PARATUBERCULOSIS (JOHNE’S DISEASE) IN LIVESTOCK
PART I: EPIDEMIOLOGY, PATHOGENESIS AND CLINICAL SIGNS

Introduction

*Mycobacterium avium* subsp. *paratuberculosis* (MAP), a facultative intracellular acid-fast bacillus, is the cause of paratuberculosis or Johne’s disease, a chronic infectious disease of cattle, sheep, goats and certain captive or free-living wild ruminant species clinically characterized by progressive emaciation and/or chronic enteritis and diarrhoea. Paratuberculosis is seen worldwide and the first case of bovine paratuberculosis in South Africa was diagnosed in 1923, followed by a single ovine case in 1967. Since 1996 more than 50 infected sheep farms were identified in the western and eastern Cape provinces, and paratuberculosis has been classified as a notifiable disease in South Africa in 1997. Although there has been suspicion of a causative link between this organism and Crohn’s disease in humans, it has not been conclusively proved. The exact cause of Crohn’s disease is not known today and it is considered an autoimmune disease with multifactorial cause, including the role of various infectious agents.

Epidemiology

Cattle, sheep, goat, white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), bighorn sheep (*Ovis canadensis*), Rocky Mountain goats (*Oreamnos americanus*), Florida Key deer (*Odocoileus virginianus*) and North American bison (*Bison bison*) have all been described to suffer from paratuberculosis. The organisms have also been isolated from the mesenteric lymph node in a raccoon (*Procyon lotor*). Outbreaks of paratuberculosis have also been described in non-ruminant species such as a colony of stump-tail macaques (*Macaca arctoides*) and wild rabbits in Scotland. In Greece the organism was isolated from samples from two mice, two rats, a hare and a fox with only one rat showing typical histological lesions. The situation as far as infection of wildlife species in South Africa is concerned remains largely unknown.

There is a correlation between the prevalence of paratuberculosis and certain soil types, with acid conditions (low pH) of soil or pasture enhancing spread of the disease, whereas alkaline (high pH) conditions inhibits its spread. The vast majority of farms with infected sheep flocks, in the Western Cape Province of South Africa, have an acid, silcrete-type soil. Low pH seems to favour iron uptake by *M. avium* subsp. *paratuberculosis*. In the study by Dieguez et al the study area had acid soils, which should favour the persistence of the bacterium and transmission of disease. However, the importance of this risk factor could not be fully clarified during this particular study. Excess iron and molybdenum, and marginal deficiencies in copper and selenium have also been implicated as favourable in the progressive expression of Johne’s disease. Survival of the organisms may possibly also favour by a silt or sand content in loamy soils.
Depending on the moisture content and the temperature the organisms may persist in pastures and may remain infective for up to 1 year, without multiplying. They are relatively susceptible to sunlight and drying, high calcium content in the soil and high pH of the soil. Continuous contact with urine and faeces may reduce the longevity of the organism, but in slurry stored in tanks these bacteria can survive for periods of 98-287 days, being influenced by the composition and alkalinity of the slurry. Organisms have been reported to survive in faecal material in soil for 55 weeks in a fully shaded environment but for a shorter period of time if the faecal material and soil were fully exposed to the weather and the vegetation was removed. Organisms survived in colostrum subjected to pasteurisation temperatures of 63°C for 30 minutes. Pasteurisation of colostrum and milk experimentally inoculated with the bacteria resulted in reduced growth, but did not eliminate the presence of viable organism.

Management practices, environmental conditions and hygiene, and manure handling practices may be correlated with the prevalence of infection. Published findings of environmental samples that were cultured revealed positive culture results in cow alleyways (77% of herds), manure storage areas (68% of herds), calving areas (21% of herds, sick cow pen (18% of herds), water run-off (6% of herds), and post weaned calves areas (3% of herds).

Calves may become infected in utero with up to 20% of infections thought to be congenital, via ingestion of infected milk from cows harbouring the organism, or by intake of \textit{M. avium} subsp. \textit{paratuberculosis} from sources such as faecally contaminated cows’ teats, pastures or other materials, objects, equipment and vehicles. \textit{Mycobacterium avium} subsp. \textit{paratuberculosis} has been isolated from the semen of bulls, embryos and foetuses.

In infected herds the majority of exposed animals are usually sub clinically infected. These animals may shed bacteria intermittently via the faecal route. Animals exhibiting clinical disease may only represent a very small number of the total number of infected animals on an infected property. Any form of stress factors, such as seen during parturition, transport and a low plane of nutrition may convert sub clinical cases into overt clinical cases. These risk factors may differ from region to region, and between infected herds. Several recent articles have been published on the results of investigation into the epidemiology of paratuberculosis, and mostly in dairy herds.

In a study by Dieguez\textit{ et al} they reported that the use of colostrum from cows with a previous diagnosis of paratuberculosis; and the housing of replacement calves under six months old together with adult cattle were the highest management risk factors. There were no significant association between paratuberculosis and animals less than six months old being fed herbage treated with manure from the farm. They found a significant relationship between the infection status of a herd defined as negative, positive or highly positive, and the proportion of farms, which had high bulk tank somatic cell, counts. The higher the infection status of a herd, the higher the proportion with a
high bulk tank somatic cell counts. However when analysing the incidence of clinical mastitis a significant difference was only observed between negative farms and highly positive farms. Relationships were observed between the infection status of the herds, the incidence of mammary infections and the rate of culling. No significant relationships were observed between the infection status of the herds and their reproductive performance. These authors emphasised the importance of adequate biosecurity, and importance of adequate housing for calves and breeding heifers.

In a different study by Tavorpavich et al they found that management practices related to milk and manure handling likely influenced the high seroprevalence of mycobacterial infection. Feeding of unsalable milk to calves, flushing of cows walkways with recycled lagoon water, handling of feed with manure handling equipment, and exposing heifers ≤ 6 months old to manure of adult cows correlated with an increased risk of a high seroprevalence of mycobacterial infection. Exposure (direct or indirect) to lagoon water was identified as a risk factor for calves. A significant relationship between herd size and high seroprevalence of infection was also detected. Large size of diary herds was associated with a higher seroprevalence in infected herds. Management practices such as separation of calving areas from housing for lactating cows, frequency of bedding changes in calving area, and interval from parturition until separation of newborn calves from their dams were not significantly associated with the seroprevalence of infection.

In a different study by Tiwari et al involving a large number of randomly selected Canadian dairy herds, a number of factors, which were significantly and positively associated with the count of seropositive cows, were identified. These included having more than one cow in the maternity pen, housing of pre-weaned calves in groups in winter, the purchase of open heifers during the previous 12 months, direct (nose to nose) contact with beef cattle, BVDV-seropositive herds and BVD vaccination not done properly in calves (vaccinated older than 6 months of age, not receiving a booster 2-4 weeks following vaccination with a killed vaccine, or not vaccinated with a modified live vaccine). Vaccination with a modified live vaccine followed by another modified live vaccination after 6 months was associated with 0.4 times fewer MAP-seropositive cows.

In a study by Nielsen et al the four most significant management-related risk factors in 97 Danish dairy herds were identified as: the housing of cows in bed stalls compared to housing in tie stalls; low level of hygiene in the feeding area of calving areas; low amounts of straw in the bedding of the calving area; high animal density among young stock > 12 months of age.

A study conducted by Dhand et al to investigate the risk factors for ovine Johne’s disease (OJD) involving 92 infected Merino sheep flocks in Australia revealed higher infection rates in sheep whose dams had been in poor condition and kept at high stocking rate during lambing, animals which had experienced a longer period of growth retardation during their lifetime. Flocks vaccinated for > 2 years (rather than only 1-2 years) with a killed paratuberculosis vaccine had significantly lower infection rates. Practices such as culling low body weight sheep; selling sub-flocks experiencing high losses, sharing of roads between neighbouring farms, and greater frequency of application of super
phosphate fertilizers were associated with higher infection rates. Infection was seen to be higher in flocks experiencing high mortalities; in weathers compared to ewes; and in 3-year-old sheep compared to 4-year-old sheep.

Pathogenesis

Neonates are most susceptible to infection and this age-dependant susceptibility of young animals to infection with *M. avium* subsp. *paratuberculosis* is due to:

- A higher intestinal acidity in suckling calves compared to that of adult ruminants, which favours the survival of organisms.
- The presence of lactoferrin and transferrin in colostrum, which serves as a source of iron for the production of mycobactin by the mycobacteria.
- Maternal antibodies in the milk of infected animals, such as opsonin, may enhance the uptake of *M. avium* subsp. *paratuberculosis* by M-cells and enterocytes.

Following ingestion of bacilli they are believed to enter the intestinal tissues through the M-cells located in the Peyer’s patches. This is facilitated by fibrinonectins binding to fibrinonectin attachment proteins present on the mycobacteria, and integrins present on the M-cells. Once the bacilli have crossed the epithelial layer they are actively phagocytosed by macrophages. These macrophages contain several receptors, which seem to favour the uptake of the bacilli and these include the complement receptors CR1, CR3 and CR4, immunoglobulin receptors (FCr), mannose receptors and scavenger proteins. It seems that complement opsonization of bacilli is playing an important role in the uptake of these organisms by macrophages.

Organisms are then restricted intracellularly in macrophages where they are resistant to digestion by phagolysosomes being able to multiply without inhibition and remaining intact for up to five weeks. These mycobacteria do not produce toxins, but both immune-inhibitory and activating molecules are released as consequence of their multiplication in the macrophages. Macrophages also present the bacteria as antigens to the lymphocytes of the gut-associated lymphoid tissue (GALT), resulting in the sensitisation of the intestinal immune system to these bacteria. Paratuberculosis seems to be a very dynamic disease with fluctuations between periods of effective cell mediated immunity when the host is successfully coping with the ineffective organisms, to periods dominated by humoral immune responses at which times the hosts seem to lose its resistance to the organisms. Periods of remission and exacerbation of the disease may therefore be seen during the course of the disease.

Apart from the GALT, the organisms may also be taken up by the tonsillar lymphoid tissue and infection may become localized in specific mesenteric lymph nodes. The mesenteric and ileocaecal lymph nodes are consistently infected, 6-14 months after initial infection, and some organisms may then spread to the caecum, colon, liver and other tissues.
Infection may result in chronic and severe granulomatous inflammation in the intestinal mucosa, which leads to decreased assimilation and absorption of nutrients; and exudation into the lumen of the intestinal tract in turn giving rise to diarrhoea and the wasting syndrome.

Some textbooks describe paratuberculosis to progresses through three stages. In first phase (stage I) there seems to be no significant faecal shedding of the organism, or development of overt clinical disease. The second phase (stage II), is reported as a sub clinical excretory phase with the organism being shed intermittently into the environment via faeces. The terminal phase (stage III) is characterized by clinical disease and intermittent to continuous large scale shedding of bacilli, diarrhoea and weight loss is encountered. Factors such as the age of the animal at the time of exposure and the number of organisms ingested at the time may determine the length of these stages.

Several very recent articles, including review articles have been published on the strategies for survival, of the organisms in the host, and all the pathways and molecules involved. It will be beyond the scope of this article to go into any detail of the complex aspects of this topic.

Clinical signs

Clinical signs are usually seen in older groups of animals, mostly older than two years. Animals, in infected herds, may be categorized into clinical cases; asymptomatic silent shedders; sub clinical non-shedding carriers; or uninfected animals. Clinical bovine paratuberculosis is characterized by progressive weight loss and continuous or intermittent chronic non-responsive diarrhoea despite a generally good appetite. Faeces are usually passed without straining, soft to watery in consistency and foul smelling. It may occasionally contain casts of mucous.

The disease usually has a long incubation period and slow course but infected animals can be identified before the full blown clinical stage by a reduced milk yield. Clinical disease may sometimes be of sudden onset and particularly in the high producing group of animals. Clinical signs which may be seen include: decreased milk production, rough hair coat or alopecia, sub-mandibular oedema (bottle jaw), absence of a temperature reaction, severe dehydration, emaciation and weakness, followed by death after a course of several weeks to months. However, the annual mortality rate is usually low and may be less than 1 percent although up to 50 percent of the animals in a herd may be infected. In some animals secondary complications may include mastitis and infertility.

Economic losses due to paratuberculosis may be very significant. Many figures have been quoted and published through the years. A very recent study by A Tiwara et al in Canadian dairy farms reports losses of $2992 annually in an average herd comprising 61 cows, of which 12.7% are seropositive. This amounted to $49 per cow and culling, decreased milk production, mortality and reproductive losses accounted for 46%, 9%, 16% and 29% of the losses respectively.
References


6-F.M. Leontides et al. Isolation of *Mycobacterium avium subspecies paratuberculosis* from non-ruminant wildlife living in the sheds and on the pastures of Greek sheep and goats. Epidemiol. Infect. 2008 May;136(5).


