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Diagnostics of Bovine paratuberculosis (Johne's disease)

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Introduction

Bovine paratuberculosis (Johne's disease) of cattle occurs worldwide, and is caused by the bacteria *Mycobacterium avium subsp. paratuberculosis* (MAP), a facultative intracellular acid-fast bacillus. Domestic and wild ruminants are susceptible to natural infection and the most commonly affected. Clinical disease is rarely reported in camelids, rabbits, equids, swine and captive primates. In 2017 clinical paratuberculosis was described in a dog in South Africa. There has been a link between MAP and *Crohn's disease* in human patients, raising concerns for a possible public health risk. However, there is currently still a lack of definitive evidence that MAP is the primary cause of Crohn's disease. Based on current research, it is more likely that MAP is coincidental or possibly plays an opportunistic role in human disease.

The Agent

Based on DNA hybridisation studies and other genotypic and phenotypic tests, the etiologic agent of Johne's disease is classified as a subspecies within the *Mycobacterium avium* complex which includes

- *M. avium subspecies hominissuis.*
- ***M. avium subspecies paratuberculosis.***
- *M. avium subspecies silvaticum.*
- *M. avium subspecies avium.*

Two major groups are recognised within *M. avium subspecies paratuberculosis:* cattle strains (C strains) and sheep strains (S strains). Whole gene sequencing has determined that C strain/Type II lineages contain the cattle strains and a subcategory of bison strains (USA and Indian bison types). The S-group sheep strains contain two sublineages, Type I and Type III, and S strain bovine paratuberculosis cases are reported.

ON THE OTHER HAND, type II "C" strains are the most common isolates associated with bovine Johne's disease. Cattle strains have a broad host range and have been isolated from domestic and wildlife species as well as non-ruminants.

Pathogenesis

Cattle usually become infected via the faecal-oral route at a very young age, acquiring the infection directly by ingestion of faeces on faecal contaminated feed or indirectly via MAP-contaminated milk/colostrum or water. Organisms may also be present in semen or urine and cross the placenta, particularly during advanced disease. There is an age-dependent susceptibility to MAP infection, with neonates being most susceptible. The infectious dose required for transmission in adults is considerably higher than for neonates.

MAP accesses the small intestinal mucosa via microfold (M cells) or epithelial cells overlying submucosal Peyer's patches. Once within the mucosa, MAP targets and invades macrophages, inhibiting phagosome-lysosome fusion, which is central to MAP survival and persistence within the host.

MAP-infected cattle initially develop a pro-inflammatory immune response in an attempt to clear the infection. This pro-inflammatory response is replaced by a powerful but ineffective MAP-specific antibody response in animals that fail to clear the bacteria. This phase heralds the transition from subclinical to clinical disease with active faecal shedding. Host cellular immune responses drive the granulomatous enteritis, which ensues with thickening and corrugation of the intestinal wall. With the progression of clinical disease, the population of MAP within the intestinal mucosa increases and live MAP are shed in the faeces more frequently and in more significant numbers. With this disease progression, there is also an extension of these proliferating bacterial engorged macrophages from the primary sites of infection in the ileum and ileocecal junction down the proximal and distal colon.

The highest risk of introducing any strain of MAP onto a farm is by introducing infected animals, be that cattle, sheep or goats. With the increased prevalence of ovine Johne disease (OJD), contact between sheep and cattle poses a significant risk of introducing infection into cattle herds. Other factors that increase the likelihood of sheep (S) strain spread from sheep to cattle include:

- Increased stocking densities
- Co-grazing cattle, especially cattle under one-year-old, with sheep shedding the bacteria
- Grazing cattle on pastures contaminated with S-strain
- Climatic conditions facilitate the survival of S-strain in the environment (such as higher rainfall areas).
- Indirect exposure of cattle to run-off from pasture contaminated with S-strain in sheep (whether from same or neighbouring properties)

Clinical Signs

Bovine Johne's disease is classified into four clinical stages.

- Silent infection.
- Subclinical infection.
- Clinical disease.
- Advanced clinical disease.

During the **silent** phase of the disease, which occurs in calves, heifers and young cattle up to 2 years of age, no clinical signs are observed, and no effect on body weight or body condition is noted. Still, a few animals may shed bacteria with positive faecal culture and/or positive faecal PCR detection.

In the following **subclinical phase,** faecal shedding may still be variable but progressive. These infected adult carrier animals do not exhibit clinical signs directly related to MAP infection but commonly develop concurrent conditions such as mastitis or infertility. Of particular importance is that this subclinical period may last for 2-5 years before animals become clinical. This phase poses a significant risk for the spread of the bacteria to susceptible herd-mates.

Clinical disease is most commonly observed in the 2 to 6-year-old age group. During this phase, affected cattle often retain a normal appetite but gradually lose weight with intermittent diarrhoea. This phase is then followed by progressive diarrhoea, which becomes persistent over 2-3 weeks with dehydration and increased thirst. Faeces are soft, sloppy, watery and homogenous without offensive odour.

In **advanced clinical disease,** animals become hypoproteinaemic due to protein-losing enteropathy as a consequence of granulomatous enteritis, with cachexia and emaciation (body condition score <1.75). The diarrhoea becomes severe with the "water hose" passage of faeces.

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Severe dehydration and emaciation eventually lead to death or euthanasia. In infected herds, although there may only be a few animals in the clinical phases of the disease, a much greater number of animals (15 to 20 times) are likely to be MAP infected. Differential diagnoses based on clinical signs include bovine viral diarrhoea virus (BVDV), salmonellosis, coccidiosis, gastrointestinal helminthiasis, intestinal tuberculosis, gastrointestinal lymphosarcoma, intestinal obstruction and copper deficiency.

Pathology



Figure 1: Bovine with severe faecal soiling of the hindquarter indicative of chronic diarrhea.*

Animals presented for post mortem are usually severely emaciated with extensive faecal soiling of the perineum (figure 1). The ileum, large intestine, and draining lymph nodes are the prime sites for the major lesions of Johne's disease. Gross intestinal lesions may be segmental or continuous. They can be distributed from the duodenum to the rectum, although lesions are usually most outspoken in the lower ileum and upper large intestine. Small intestinal concentric corrugations are frequently visible from the serosal surface (figure 2). The classic pathology of the opened intestine is the thickening of the mucosa, which is granular, congested and folded into transverse rugae (figure 3). Mucosal thickening is due to the accumulation of inflammatory cells, predominantly macrophages, as well as oedema fluid, in the mucosa and submucosa.



Figure 2: Bovine small intestine with concentric corrugations (arrowheads), thickened serosal lymphatics due to lymphangitis (line) and voluminous watery, homogenous faecal material (*).*



Figure 3: Bovine colon showing marked mucosal thickening with transverse rugae which mucosal congestion and surface granular appearance.*

The ileocecal and mesenteric lymph nodes are enlarged, pale, and edematous (figure 4), while serosal and mesenteric lymphastics are frequently thickened due to granulomatous lymphangitis (figure 2). In rare cases, mucosal lesions are subtle, with lymphangitis the only visible gross lesion. However, the lesion of intestinal lymphangitis is still specific enough to warrant a collection of samples to exclude Johne's disease.



Figure 4: Bovine mesenteric lymphnode which is enlarged (lymphadenopathy) with irregular cobblestoned appearance on cut surface (granulomatous lymphadenitis).*

Courtesy J. Shilling.

Ancillary pathology includes marked loss of muscle mass (low body condition score), serous atrophy of subcutaneous, intra-abdominal and cardiac fat depots, intermandibular oedema, and fluid effusion in various body cavities (hydrothorax, hydropericardium, ascites). Plaques of intimal fibrosis and mineralisation may be evident in the thoracic aorta.

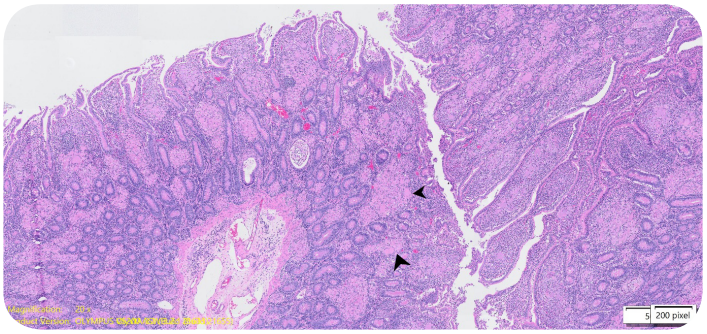


Figure 5: Histopathology (H&E stain) bovine small intestine with multifocal mucosal and submucosal clusters of epithelioid macrophages (arrowheads).

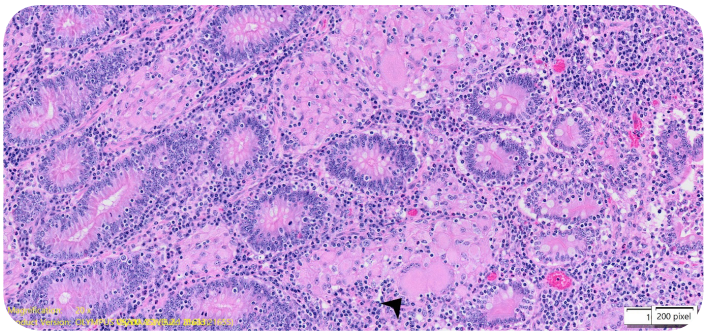


Figure 6: Histopathology (H&E) bovine small intestine, higher powered view of mucosal epithelioid macrophages and Langhans giant cells (arrowhead)

The hallmark histopathology features are transmural granulomatous enteritis, lymphangitis and mesenteric lymphadenitis, characterised by aggregates or diffuse sheets of epithelioid macrophages (activated macrophages that look like epithelial cells), and Langhans-type multinucleated giant cells (figure 5 and 6).

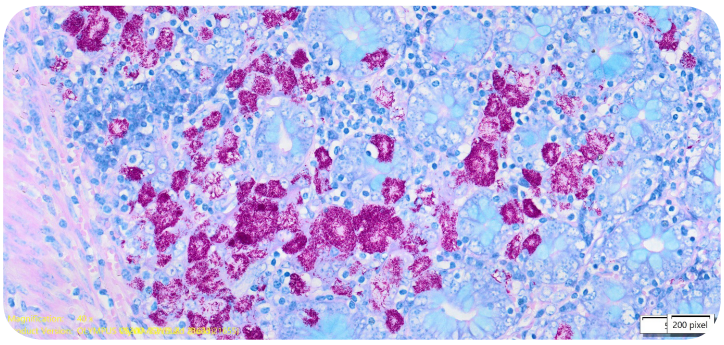


Figure 7: Histopathology (ZN stain) showing numerous acid fast bacilli within epithelioid macrophages.

MAP associated granulomas have also been described in the liver, hepatic lymph nodes, and very rarely, the kidney and lungs. Acid-fast bacilli are usually demonstrable in large numbers in these lesions (figure 7).

Diagnostic Tests for Confirmation of Infection and Freedom of Disease.

Polymerase Chain Reaction (PCR)

- High throughput Johne's disease faecal PCR (HT_J PCR) targets the genetic IS900 insertion sequence element, unique to MAP. The HT_J PCR can be run on individual or pooled (5 animals) faecal samples and so has applications as an individual animal diagnostic and herd screening test. The advantage of this assay is the speed for results (3 days) compared to faecal culture (12 weeks).

- Conventional IS900 PCR + reverse enzyme assay (REA) is used broadly as a diagnostic tool in animals and humans to distinguish MAP from other *M. avian* subspecies. The REA is primarily run on PCR products from conventional IS900 PCR and culture material but can also be run directly on intestinal content if sufficient target organisms are present. A lot more organisms are shed in the faeces/intestinal contents than can be found in intestinal mucosa or mesenteric lymph node.

- IS1311 PCR + REA. Enables strain differentiation between cattle (C) and sheep (S) strains and is used exclusively for this purpose. This PCR is usually run on formalin-fixed paraffin-embedded (FFPE) tissue scrolls, HT-J PCR DNA extract or culture material. The IS1311 PCR + REA can also be performed directly on primary samples (faeces/intestinal content) but with reduced sensitivity (a negative is meaningless).

Bacterial Culture

MAP in culture is slow-growing (takes up to 12 weeks) and requires highly specialised culture media. These bacteriums' dependence on the iron chelator mycobactin is the key distinguishing phenotypic characteristic for identification. Faecal culture is most commonly employed in live animals.

Still, the organism can also be isolated from milk, with an estimated 8-22% of subclinically infected and 35% of clinically infected cattle harbouring the bacteria in their udders. Postmortal cultures are usually performed on intestinal content, faeces, affected sections of the intestine and mesenteric lymph nodes.

Serology

The serological test commonly used for paratuberculosis in cattle is enzyme-linked immunosorbent assay (**ELISA**).

The complement fixation test (CFT) and agar gel immunodiffusion (AGID) show poorer sensitivity and specificity and are no longer recommended in cattle. The ELISA detects about 30–40% of livestock identified by faecal culture of faeces.

Histopathology

The demonstration of characteristic histological lesions in H&E stained tissues sections in conjunction with the demonstration of intra-histiocytic acid-fast bacteria on ZN stains are highly diagnostic but require confirmation via PCR (fresh tissue, intestinal content, formalin-fixed tissue scrolls, DNA extract) or MAP culture (faeces, intestinal content, fresh tissue).

Bovine Johnes Disease in South Africa

Bovine Johnes Disease is a Controlled Disease in South Africa with diagnostic testing and disease control under the Department of Agriculture, Land Reform and Rural Development (DALRRD) jurisdiction. If you have any suspicion of Bovine Johne's disease, your local State Veterinary Authority needs to be immediately informed by completing and submitting an SR1 Disease Report Form.

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MULTIPLE-CHOICE QUESTIONS

QUESTION 1

In which of the following animal species is clinical paratuberculosis most commonly described?

A. Camelids
B. Ruminants
C. Swine
D. Primates
E. Rabbits

QUESTION 2

What is the primary route of infection for MAP?

A. Indirect contamination of milk
B. At natural breeding or AI via infected semen
C. Indirect contamination of colostrum
D. Feco-oral route
E. Indirect contamination of water

QUESTION 3

What is the primary target cell of MAP once bacteria have passed through the intestinal epithelium into the mucosa?

A. Macrophage
B. Crypt epithelial cells
C. Lymphocytes
D. Plasma cells
E. Neutrophils

QUESTION 4

Which of the following mechanisms are central to MAP survival and persistence within the bovine host?

A. Host pro-inflammatory immune response
B. Ineffective MAP specific antibody response
C. Excessive host cellular immune response
D. Excessive host humoral immune response
E. Inhibition of phagosome lysosome fusion

QUESTION 5

Which of the following statements about factors that increase the risk of spread of sheep strain MAP from sheep to cattle, is untrue?

A. Decreasing stocking densities
B. Co-grazing cattle, especially animals under one year of age, with sheep shedding the bacteria
C. Grazing cattle on pastures contaminated with sheep strain MAP
D. Climatic conditions that facilitate survival of sheep strain MAP in the environment
E. Indirect exposure of cattle to run off from pasture contaminated with sheep strain MAP

QUESTION 6

How long does the sub-clinical phase of MAP usually last?

A. 1 to 3 months
B. 6 to 12 months
C. 12 to 18 months
D. 2 to 5 years
E. 6 to 8 years

QUESTION 7

Which of the following anatomical sites would be considered a prime site for the major gross lesions of Johne's disease?

A. Jejunum and mesenteric lymph node
B. Ileum and draining lymph node
C. Duodenum and draining lymph node
D. Abomasum and draining lymph node
E. Rumen and draining lymph node

QUESTION 8

Which of the following gross lesions would be considered specific enough on its own to warrant collection of samples to exclude Johne's disease?

A. Thickening of the small intestine with serosal corrugations
B. Mesenteric lymphadenopathy
C. Thick mucosal rugae in the cecum
D. Mesenteric lymph node edema
E. Intestinal serosal lymphangitis

QUESTION 9

In which of the following organs have MAP associated granulomas not been reported?

A. Liver
B. Hepatic lymph nodes
C. Tongue
D. Kidney
E. Lung

QUESTION 10

Which of the following laboratory tests are used to distinguish between the cattle and sheep strains of MAP?

A. High throughput Johnes disease faecal PCR
B. Conventional IS900 PCR + reverse enzyme assay
C. IS1311 PCR + reverse enzyme assay
D. MAP Culture
E. Elisa serology

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