

## Review Article

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## A Mini Review on Diagnosis of Johne's disease in Livestock

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### ABSTRACT

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Paratuberculosis (Johne's disease) is a chronic disease affecting livestock especially ruminants. The disease is caused by *Mycobacterium avium subspecies paratuberculosis* (MAP) an acid fast bacilli. The disease is characterized by emaciation, progressive weight loss and chronic enteritis unresponsive to treatment. This paper describes a mini review on diagnosis of Johne's disease.

### Definition

Paratuberculosis (Johne's disease) is widely distributed throughout the world and is caused by *Mycobacterium avium subspecies paratuberculosis* (MAP) (Baumgartner and Johannes, 2008). The disease mainly affects domestic and wild ruminants (Chiodini *et al.*, 1984) but MAP can also be found in numerous monogastric animals (Klee, 2002). Johne's disease (JD) characterized by emaciation, progressive weight loss and chronic enteritis unresponsive to treatment (Moges *et al.*, 2016).

### Agent

*Mycobacterium avium subspecies paratuberculosis* (MAP) is a gram positive, slow growing acid fast bacillus (Ayele *et al.*, 2001). Strains of this microbe are I (sheep),

II (Cattle) and III (intermittent) (Gwozdz, 2010). It has wide host range and able to survive outside the host for longtime (more than 9 months) and therefore it seems more insidious than any other bacteria to animal health (Begg and Whittington, 2010). Ovine and caprine paratuberculosis involves chronic inflammatory lesions of the intestinal and lymphoid organs caused mostly by one or other of the 'S'(Sheep), 'C'(Cattle) or 'Bison type' strains of MAP (Windsor, 2014). The MAP may survive high temperature short time pasteurization (Manning and Collins, 2001).

### Host

*Mycobacterium avium subspecies paratuberculosis* (MAP) affects domesticated

and wild ruminants including cattle, sheep, goats, ilamas, alpaca, camels, moose, elk, bighorn sheep, buffalo and deer (Moges *et al.*, 2016). Cattle, buffaloes, sheep and goat are the most infected species of domestic animals with JD (Rosseels and Huygen, 2008). In wild animals almost all ruminants get infected with JD including giraffe, deer and wild goats (De lisle and Collins, 1995). Camels are also prone to JD infection (Al-Ghamdi, 2013). In addition, MAP has been isolated from many non-ruminant species including rabbits, cats, foxes, weasel, badgers, bears, raccoons, woodmice and Norway rats. It also has been found in jackdaw, rook and crows (Sweeney, 2011). Horses and dogs can be infected experimentally (Van Leeuwen *et al.*, 2006).

### **Transmission**

The MAP bacterium is excreted with faeces, the most important source of infection, but is as well shedded in milk and ejaculate (Chiodini *et al.*, 1984). The transmission mainly takes place in calves via faecal-oral route by contamination of feed and udder, or by intake of infectious milk and colostrum. The MAP excreted in great numbers in the faeces and affected animals shed organisms in faeces and milk (Hines *et al.*, 2007). Soil, feed (pasture and hay) and drinking water and anything that is placed in contact with infected excrements can become a secondary source of contamination (Whitlock *et al.*, 2000). Contaminated food, water, vehicles and other equipment may be a source of infection and responsible factors for MAP transmission from one flock to another flock (Ayele *et al.*, 2001). More recent studies have shown that transplacental infection occurs in sheep and goats (Lambeth *et al.*, 2004).

### **Clinical signs**

Hemalatha *et al.*, (2013) reported that clinical signs of JD in sheep were diarrhoea, enteritis, pneumonia, debility and Stage III is clinical disease. Stage IV is

advanced clinical disease. For every clinical case, there may be 10-15 sub clinically infected sheep in the flock (Dieguez, 2008). This phenomenon is referred to as “Johne’s iceberg”. The number of observed cases is just the tip compared to the number of subclinical (or incubating) animals in the flock (Singh *et al.*, 2007).

### **Diagnosis**

A wide array of procedures and laboratory tests ranging from conventional methods like skin sensitivity test, Ziehl-Neelsen (ZN) staining and histopathological analysis of terminal ileum and mesenteric lymph nodes (Buergelt and Ginn, 2000) have been employed for its diagnosis. Diagnosis is based on clinical signs, postmortem lesions, and laboratory confirmation characteristics including direct test e.g. fecal smears, polymerase chain reaction (PCR) and indirect tests e.g., delayed-type hypersensitivity (DTH), interferon Assay, Enzyme linked immune-sorbent assay (ELISA), Agar gel immunodiffusion (AGID) and complement fixation test (CFT) (Munir *et al.*, 2014).

### **Clinical samples**

Clinical samples like faeces, milk and blood were collected aseptically in a sterile manner from an individually clinically suspected JD animal to diagnose the presence of paratuberculosis. Diagnosis best confirmed by the collection of multiple intestinal wall and mesenteric lymphnode in dead animals (OIE, 2004).

### **Postmortem diagnosis**

The primary macroscopic lesions of JD in ruminants are usually confined to the ileum caecum, colon and draining lymphnodes. The lesions are thickening of the intestinal wall, lymphatic vessels on serosal surface and

along mesentery to enlarged lymphnodes, pale and oedematous, hypertrophy of lower small intestine, thick mucosal folds (“like convulsions of cerebral cortex”) and folds erased by stretching (Sweeny, 2013).

### **Laboratory Diagnosis**

#### **Ziehl-Neelsen (ZN) /acid fast staining of faecal smears**

Sohal *et al.*, (2007) reported that staining of fecal samples for acid-fast bacilli (AFB) may reveal mycobacterial bacilli but the sensitivity of this method is low. Singh *et al.*, (2011) found that screening of 153 fecal samples by ZN staining detected 15 (57.6%) sheep and 17 (68%) goats were positive for acid fast bacilli. Vinodhkumar and Gunaseelan (2012a) reported that the ZN staining can effectively used for surveillance of JD in clinically suspected animals and when animals were in diarrheic phase. Vinodhkumar and Gunaseelan (2012b) reported that 56 sheep were positive by absorbed ZN staining technique. Geetha *et al.*, (2014) reported that sensitivity and specificity of Z-N staining technique in identification of MAP infection compared with PCR were 5.8 and 100 percent respectively.

### **Culture**

Culture traditionally has been used as the definitive diagnostic for JD (Stevenson, 2008). It is used for fecal samples and tissues acquired postmortem but is unsuitable for milk or blood samples for which it has poor sensitivity and specificity (Irrenge *et al.*, 2009). Most diagnostic laboratories employing culture as an automated liquid culture system such as BACTEC 460 radiometric system, BACTEC MGIT 960 fluorescence detection system or the TREK ESP pressure detection system (Stevenson, 2008). These systems have the advantage over

solid medium culture in that they reduce the incubation required to detect growth from 12-20 weeks to 8-10 weeks for Type II (cattle type) strains. Cultures grown using these systems also have to be tested using a MAP-specific PCR to confirm that they are MAP (Whittington, 2009).

### **Molecular diagnosis**

#### **Polymerase chain reaction (PCR)**

The insertion element IS 900 has been routinely used to detect the presence of MAP as it was shown to be present only in MAP (Collins *et al.*, 1989). The PCR has been investigated as a diagnostic method for JD since the identification of the MAP-specific insertion sequence IS 900 (Green *et al.*, 1989). The IS 900 has a unique nucleotide sequence which can be specifically detected by hybridization or PCR techniques. Sequences of IS 900 proved to be highly sensitive and specific markers of MAP among other slowly growing, acid fast bacteria, because IS 900 has been detected in field isolates of MAP from several hosts but never in other bacterial species. The MAP strains harbor multiple copies of IS900 at several sites of their chromosomes (Millar *et al.*, 1995). He reported that when primer p90 and p91 for IS 900 were used for amplification of MAP DNA, they specifically recognize a 413 bp sequence of MAP. Vansick *et al.*, (2004) used different sets of primer for efficient detection of paratuberculosis. Mobius *et al.*, (2006) reported IS 900 remains a favourite target sequence for amplification of MAP specific loci.

#### **Detection of humoral immune response**

The commonly used immunological tests to detect humoral immune response to MAP infection are CFT, AGID and ELISA (Collins *et al.*, 2006).

### **Enzyme linked immunosorbent assay (ELISA)**

Clarke (1996) reported that variable sensitivity of ELISA detection of in multi-bacillary and pauci-bacillary condition of JD. Perez *et al.*, (1996) reported that vowing to high specificity of serological tests in sheep, they could be used for the screening and control of JD in sheep flocks. The ELISA like PCR had a lower sensitivity compared to tissue culture and was not over sensitive (Kumar, 2002).

Singh *et al.*, (2007) reported that the test sensitivity varies and depends on the stages of disease represented within the herd or flock and the antigens used in the serologic test. The sensitivity of ELISA has been reported to be 87-100 percent for sheep showing clinical signs. The ELISA is considered as a standard procedure of detecting antibodies for MAP cases (Gupta *et al.*, 2012). The sensitivity of ELISA in serum of animals is 7% in the silent stage, 15% in the subclinical stage and 85% to 98% in the clinical stage (Gilardoni *et al.*, 2012). The sensitivity of ELISA in MAP cases it increases with disease progression (Donat *et al.*, 2014).

### **Detection of cell-mediated immunity**

The cell-mediated immune (CMI) response is considered to be a key determinant of immunity to all mycobacterial infections with the humoral response giving little or no protection (Chiodini and Herman, 1996). He reported that the CMI response occurred early in infection and therefore tests to detect CMI had the potential to detect infected animals before shedding of MAP occurs.

### **Delayed type hypersensitivity (DTH)**

Delayed type hypersensitivity is measured by injecting intradermal antigens (Johnin or

Avian Purified Protein Derivative) in skin to detect cell mediated immunity. Reaction is allowed to occur and after 72 hours and increased in skin thickness of more than 4 mm will give the indication of positive result (Manning and Collins, 2001).

### **Gamma – interferon assay (IFN-Y)**

Buffy coat (leukocytes) is collected from heparinized blood and exposed to antigen to measure CMI by the release of gamma interferon (Manning and Collins, 2001). This method is successfully used for detection of cytokines for the indication of CMI to check exposure of animal to MAP (Nielsen and Toft, 2008). This test shows a low sensitivity when used to detect infection in a herd with mixed infection of tuberculosis and paratuberculosis (Alvarez *et al.*, 2009). The advantage of the IFN-Y test is the significant secretion of IFN-Y occurs during the early stages and may be used to detect animals in the subclinical stage (Stabel and whittlock, 2001).

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