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ISSN: 2594-3146

# Journal of Agriculture and Forestry University

Volume 2 2018

**Agriculture and Forestry University**
Rampur, Chitwan

ISSN: 2594-3146

# Journal of Agriculture and Forestry University

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| | |
|---|---|
| Frequency of Publication | Annual |
| Editorial Policy | A medium of publishing original scientific papers |
| Official Language | English |
| ISSN | 2594-3146 |
| Subject of Interest | Plant Science, Animal Science, Veterinary Science, Forestry, and Social Science |

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**Correct citation**: Authors detail with surname of first author, first name, followed by first name and surname of other authors in sequence (2018). Title of the article, Journal of AFU (Volume 2): pages, Agriculture and Forestry University, Chitwan, Nepal.

**Agriculture and Forestry University**
**Rampur, Chitwan, Nepal**

**Journal of Agriculture and Forestry University (JAFU)**

**Volume 2** **2018**

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**Review Article**

# CURRENT DIAGNOSTIC TECHNIQUES OF *Mycobacterium avium subsp. paratuberculosis* IN DOMESTIC RUMINANTS

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## ABSTRACT

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes Johne's disease (JD), a chronic wasting disease in cattle with important welfare, economic and potential public health implications. Current laboratory tests are unable to recognize all stages of the disease, which makes it difficult to diagnose and control the disease. Methods such as fecal smear, acid-fast stain, bacterial culture and polymerase chain reaction (PCR) tests are used as direct tests while detection of host response that includes clinical signs in combination with gross and microscopic pathology and immunologic markers of infection that include antibody response to *MAP* (serology), delayed-type hypersensitivity (DTH) reaction, lymphocyte proliferation, and increased cytokine (IFN-γ) production- are the indirect ways to diagnose MAP infection. For effective control, early and confirmatory diagnosis is quite important. Despite considerable research effort, all methods are fraught with difficulties that have impeded the control and eradication of paratuberculosis. This article briefly reviews the recent diagnostic tests available for diagnosis of MAP which can facilitate clinician's ability to apply the results and evaluate the test sensitivity and specificity to compare the accuracy of different tests in detecting MAP.

**Key words:** Paratuberculosis, molecular, culture, ELISA, PCR, MAP

## INTRODUCTION

The MAP (*M. paratuberculosis*) causes paratuberculosis or Johne's disease, an intestinal granulomatous infection, which is an organism first observed by Johne & Frothingham in 1895 (OIE, 2008). First recognized in cattle, then in sheep and later in goats, paratuberculosis is found most often among domestic and wild ruminants and has a global distribution. The disease has also been reported in horses, pigs, deer and alpaca, and recently in rabbits, stoat, fox and weasel (Beard et al., 1999; Greig et al., 1999). The major effects of the infection on the animal level can be reduced milk yield (Kudahl et al, 2004), premature culling and reduced slaughter value (Benedictus et al., 1987), and losses due to continued spread of the infection (Kudahl et al., 2007). The MAP is responsible for huge losses in animal production (Ott et al., 1999) and has significant impact on food safety as well as association with Crohn's disease (Singh et al., 2008) in humans. The organism appears to survive pasteurization and could enter the human food chain through dairy products, meat and untreated water supplies (Grant, 2005). Overall, economic losses from this disease has been reported as 12 – 15% (Manning & Collins, 2001). Due to overwhelming expenses of diagnosis and treatment, economic impact of Johne's disease is major due to early culling of infected animals, reduced milk yield and wasting (Over et al., 2011). Thus, early detection of MAP is prime factor for effective control.

The MAP is usually resistant to chemotherapeutic agents in vitro and treatment of infected animals has not been successful (Maroudam et al., 2015). The OIE recommended kits (Table 1) on MAP control are "test and culling" of the MAP positive animals.

Incubation period of the disease is long and variable before manifestation of clinical signs (Salgado et al., 2005). Prevalence of the infection vary world-wide (Kennedy & Benedictus, 2001), but most notably the apparent prevalence vary by the test and test strategies used in the prevalence studies conducted (Nielsen & Toft, 2008). Reduction of contact with manure is important because MAP is most frequently transmitted by the fecal-oral route (Tiwari et al., 2006). In infected herds, calves are likely exposed to manure from adult cattle that shed the bacteria in their feces and to contaminated water, feed, or milk (Mortier et al., 2015). Control of the infection can be obtained via timely detection and culling of infectious animals and reduction of transmission from these animals (Nielsen & Toft, 2008).

For effective control, early and confirmatory diagnosis is highly important. A range of diagnostic techniques are used to detect infection with MAP in cattle, but their performance can vary widely depending on the stage of MAP infection (Nielsen & Toft, 2006; Whitlock et al., 2000).

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**Table 1. The nutshell of all important diagnostic techniques (OIE, 2008)**

| Diagnostic Test | Advantage | Sensitivity | Specificity | Limitation |
|---|---|---|---|---|
| Organism detection (Fecal Culture) | Slow and expensive | 50% | 95% | Low sensitivity |
| Agar Gel Immuno diffusion (AGID) | Simple, fast and inexpensive | 18.9-95% | - | Low sensitivity in early stages |
| Antibody Detection (serum ELISA) | Rapid and economical | 15-85% | 97-100% | Can be detected only in the later stages of disease by the time the entire environment could have been contaminated |
| Antibody Detection (milk ELISA) | Rapid and economical | 21-64% | 80-99% | |
| Polymerase Chain Reaction (PCR) | Rapid and high specificity | 29-60% | 97-99.3% | Expensive and limited applicability in prevalence studies |
| IFN-γ assay | Rapid and sensitive, early diagnosis | - | - | Low specificity |

**Table 2. Different stages of Paratuberculosis (Stevenson, 2010)**

| Features | I: Silent infection | II: Inapparent carrier | III: Clinical disease | IV: Advanced clinical disease |
|---|---|---|---|---|
| Replication of MAP | Slow proliferation in jejunal and ileal mucosa and spread to regional lymph nodes | Continued replication in infected tissues | Infection becoming disseminated. MAP present in extra intestinal sites | Widespread proliferation and replication of MAP |
| Shedding | Intermittent shedding of the organism at low levels in feces | Most animals shed the organism in feces and possibly in milk | Shed increasing numbers of MAP in feces and milk | Shedding large numbers of MAP in feces and milk - >1000 cfu/g feces = super shedders |
| CMI response | Th1 CMI responses initiated to control infection | Increasing CMI response. Gradual switch from Th1 to Th2 | May be detectable | Possibly energy |
| Humoral immune response | None | Increasing antibody response IgG2, IgG1 | Predominantly strong antibody response | Predominantly strong antibody response |
| Clinical signs | None | None | Gradual weight loss and diarrhea | Emaciation, profuse diarrhea, bottle jaw, cachexia |
| Histopatho-logical changes | None detected | Detectable granulomas if multiple tissues examined | Abundance of lymphocytes, epithelioid macrophages and giant cells in infected tissues, blunted villi | Abundance of lymphocytes, epithelioid macrophages |

A common misconception is that no accurate diagnostic tests exist for paratuberculosis, but today, there are available a great diversity of accurate and affordable tests for paratuberculosis, than for most other ruminant infectious diseases, including brucellosis and tuberculosis (Collins, 2011). There are several suitable diagnostic tests for virtually every paratuberculosis testing need and the present-day challenge for practitioners is to select the appropriate test for the intended purpose.

It is important to define the disease stage that is being targeted for each test method, but it can be difficult to extract these data from test evaluation studies (Britton et al., 2016). Diagnostic tests will generally tend to perform better in individual animals in the later stages of the disease. This may not be true for immunological tests, where anergy of either cell-mediated or antibody responses to MAP has been noted in animals with a heavy bacterial burden. At the herd level, tests will tend to perform better as the proportion of individuals in more advanced stages of disease increases (Rideout, 2003).

This article briefly reviews the recent diagnostic tests available for diagnosis of paratuberculosis, methodologic features critical to performance of each test are discussed to facilitate clinician's ability to apply the results and evaluate the test sensitivity and specificity to compare the accuracy of different tests in detecting paratuberculosis.

***Mycobacterium avium* subsp. *paratuberculosis* diagnosis methods**

According to Rideout (2003), the tests for JD can be divided into two categories:

- those that detect the organism and
- those that assess the host response to infection.

The first category includes fecal smear and acid-fast stain, culture, and polymerase chain reaction (PCR) tests. There are no tests of metabolic products or unique antigens of MAP. The second category, detection of host response, includes clinical signs in combination with gross and microscopic pathology and immunologic markers of infection, which include antibody response to MAP (serology), delayed-type hypersensitivity reaction, lymphocyte proliferation, and increased cytokine (IFN-γ) production. The table given below illustrate the summary of current and emerging ante-mortem MAP diagnostic tests (Table 3). Most of the development and evaluation of diagnostic tests has occurred in domesticated cattle and sheep. Despite considerable research effort, all methods are fraught with difficulties that have impeded the control and eradication of JD (Rideout, 2003).

**Table 3. Summary of current and emerging ante-mortem MAP diagnostic tests (Whitlock et al., 2000)**

| Name of tests | | Principle of detection | | Sample type |
|---|---|---|---|---|
| | | Direct[a] | Indirect[b] | |
| Current tests | Culture | + | | Feces/tissue |
| | PCR | + | | Cultured colony/feces/tissue/milk |
| | AGID | + | | Blood |
| | Antibody ELISA | | + | Blood/milk |
| | IFN-γ assay | | + | Blood |
| Emerging tests | Phage assay | + | | Feces/blood/milk |
| | Antibody ELISA using novel antigens | | + | Blood/milk |
| | IFN-γ assay using novel antigens | | + | Blood |
| | Specific protein assay | | + | Feces/blood |
| | Transcriptomic analysis | | + | Blood |
| | miRNA analysis | | + | Blood/tissue |
| | Microbiome analysis | | + | Feces |

[a] detection of the organism; [b] detection of the host immune response

The diagnostic tests have different sensitivities and specificities, and consequently different pros and cons in different stages and for different purposed, as summarized in table 4. Indirect ELISAs and culture have been used in practice for decades, and therefore, significant experiences and numerous evaluations have included these tests. Newer tests like PCR and IFN-γ have provided less and still insufficient data to provide consistent evidence of their potential use, although PCR can be used more, and soon provide sufficient data to allow better interpretation in practice.

**Table 4. Summary of sensitivity (Se) and specificity (Sp) of the most common diagnostics used for different purposes associated with MAP control (Nielsen, 2014)**

| | Purpose of testing – detection of: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MAP infected | | MAP infectious[1] | | Affected by MAP[2] | | MAP excretion in milk | |
| | Se | Sp | Se | Sp | Se | Sp | Se | Sp |
| Culture | + | ++ | +++ | ++ | +++ | +++ | + | - |
| PCR | + | + | +++ | + | +++ | +++ | + | - |
| IgG ELISA | + | +++ | +++ | ++ | +++ | +++ | + | - |
| IFN-γ | + | + | + | + | ? | ? | ? | ? |

[1]Presumes that testing is done just prior to the animal becoming infectious.
[2]Combined with clinical sign e.g. low/reduced milk yield; loss of body condition; wastings and diarrhea.

## Clinical signs, Bacterioscopy and pathological examination

MAP infection in cattle causes a chronic wasting disease characterized by a progressive loss of body condition and watery diarrhea (Clarke, 1997). Clinical signs usually occur >24 months of age (Sweeney, 2011), but only 10 - 15% of infected cattle developed clinical signs (Olsen et al., 2002).

Gross pathological changes in ruminants with Johne's disease include thickening and corrugation of the intestinal wall, dilatation of lymphatic vessels and enlargement of mesenteric lymph nodes (Buergelt et al., 1978). Granulomatous inflammation evident on histopathological examination is found primarily in the ileum and draining lymph nodes, but may involve the large intestine and other sites (Brady et al., 2008). Histological sections of the ileum and associated lymph nodes should be stained with Haematoxylin and Eosin (H&E) and by the Ziehl-Neelsen (ZN) method.

As histopathological examination is usually a post-mortem procedure, it is possible to obtain samples by biopsies. It is worth to mention that early pathological changes can be difficult to identify in young animals. 'Tuberculoid' lesions in sub-clinical infection are associated with scant acid-fast bacilli (Tanaka et al., 2005), whereas 'lepromatous' lesions in cattle with clinical disease have abundant acid-fast bacilli (Lee, Stabel, & Kehrli, 2001) and are correlated with a rise in serum antibodies (Olsen et al., 2002; Sigurardóttir et al., 2004).

## Bacterial Culture of MAP – Solid media and Liquid media

The identification of viable MAP by bacterial culture is considered the reference diagnostic test (i.e., the gold standard). Feces, colostrum, milk or intestinal mucosal scrapings can be used as samples (OIE, 2008). MAP is a particularly slow growing organism, particularly requires mycobactin for growth and may take months (Olsen et al., 2002). Non-automated methods of culture use solid nutrient media, such as Herrold's Egg Yolk or Löwenstein–Jensen medium (Pearson, 2014). Automated culture methods use liquid media, which generate a non-specific signal in response to microbial growth (Pozzato et al., 2011; Trefz et al., 2013). An example of this is the radiometric BACTEC 460 system (Becton Dickinson Inc.), which contains a precursor radiolabeled with C14 that detects changes in $CO_2$ concentration due to bacterial respiration. Other automated systems use fluorometric, barometric and colorimetric methods (Stich et al., 2004). Cattle (C) and sheep (S) strains of MAP have different optimal culture requirements (De Juan et al., 2006) and culture of the S strain is particularly challenging (Whittington et al., 2001); the two strains can be distinguished by molecular analysis (Stevenson et al., 2009). The sensitivity of fecal culture is ~70% in clinically affected cattle, but only 23–29% in sub-clinically affected cattle, which may shed MAP intermittently and in lower numbers (Nielsen & Toft, 2008). Isolation from gastrointestinal tissue samples is more sensitive than fecal culture because it can detect infected but non-shedding cattle (among 994 slaughtered dairy cattle, 16.1% were positive by culture

from the ileum and ileal lymph nodes, whereas 3.6% were positive on fecal culture) (McKenna et al., 2005). At the clinical stage, the microbiological culture is good, sensitive and specific diagnostic method since the animals are usually large fecal shedders of MAP (Gilardoni et al., 2012). Detection of MAP by culture is almost 100% specific, but may yield false positive results due to the 'pass-through' effect in uninfected cattle that ingest MAP from the environment (Whittington et al., 2001).

The advantages of bacterial culture are the accurate diagnosis by isolation of MAP and its quantification as colony forming unit per ml (CFU/ml), which allows classifying the animals according to their level of excretion, a useful way of establishing a program for removal of infected animals from the herd. The disadvantages are the high cost and the long incubation period that causes an epidemiologically dangerous delay in taking measures (Gilardoni et al., 2012).

## Molecular detection by Polymerase chain reaction (PCR)

The characterization of the IS900 insertion sequence (Collins et al., 1989) which has 1,451 base pairs and is present with 15 to 20 copies in the MAP genome, has enabled the specific identification of minimum amounts of bacterial DNA by the polymerase chain reaction (PCR) technique (Motiwala et al., 2006). The polymerase chain reaction (PCR) offers a rapid, cost-effective, high-throughput method of assessing MAP status in a range of samples, including cultures, feces, tissue and milk (Clark et al., 2008; Pozzato et al., 2011). PCR can be tailored to detect different genetic targets, such as insertion sequence (IS) *IS900* or *IS1311* (Marsh et al., 1999). PCRs for *IS900* are commonly used to screen samples for MAP because it is a sensitive target, but other mycobacteria can harbour *IS900*-like sequences and the assay may not be 100% specific (Cook & Britt, 2007). PCR may be more sensitive than fecal culture, but this is difficult to ascertain because culture is the current gold standard test (Clark et al., 2008). Using Bayesian analysis, the sensitivity and specificity of direct PCRs on fecal samples were estimated to be 29% and 99.3% (Wells et al., 2006) and 60% and 97% (Alinovi et al., 2009), respectively.

The advantage of PCR is the timely detection of MAP, without the need of viable bacteria in the sample. Multiplex PCR provides information from several loci in a single reaction. The advantage of RT-PCR is that it allows the immediate observation of the target amplification, quantification and has greater sensitivity than bacterial culture (Bögli-Stuber et al., 2005). The use of *IS900* in this type of PCR is sensitive to detect very low numbers of MAP, but inadequate for accurate quantification of CFU in the sample, since it is present in many copies within the bacterial genome.

The disadvantage of PCRs is their high cost. The possibility of false positive results (by contamination during the development of the technique) and/or of false negatives (by possible inhibitory components on the Taq polymerase), required control by use of appropriate internal negative and positive controls within each batch of samples. All different types of PCR previously described show risks of contamination (Möbius et al., 2008; Moravkova et al., 2008).

Although PCR is suitable for confirming clinical cases of Johne's disease, it is currently considered to have limited applicability in prevalence studies and eradication programs (Pearson, 2014).

## Detection of the host immune response (Indirect Diagnosis)

Indirect diagnosis can be made by assessing the animal's immune response, which depends on the stage of disease. Subclinical stages are typically characterized by high cellular immune response, clinical stages by a humoral immune response (Stabel, 2000) and advanced stages by anergy, where diagnostic tests of cellular immunity become negative and serological tests are less reliable. The ELISA is, at present, the most sensitive and specific test for serum antibodies to MAP, and several absorbed ELISA kits are commercially available (OIE, 2008).

### Cellular Immune response

The first immune response after MAP entrance is mediated by cells, specifically T lymphocytes. The diagnostic tests that evaluate this response are the intradermal reaction (*in vivo*) and the detection of gamma interferon production (*in vitro*). The tests that evaluate the cellular immune response allow to detect subclinical infected animals much earlier than serological tests or the bacterial culture, whereas cellular immune response has no value in clinical phase of PTB. The discriminatory power of both tests is low due to their cross-reaction with other environmental mycobacteria (Gilardoni et al., 2012).

**a. *In vivo*: intra-dermal reaction (IDR)**

As the animal first come into contacts with MAP, it develops a type IV delayed hypersensitivity which can be detected by IDR. IDR allows to identify cattle carrying MAP, without interfering with the controls of health prophylaxis and eradication of tuberculosis. IDR has an estimated sensitivity of 54 %, a specificity of 79 % (Kalis et al., 2003), low positive predictive power (22%), and good negative discriminatory power (95%) (Gilardoni et al., 2012).

The advantages are that it is easy to perform in the field, and that there is a chance of early detection of infected animals, since cellular immunity is developed before bacilli excretion and development of the humoral immune response, allowing the detection of infected animals in subclinical stages much earlier than the serological tests or bacterial culture (Gilardoni et al., 2012).

The disadvantages are its low sensitivity and its low specificity (due to probable cross-reactions). Since the positive reactions indicate sensitization of the animals to MAP or to the *M. avium* complex, this technique should be used only as a preliminary test, before the initiation of control programs (OIE, 2008).

**b. *In vitro*: detection of interferon gamma (IFN-γ Assay)**

Interferon (IFN)-γ assay can be used to detect a cell mediated immune (CMI) response to MAP infection (Jungersen et al., 2002; Kalis et al., 2003). This test evaluates the specific production of cytokine IFN-γ by T lymphocytes after stimulation with PPD. An increase in IFN-γ levels may occur prior to the onset of fecal shedding in cattle infected with MAP (Huda et al., 2003; Jungersen et al., 2002). However, the IFN-γ response diminishes as the disease progresses and positive results do not correlate with fecal shedding of MAP. Quantitative detection of IFN-γ can be used in animals aged 1 to 2 years old (Speer et al., 2006). In a Danish study, the mean age for IFN-γ positivity in infected cattle was 26 months, whereas the mean age for fecal culture positivity was 37 months (Huda et al., 2003). In animals in the subclinical stage, the sensitivity of this test is higher than that of the serological tests, but low in absolute terms (41 %) (J. R. Stabel, 1996). It can even decrease to about 20 % in herds with mixed infections (tuberculosis and PTB), these differences could be due to the host species or the strains present in each herd (Álvarez et al., 2009). Walravens et al., (2002) concluded that this test does not allow an accurate diagnosis in the first six months post-infection.

The reported sensitivities and specificities of the IFN-γ test in detecting MAP infected cattle vary widely (Nielsen & Toft, 2008) and can be influenced by age, exposure to environmental mycobacteria, assay interpretation criteria and the batch of purified protein derivative (PPD) used as the antigenic stimulant (Huda et al., 2003; Jungersen et al., 2002; Kalis et al., 2003).

The advantage of the IFN-γ test is the significant secretion of IFN-γ during the early stages of PTB and may thus be an attractive tool to detect animals in the sub-clinical stage. However, it has several disadvantages: i) the possible cross-reactions, ii) the need to process the sample quickly since cells must be alive (Stabel, 1996), iii) its high cost and iv) its low sensitivity. For all these reasons, this test is not widely used, although it can be used in control programs in order to reduce transmission to adult animals and to identify infected animals before they develop the disease (Stabel, 1996). Thus, the test can be used as screening test.

**Humoral Immune response**

In the later stages of the disease and especially with lepromatous lesions show high concentration of antibodies specific to MAP, which can be detected by complement fixation (CF), agar gel immunodiffusion (AGID), and enzyme-linked immunosorbent assay (ELISA). The last two techniques are fast, inexpensive, easy to implement and do not require much equipment. In addition, ELISA may be automated. In contrast, CF is difficult to perform and is carried out only by reference laboratories. In cattle, ELISA is more sensitive than CF and AGID.

In general, the tests to assess the humoral immune response have the disadvantage of being variable in individual responses due to the stage of disease and anergy (Manning & Collins , 2001; Whittington & Sergeant, 2001). The sensitivity is high in animals in the advanced clinical stage and large fecal shedders, but irrelevant to identify animals in the subclinical stage (Nielsen, 2008). To conclude, the various diagnostic tests that evaluate the humoral immune response in the sub-clinical stage of PTB have low sensitivity and specificity due to the late appearance of antibodies (Gilardoni et al., 2012). The tests which evaluate humoral immune response are described and compared as below:

**a. Complement fixation**

This test has been widely used in the past, being adequate to identify animals with clinical signs suggestive of PTB, but not specific enough to be used in control programs. However, it is often applied in international export of cattle (OIE, 2008). The technical protocols are variable, but it is generally dilution of sera samples plus specific antigen OIE, 2008; Pearson, 2014).

**b. Agar Gel Immunodiffusion**

AGID is based on the precipitation of immune complexes formed by the antibodies of infected animals with a soluble antigen from a protoplasmic extract of MAP in a gel matrix of agar. It is a simple, fast and relatively inexpensive method, but has low sensitivity in the early stages of PTB and therefore it is considered a good diagnostic method in animals in advanced clinical stages. It can be used as a rapid confirmatory test of suspected cases. The sensitivity is good in advanced clinical PTB (90 % - 95 %), but low in subclinical stages (30 % - 18.9 %) (Ayele et al., 2001). This test is getting less popularity now a day.

**c. Enzyme-linked immunosorbent assay (ELISA)**

ELISA is the diagnostic test most commonly used for the serological diagnosis of PTB (Gilardoni et al., 2012) to detect antiMAP antibodies can be applied to serum samples (Clark et al., 2008), individual milk samples (Nielsen et al., 2013) and bulk milk tank samples (Nielsen et al., 2000). ELISAs provide rapid results and offer a cheaper alternative to fecal culture (Roussel et al., 2007). However, cattle in the first stage of infection can be difficult to detect with antibody ELISAs (Nielsen, 2009). The appearance of an antibody response detectable by ELISA is associated with progression of disease, increased infectivity and histological lesions (Clark et al., 2008; Nielsen & Toft, 2006; Vazquez et al., 2013). The sensitivity of serum ELISAs is 50–87% in cattle with clinical signs, 24–94% in cattle with no clinical signs but shedding MAP and 7–22% in cattle infected with the organism but with no clinical signs and not shedding (Nielsen & Toft, 2008). ELISA carried out in milk can detect about 12% less positive animals than carried out in serum (Hendrick et al., 2005). The specificity of ELISAs depends upon the particular test, exposure to environmental mycobacteria, concurrent infection with *Mycobacterium bovis*, intradermal tuberculosis (TB) testing and MAP vaccination (Thomsen et al., 2012). The sensitivity of commercial ELISA kits is between 9 % and 32 % for low fecal shedders and between 47 % and 63 % for moderate fecal shedders (Whitlock et al., 2000). According to Nielsen & Toft (2008), the specificity of ELISAs is 40–100% for serum and 83–100% for milk. To conclude, the combination of ELISA and bacterial culture, in order to interpret the results in parallel (bacterial culture level and stability of ELISA) provides high sensitivity in low-prevalence herds (Nielsen, 2008).

The traditional ELISA test has several advantages, such as easy automation, repeatability, objective interpretation of the results, possibility to evaluate multiple samples together and possibility to modify the cutoff according to the sensitivity or specificity required (Gilardoni et al., 2012). It has very good sensitivity and specificity in clinical stages and is relatively inexpensive. It is a good method to assess the prevalence of PTB in the herd, although several researchers have found that the prevalence of bovine tuberculosis decreases the sensitivity and specificity of the test for PTB (OIE, 2008). The disadvantage is that the antigenic variability in different ELISA tests of serum and the different ages of the animals tested can lead to errors in sensitivity and specificity (Gilardoni et al., 2012). This test is widely used these days.

**d. Flow cytometry**

Flow cytometry allows to detect animals with subclinical infection and differentiate between MAP, *Mycobacterium scrofulaceum* and *M. avium* subsp. *avium*. Using this technique, Eda et al. (2005) detected antiMAP IgG in calves at 240 days post-experimental inoculation, without cross-reactions, and with a sensitivity of 95 % and a specificity of 97 %. This technique is rapid (less than 4 hours) and objective, but expensive and complex to execute given the kind of sophisticated equipment required.

## CONCLUSION

Current diagnostic tests, when performed on ruminants at different stages of MAP infection, vary in accuracy and it remains easier to identify ruminants with clinical signs (advance stage) than to detect subclinical infections (early stage). Current diagnostic tests also lack the sensitivity to identify infected animals at early (asymptomatic) stages of the disease. Therefore, considerable research focus has been given in developing improved diagnostic assays, to diagnose MAP in early stage of infection.

Due to the immunological complexity and the prolonged subclinical period of the disease, it is difficult to determine only one reference diagnostic test, especially if a diagnostic test with high sensitivity and specificity is expected. The limitations of each diagnostic test determine the use of two or three of them, repeated in time in the same animal to establish the stage of the disease both in the animal and the herd. For this reason, and to prevent PTB transmission, detection of infected animals in the silent or sub-clinical periods is important.

The diagnostic procedure of MAP is complex and there is no such single diagnostic tool available with a good accuracy, sensitivity and specificity. Thus, More focus is required to understand the characteristics of this organism, pathogenesis and to develop molecular diagnostic techniques. Due to limiting factors of current diagnostics, selection of multiple laboratory tests to diagnose the disease can be of confirmatory approach.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge DOREX, AFU for support, and are also thankful to the lab crew of department of veterinary medicine and public health, for their help and support during literature review.

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