ABSTRACT
Paratuberculosis (PTB), or John's disease, is a chronic infectious granulomatous enteritis of ruminants, caused by Mycobacterium avium subspecies paratuberculosis (MAP). The disease has wide host range that affects domesticated and wild ruminants. The bacterium is transmitted by fecal oral route, fomites and vertical transmission. MAP localizes in the mucosa of the small intestine and to its associated lymph nodes and to lesser extent, in the tonsils and suprapharyngeal lymph nodes leading to infiltration the intestinal sub-mucosa. This causes corrugation or thickening of intestinal mucosa, diarrhea and progressive cachexia, which may cause death to the animal. The disease has three forms, silent, subclinical and clinical forms. The disease has strong economic impact especially the subclinical form and public health relevance (possibly transmitted via meat & even pasteurized milk & its products) because of its possible association with Crohn's disease. Necropsy with culture and histopathology on multiple tissues is the gold standard for definitive diagnosis. There are no effective drugs for treatment or effective vaccines for protection against John’s disease. Paratuberculosis control program is time consuming and economically relatively costly, hence prevention of a herd or flock from new infection should be the first preventive strategy. Purchasing replacement cattle from disease free herds and other bio-security measures could be helpful to achieve our control strategies.

KEYWORDS: Paratuberculosis (PTB), avium subspecies paratuberculosis, affects domesticated, wild ruminants.

INTRODUCTION
Ethiopia is known for its high livestock population, being the first in Africa and tenth in the world [1]. The recent livestock population estimates that the country has about 52.1 million heads of cattle, 24.2 million sheep, 22.6 million goats and 44.9 million poultry [2].

The livestock subsector has an enormous contribution to Ethiopia’s national economy and livelihoods of many Ethiopians, and still promising to rally round the economic development of the country. Livestock plays vital roles in generating income to farmers, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP. It also contributes 15% of export earnings and 30% of agricultural employment [3].

However, there are different constraints to livestock production. The main constraint to increasing livestock productivity and output includes lack of adequate supplies of good quality livestock feed, lack of knowledge and diseases. The disease constraints include a wide range of viral, parasitic & bacterial diseases. Of the bacterial diseases John’s disease (JD) or Paratuberculosis is among the well known and leading ones [4].

JD has recently emerged as one of the most widespread, highly prevalent and economically devastating infectious diseases of livestock around the world [5]. The disease is named after Dr. Heinrich A. Johne’s German veterinarian who first described the disease in a dairy cow in 1895. Mycobacterium avium subsp. paratuberculosis is the causative agent of John’s disease [6].

Paratuberculosis is globally distributed among different livestock. Mycobacterium avium subsp. paratuberculosis is highly resistant to environmental stresses like temperature and drying, and is able to persist for years in farm soil [7]. Infected animals (sub-clinical and clinical) excrete huge quantities of bacilli in their feces, milk, semen, and uterine fluids. Animals get infection of Mycobacterium avium subsp. paratuberculosis through fecal oral route and either semen or in fetal life during pregnancy or at birth through suckling of mother.
(colostrums and milk) or later in life through contaminated feed and environment.[8]

The animals that infected by Mycobacterium avium subsp. Paratuberculosis shows different clinical sign includes diarrhea, progressive weight loss, and decreased milk production. Paratuberculosis is an important disease both economically and socially. Economic losses occurs due to subclinical stage of disease, in the form of progressive weight loss, reduced carcass value, poor reproductive performance, reduced milk production, premature culling, reduced fertility and eventual death.[9]

Mycobacterium avium subsp. Paratuberculosis has been isolated from humans, including notably the intestinal tissues of children with Crohn’s disease, which is an ‘inflammatory bowel disease’ (IBD), most commonly affecting the small and large intestine.[10] Currently, there are no antimicrobials approved for the treatment of John’s disease. However, the disease is easily controlled and prevented by using culling of chronically infected animals, animal management and control of super-shedders. The eradication of the disease is a difficult goal to achieve, due to low specificity and sensitivity of available diagnostic tests. Therefore, the main objective of this seminar paper was.

 ✓ To provide a concise review on the disease, bovine paratuberculosis and.
 ✓ To describe its economic and public health significance.

REVIEW ON BOVINE PARATUBERCULOSIS
Definition
Bovine paratuberculosis or John’s disease (JD) is chronic, contagious, invariably fatal enteritis, which can affect domestic and wild ruminants. It is characterized by a loss of physical conditions and weight, emaciation, decreased milk production and diarrhea which results in heavy economic losses.[10]

Etiology
Paratuberculosis is caused by Mycobacterium avium subsp. paratuberculosis (MAP), which is a slender, gram positive, slow growing, acid-fast, non-spor forming, aerobic and facultative obligate intracellular bacterial pathogen.[11] It is a member of the M. avium complex (MAC), which also includes opportunistic pathogens of humans, as well as innocuous (not harmful), environmental bacteria.[12]

Its main features are the requirement for mycobactin (a lipid soluble substance that permits iron utilization) in growth media, and slow growth, which may takes up to 26 weeks before colonies can be observe on solid medium. These complicate its cultivation and diagnosis.[13] Like the other mycobacteria, MAP is a thick complex cell wall, relatively impermeable and rich in lipids. This confers acid-fast properties and may enhance its survival in the external environment, which enable it to persist for more than a year.[14]

Epidemiology
Paratuberculosis is a globally distributed chronic disease except in Sweden and some States of Australia. Nevertheless, as it is non-notifiable Disease, its presence is generally, underestimated in certain countries such as New Zealand, Australia, the United Kingdom and Mediterranean Countries. It is considered as one of the most important infectious diseases that threaten bovine and ovine industries. On the African continent, it has been discovered in almost all countries.[15]

Understanding the epidemiology of John’s disease has been very important in the development of prevention and control strategies.[16]

Occurrence
Paratuberculosis is occurs worldwide most commonly in ruminant like cattle, and to a lesser extent in sheep and goats.[17] The disease is wide spread in cattle in Europe and has been spread to many countries by the export of infected clinically normal pure bred stock .It is of major importance in cattle, and sheep in temperate climates, and some humid, tropical areas. The incidence is greatest in animals kept intensively under climatic and husbandry conditions, which are conducive to the spread of infection.[18]

MAP is able to survive pasteurization temperature and is resistant for chlorine treatment. Hence, the opportunity exists for humans to become infected with this organism via milk, milk product, and beef and water supply.[9] Moreover, it is more heat resistant than other Mycobacterium spp. and Coxiella burnetti (the current target of pasteurization) and is able to survive at the current pasteurization standards.[19]

Host Range
MAP has wide host range and able to survive outside the host for long time (more than 9 months) and therefore it seems more insidious than any other bacteria to human and animal health.

Paratuberculosis affects domesticated and wild ruminants including cattle, sheep, goats, llamas, alpaca, camels, moose, elk, bighorn sheep, buffalo and deer. In addition M.aviumsubsp. paratuberculosis has been isolated from many non ruminant species including rabbits, cats, foxes, weasels, badgers, bears, raccoons, wood mice and Norway rats. It also has been found in jackdaw, rook and crows. Horses and dogs can be infected experimentally.[20]

Morbidity and Mortality
The incidence of paratuberculosis seems to be increasing worldwide. Among ruminants, infections are most common in dairy cattle; approximately 20-50% of the herds are infected in many dairy-producing countries. In an endemic herd, only a minority of the animals develops clinical signs; most animals either eliminate the infection or become asymptomatic carriers. The mortality rate is
about 1%, but up to 50% of the animals in the herd can be asymptomatically infected, resulting in losses in production. Once the symptoms appear, paratuberculosis is progressive and affected animals eventually die. The percentage of asymptomatic carriers that develop overt disease is unknown.\textsuperscript{[21]}

**Sources and Transmission of Infection**

Clinically or latently infected animal excrements constitute the main source of infection. *M.paratuberculosis* excreted in great numbers in the faeces. Soil, food (pasture, hay and straw), drinking water, and anything that is placed in contact with infected excrements can become a secondary source of contamination.\textsuperscript{[15]}

The main transmission route of paratuberculosis is via oral uptake of MAP by susceptible animals from a contaminated environment. Faecal shedders build up the infectious dose by intermittent shedding of MAP in faeces, which may occur already before the age of 2 years.\textsuperscript{[22]}

Vertical transmission of MAP is most common, however, horizontal transmission from either calf-to-calf or calf-to-contaminated wildlife and contaminated wildlife-to-calf, potentially including insects, has also been observed. While wildlife reservoirs of disease represent a potential source of infection, MAP most often enters a cattle herd through the acquisition of an infected cow.\textsuperscript{[23]}

**Risk Factors**

The risk factors that influence the initiation, spread, maintenance, and control of bovine paratuberculosis are related to the animal population, environment, and the biology of disease.\textsuperscript{[24]}

**Animal risk factor:** age of the animal is a major determinant of occurrence of clinical signs and of shedding of detectable levels of MAP: the younger the cattle are when infected, the quicker they develop clinical signs and the more likely they are to shed detectable levels of Map.\textsuperscript{[25]}

In experimental infection, a relationship between the dose given to the animals and occurrence of lesions and clinical signs was established with larger doses resulting in earlier disease development. It is also recognized that factors such as stress may influence the development of the disease.\textsuperscript{[26]}

**Environment:** the ability of *M. paratuberculosis* to survive in the environment for an extended period significantly affects how Johne’s disease needs to be managed. The organism has been found to survive up to 55 weeks in a dry, fully shaded environment. However, moisture, application of lime, UV radiation and temperature fluctuations can all significantly affect the recovery of viable *M. paratuberculosis*. It has been suggested that dormancy may also play a role in the survivability of this organism.\textsuperscript{[27]}

**Pathogen risk factors:** *M. avium subsp. paratuberculosis* is an obligate pathogen and parasite of animals, however, and the organism can survive for long periods outside the host, enabling it to persist and spread in the grassland environment and to withstand a periodic lack of suitable hosts.\textsuperscript{[24]}

**Pathogenesis**

After oral ingestion, the organism localizes in the mucosa of the small intestine and to its associated lymph nodes (payers patches) and to lesser extent, in the tonsils and supravpharyngeal lymph nodes. The primary site of bacterial multiplication is the terminal part of the small intestine and the large intestine. In early stage of infection, the organism is found in phagocytes macrophage in the intestine. Once inside the phagosome of an infected macrophage the organism interferes with the normal course of phagosome maturation into phagolysosome. Macrophage now lyses escaping the bacteria to be accumulated in the intestinal mucosa.\textsuperscript{[28]}

A phagolysosome is a cytoplasmic body formed by the fusion of a phagosome or ingested particle by phagocytes. The organism is phagocytosed by macrophages, which in turn proliferate in large numbers, infiltrate the intestinal sub mucosa, and cause corrugation (thickening) of intestinal mucosa.\textsuperscript{[29]}

The host immune system begins a series of attacks against MAP infected macrophages, including the rapid development of activated T cells, CD4\(^+\)/T cells, and cytolytic CD8\(^+\)cells. These cells interact with the persistently infected macrophage and with each other through a complex network of cytokines and receptors. Despite these aggressive efforts to clear the infection, MAP persists and the constant struggle of the immune system leads to pronounced injury to the intestinal epithelial cells. The loss of putatively protective CD4\(^+\)/T cells leads to a lack of control of mycobacterial replications subsequently granulomatous, typical symptom of bovine paratuberculosis develops.\textsuperscript{[30]}

**Clinical Findings**

Clinical signs usually develop months to years after the initial exposure, depending on the species and strain of MAP. The incubation period of John’s disease is long. In cattle, the incubation period is generally 3–10 years while in sheep and goats the period of subclinical disease tends to be shorter, although there is no defined period and individual animals vary enormously in disease progression and outcome.\textsuperscript{[31]}

The clinical sign of bovine paratuberculosis have three stages: - **Stage 1:** it is silent, subclinical and non-detectable infection seen typically in calves, heifers and young stock less than two years of age. In adult animals exposed to small doses of disease-carrying organism.\textsuperscript{[16]}

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Stage II: Subclinical infection typically occurs in older heifers or adults. Animals at this stage appear healthy but shedding adequate numbers of *M. paratuberculosis* organisms in their manure to be detected on fecal culture. Blood tests will detect some animals but not all animals at this stage. Stage III: is clinical John’s disease and animal with advanced infection. Cattle at this stage Continuous have watery pea-soup diarrhea leading to a cachexia syndrome.

Pathology

Macroscopic lesions

The most prominent gross lesions are thickening, oedema and corrugation of the wall of the small and large intestines. Lesions are localized in the Jejunum, ileum and ileo-caecal valve and sometimes in the caecum. Intestines appear slightly more voluminous, inflamed and appearing prominent and folded in a state of repletion. Mesentry is pale, white and moist. When examining diseased intestine, a considerable thickening and corrugation of the mucosa is observed, revealing deep, stiff folds, which give the mucosa a convoluted brain-like appearance.

The primary macroscopic lesions of John’s disease in ruminants are usually confined to the ileum, caecum, colon and draining lymph nodes. The earliest lesions are thickening and "cording "of lymphatic vessels on serosal surface and along mesentery to lymph nodes, mesenteric lymph nodes enlarged, pale and edematous, hypertrophy of lower small intestine, thick mucosal folds ("like convolutions of cerebral cortex"), and folds cannot be erased by stretching.

The macroscopic lesion characteristic of bovine PTB is a thickening of the intestinal wall, as well as the corrugation of the mucosa affecting different intestinal locations.

**Microscopic lesions**

Ziehl–Neelsen-stained smears of faeces or intestinal mucosa are examined microscopically. A presumptive diagnosis of paratuberculosis can be made if clumps (three or more organisms) of small (0.5–1.5 µm), strongly acid-fast bacilli are found. The presence of single acid-fast bacilli in the absence of clumps indicates an inconclusive result. The disadvantages of this test are that it does not differentiate among other mycobacterial species and only a small proportion of cases can be confirmed on microscopic examination of a single faecal sample.

**Importance of Disease**

Economic importance: Johnne’s disease has become the most important disease of domestic livestock. JD is listed as reportable disease of OIE, disease free certification is necessary for the export of animals and their products. The economic losses associated with John’s disease causes considerable and huge economic
losses in developed and developing countries. Economic losses occur due to subclinical stage of disease, in the form of progressive weight loss, reduced carcass value, poor reproductive performance reduced milk production, lower slaughter value, and premature culling, reduced fertility, reduced body condition and eventual death.\(^{38}\)

The economics costs, as well as animal health and welfare considerations, are sufficient to define JD as a priority for the livestock industry. However, the disease has taken on even greater importance with the possible implication of MAP as a causative agent or contributing factor in Crohn’s disease, an inflammatory bowel disease of humans.\(^{39}\)

Public health significance: Public health issues have been raised about the transmission of \textit{M. paratuberculosis} from animals to humans through animal products (dairy foods, meat, contaminated surface water) and the potential for subsequent infection and perhaps disease. Crohn’s disease, a debilitating chronic inflammatory bowel disease, is thought by some to be linked to \textit{M. paratuberculosis}.\(^{5}\)

MAP has a strong zoonotic potential while existing as a sharp veterinary pathogen. MAP has been isolated from humans, including notably the intestinal tissues of children with Crohn’s disease, which is an ‘inflammatory bowel disease’ (IBD), most commonly affecting the small and large intestine.\(^{40}\)

There are conflicting data on the involvement of the causative organism in Crohn disease, chronic granulomatous enteritis of unknown cause in people. However, \textit{M paratuberculosis} consistently detected by PCR in people with Crohn disease. This fact, coupled with its broad host range, including non-human primates, indicates that paratuberculosis should be considered a zoonotic risk until the situation is clarified.\(^{28}\)

An earlier review\(^{41}\) concluded, "The association of MAP and Crohn's disease is no longer in question. However, its role in causation of Crohn's disease remains to be defined". The most probable transmission route of MAP from animals to humans is milk and milk products. The presence of MAP in raw milk is concluded by the fact that there is evidence that the organism can survive the milk pasteurization process under certain conditions.\(^{42}\)

**Diagnosis**

The major difficulty encountered in the diagnosis of paratuberculosis is the exact identification of Subclinical cases. Infected animals may not show symptoms of the disease for three to five years after infection and by the time clinical signs are manifested animals have already enough time to contaminate the environment. Moreover, the intracellular and slowly progressive nature of \textit{M. paratuberculosis} complicates the diagnosis process.\(^{43}\)

Diagnostic tests for JD are divided into two categories: those that detect the organism and those that assess the host response to infections. The first category includes acid-fast stain on fecal smear, culture, and polymerase chain reaction tests. The second category, detection of host response, includes clinical signs in combination with gross and microscopic pathology and serological tests, which includes complement fixation Test (CFT), agar gel immune-diffusion (AGID), ELISA; gamma-interferon test.\(^{43}\)

**Histopathological Diagnosis**

Criteria accepted for a positive histological diagnosis include the presence of epithelioid and/or Langerhans’-type giant cells, and the demonstration of intracellular acid-fast bacilli in these cells. Histological lesions of Johne's disease are characterized by the presence of aggregations of large macrophages with abundant granular cytoplasm, often referred to as epithelioid cells, in the intestinal mucosa and sub mucosa, lymphatic’s and in the cortex of mesenteric lymph nodes. Multinucleate giant cells are seen in the intestinal mucosa and cortex of the mesenteric lymph nodes of cattle and small ruminants.\(^{15}\)

**Necropsy Finding**

Paratuberculosis cannot be diagnosed on superficial examination of the intestines for signs of thick-ening. The intestines should be opened from the duodenum to the rectum to expose the mucosa. There is not always a close correlation between the severity of clinical signs and the extent of intestinal lesions. The mucosa, especially of the terminal ileum, is inspected for pathognomonic thickening and corrugation.\(^{23}\)

Lesions are confined to the posterior part of the alimentary tract and its associated lymph nodes. The terminal part of the small intestine, the cecum, and the first part of the colon are usually affected. In advanced cases, the lesions may extend from the rectum to the duodenum. Typically, the intestinal wall is three or four times normal thickness, with a corrugated mucosa and prominent thickened serosal lymphatic.\(^{24}\)

**Differential Diagnosis**

The clinical disease must be differentiated from diseases, which cause chronic diarrhea in adult cattle. The chronic nature of Johne's disease is usually sufficient to differentiate it from the other common enteritis of cattle. Salmonellosis, coccidiosis, and gastrointestinal helminthiasis are usually acute and the latter two occur principally in younger animals and are distinguishable on fecal examination for oocysts and helminthes eggs.\(^{24}\) Secondary copper deficiency (chronic molybdenum poisoning) is likely to be confused with Johne's disease in cattle, but is usually an area problem affecting large numbers of animals and responds well to the administration of copper. Tuberculosis is likely to be confused with John’s disease in cattle, but tuberculosis granulomas may be found in any of the lymph nodes.\(^{14}\)
Treatment

The treatment of paratuberculosis has generally been unsuccessful because of intracellular sequestration of the organism in macrophages, chemically resistant mycobacterial cell wall that resist to destruction or penetration and the ability to neutralize antibacterial chemicals produced inside macrophages.\textsuperscript{[17]}

Currently, no antimicrobials are approved for the treatment of John’s disease although costly; combination of different drugs has been practiced as treatment measure, mostly with isoniazid, clofazimine and rifampin.\textsuperscript{[43]}

\textit{M. paratuberculosis} more resistant to chemotherapeutic agents in vitro than \textit{M. tuberculosis} so that prospects for suitable treatment are poor. Because of this lack of efficacy and the failure of any of the antimicrobials to provide a bacteriological cure, treatment is not recommended.\textsuperscript{[24]}

Control and Prevention

A clear understanding of the epidemiology of John’s disease is pivotal in the creation of prevention and control strategies. To control the disease in an infected herd, early detection and removal of infected cattle and protection of newborn calves from contaminated feces are vital. Nursing calves are to be raised on a clean, uncrowned pasture. There are no drugs for treatment of JD or effective vaccines for protection (because vaccines are unable to fully protect and prevent shedding of the microorganism in vaccinated group of animals). In ruminants the control of JD is challenging because of the ubiquitous nature of the organism, the long incubation period, most cases are subclinical, and the laboratory tests available lack sufficient sensitivity to identify infected animals, which allows the infection to spread within and between herds. Control is further constrained by the lack of economically feasible and practically useful treatments for this disease.\textsuperscript{[38]}

The implementation of herd or flock level control programs, establishment of test negative or low-risk herds or flocks, and reduction of environment and feed contamination with \textit{MAP} are attainable goals. Because of the inaccuracy of the diagnostic tests available, it is impossible to eradicate the disease, other than by complete depopulation of the herd and restocking with non-infected animals.\textsuperscript{[17]}

The eradication of the disease is a difficult goal to achieve, due to low specificity and sensitivity of available diagnostic tests. The prolonged incubation time of the infection and the onset of shedding in the absence of clinical symptoms, allow the disease to spread before its successful identification.\textsuperscript{[9]}

Paratuberculosis control program is time consuming and economically relatively costly, hence prevention of a herd or flock from new infection is the first option to be adopted. This practice can be achieved by care when purchasing replacement cattle to prevent introduction of John’s disease into a healthy, disease-free herd. Obtain mature cattle from John’s test-negative herds or herds with no history of John’s disease. Isolate cattle purchased from non-tested herds until they are proven negative by fecal cultures. Because of there is no effective vaccine and no cure, prevention is the key to control.\textsuperscript{[23]}

CONCLUSION AND RECOMMENDATIONS

Bovine paratuberculosis caused by \textit{MAP}, is a chronic debilitating disease, which can affect domestic and wild ruminants worldwide. It is both economically and socially important disease, which causes heavy loss of livestock productions through heavy culling of infected animals, loss of physical condition, emaciation, decreased milk production and diarrhea. Paratuberculosis, in addition to its economic importance, it is of public health significance, as it is supposed to cause Crohn’s disease, a debilitating chronic inflammatory bowel disease in human. Animal products like meat, milk (even pasteurized milk) and milk products are supposed to be the potential vehicles for the pathogen & therefore the risk to human infections. In adult cattle, the bacteria cause enteritis, and inflammation of the small intestine that becomes thickened and corrugated. The greater the numbers of infected animals that shed the organism, the greater the exposure risk to other cattle in the herd. The definitive diagnosis of paratuberculosis requires the isolation and identification of the bacteria. Necropsy with culture and histopathology on multiple tissues is the gold standard for definitive diagnosis. There are no effective drugs for treatment or effective vaccines for protection against John’s disease. The chronic nature of the disease and the subclinical shedders together with the other factors make paratuberculosis control program time consuming and economically costly, hence prevention of a herd or flock from new infections is the first option to be adopted.

Therefore, based on the above conclusion, the following recommendations were forwarded.

- An integrated, bottom-up approach to on-farm disease control is required that involves the collaboration of all the stakeholders in the animal health sectors
- Training programs are recommended for practicing veterinarians and laboratory personnel to ensure a uniform base of knowledge and practice regarding the disease.
- Disease status is not well studied in Ethiopia, so efforts should be made towards studying the disease.
- There are still open questions to be resolved regarding the zoonotic aspects of the disease, so it could be a potential research area for both veterinary and medical health professionals.
- Improvements towards the current vaccines is mandatory as they are unable to fully protect the...
disease as well as shedding of the agent, which results in silent economic loss and progression of the disease.
- Test and slaughter of positive animals should be taken into consideration due to the chronic nature of the disease & low rate of success of treatments
- Hygienic measures are recommended.

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