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Mycobacterium avium subsp. paratuberculosis in Colombia (1924-2016): A review¤

***Mycobacterium avium* subsp. paratuberculosis en Colombia (1924-2016): Revisión de literatura**

***Mycobacterium avium* subsp. paratuberculosis na Colômbia (1924-2016): Revisão de literatura**

Nathalia Correa-Valencia, MV, MSc, DrSc(c); Yadi Marcela García-Tamayo, MV; Jorge A Fernández-Silva*, MV, MSP, Dr. med. vet.

Línea de Investigación en Epidemiología y Salud Pública Veterinaria, Grupo de Investigación CENTAURO, Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia.

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Abstract

Mycobacterium avium subsp. *paratuberculosis* (MAP) is an acid-fast, gram-positive bacillus. MAP is the causal agent of paratuberculosis (PTB) or Johne's disease, an infectious disease affecting domestic ruminants and some wild species. Its importance as a potentially zoonotic agent due to its relation to Crohn's disease (CD) in humans is still under debate and investigation. The aim of the present systematic review is to summarize original studies on MAP carried out in Colombia since 1924, as well as to establish strengths, weaknesses, and future research opportunities in the country with emphasis on diagnosis and epidemiology. The initial search for existing publications reporting original studies on MAP, PTB, and the relationship between MAP and CD was carried out in the available databases and national libraries. After compilation of the available studies (n = 20), the relevant data was extracted (year, province of report, species studied, diagnostic tests used, study design, summary of results, and authors). Recommendations for future research opportunities on MAP in Colombia are made.

Keywords: *Buffalo, cattle, epidemiology, goats, Johne's disease, sheep.*

Resumen

Mycobacterium avium subsp. *paratuberculosis* (MAP) es un bacilo ácido resistente, gram-positivo. MAP es el agente causal de la paratuberculosis (PTB) o enfermedad de Johne, una enfermedad infecciosa que afecta rumiantes domésticos y algunas especies salvajes. Su importancia como agente zoonótico, debido a su relación con la enfermedad de Crohn (CD) en humanos, está aún en debate y bajo investigación. El objetivo

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* Corresponding author: Jorge Arturo Fernández Silva. Línea de Investigación en Epidemiología y Salud Pública Veterinaria, Grupo de Investigación CENTAURO, Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia. E-mail: jorge.fernandez@udea.edu.co

de la presente revisión es exponer los estudios originales sobre MAP llevados a cabo en Colombia desde 1924, así como establecer sus fortalezas, debilidades y oportunidades de investigación futura, con énfasis en los puntos de vista diagnóstico y epidemiológico. La búsqueda inicial de las publicaciones existentes sobre estudios originales realizados acerca de MAP, PTB y la relación MAP y CD fue realizada en las bases de datos disponibles y en bibliotecas nacionales. Luego de la compilación de los estudios disponibles (n = 20), los datos relevantes fueron extraídos (año, provincia de reporte, especie estudiada, prueba diagnóstica usada, diseño del estudio, resumen de resultados y autores). Se hacen recomendaciones para futuras investigaciónes de MAP en Colombia.

Palabras clave: *búfalos*, *cabras, enfermedad de Johne, epidemiología, ganado bovino, ovejas.*

Resumo

Mycobacterium avium subsp. *paratuberculosis* (MAP) é um ácido forte, bacilo gram-positivo. O MAP é o agente causador da paratuberculosis (PTB) ou doença de Johne, uma doença infecciosa que afecta os ruminantes domésticos e algumas espécies selvagens. Sua importância como um agente zoonótico por causa de sua relação com a doença de Crohn (CD) em humanos ainda está em discussão e sob investigação. O objetivo desta revisão é apresentar a estudos mapa original realizado na Colômbia desde 1924, e estabelecer pontos fortes, pontos fracos e oportunidades para futuras pesquisas no país com ênfase no diagnóstico e epidemiológico pontos de vista. A busca inicial da literatura sobre estudos originais sobre MAP, PTB e a relação MAP e CD foi feita nas bases de dados disponíveis e bibliotecas nacionais. Após a compilação dos estudos disponíveis (n = 20), os dados relevantes foram extraídos (ano, relatório província, espécies estudadas, teste de diagnóstico utilizado, desenho do estudo, os resultados resumo e autores). Foram feitas recomendações sobre futuras oportunidades de pesquisa em MAP em Colômbia.

Palavras-chave: *búfalo, cabras, doença de Johne, epidemiologia, gado, ovelhas.*

Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is an obligate intracellular, gram-positive, acid-fast bacterium, causing a persistent infection of host macrophages leading to a strong immune response (Harris and Barletta, 2001). This bacterium has a remarkable tropism for the intestine, a characteristic not seen in any other mycobacterial species (Clarke, 1997), and can impact a wide variety of domestic and wild species (Stief *et al.,* 2012; Sweeney *et al,* 2012; Carta *et al.,* 2013; Kukanich *et al.,* 2013) and humans (Rani *et al.,* 2010; Rosenfeld and Bressler, 2010; Cossu *et al.,* 2011; Chiodini *et al.,* 2012). Recent data from whole-genome comparison studies support the classification of MAP isolates into the two major strain types, I (Sheep type; S) and II (Cattle type; C; Alexander *et al.,* 2009). These strains show differences related to the ease of primary isolation, incubation time for primary growth on solid and liquid media, and host preference or range, among others (Stevenson, 2010a).

Paratuberculosis (PTB) or Johne's disease (JD), a slow-developing and incurable infectious animal disease (caused by MAP) is characterized by chronic granulomatous enterocolitis. This disease has a variable incubation period from 6 months to over 15 years (Clarke, 1997). The PTB is transmitted between animals by a fecal-oral route, but intra-uterine and trans-mammary pathways occur (Sweeney, 1996; Lambeth *et al.,* 2004; Whittington and Windsor, 2009). Animals from 0 to 6 months of age are thought to be most susceptible (Windsor and Whittington, 2010). Experimental infection studies have demonstrated that goats are naturally less resistant to PTB compared to sheep and cattle (Stewart *et al.,* 2007). Chronic, progressive weight loss and chronic or intermittent diarrhea are the primary clinical signs of bovine PTB (Clarke, 1997). Symptoms are vague and unspecific in goats and sheep and, like many other diseases, are only characterized by weight loss (Djønne, 2010). Diarrhea is a common clinical sign in cattle but not in small ruminants (Clarke, 1997; Begg and Whittington, 2010; Robbe-Austerman, 2011). The clinical disease is most frequent among cattle 2-5 years old, although younger and older cattle (0-13 years old) can be affected (Nielsen and Toft, 2008). In other domesticated and wild ruminants, the course of infection and clinical disease are poorly described (Djønne, 2010). In sheep,

clinical signs are limited to weight loss, which can occur from 2 years of age and animals succumb to MAP infection from 3-5 years of age (Lugton, 2004; Begg and Whittington, 2010). In goats, the clinical development of the disease is similar to that in sheep (Djønne, 2010; Manning and Collins, 2010; Robbe-Austerman, 2011). Parturition, lactation, or other stresses may provoke clinical manifestations (Clarke, 1997; Fecteau and Whitlock, 2010).

Several tests are used to diagnose MAP infection in cattle, sheep, goats, and humans. The most common are histopathology (on intestinal tissue and regional lymph nodes using Ziehl Neelsen (ZN) stain, enzyme-linked immunoassay (ELISA; in serum and milk), microbiological (in tissues, feces, and environmental samples), and the detection of MAP-DNA by polymerase chain reaction (PCR; in feces, milk, tissue, and blood). Less common tests include interferon gamma (IFN-γ) assay and intradermal Johnin test (IJT).

In all affected species, the necropsy findings are commonly restricted to the ileum and the mesenteric and ileocecal lymph nodes. In most cases, congestive and "wrinkled" surfaces of the ileum, cecum, and colon are observed (Manning and Collins, 2001; Olsen *et al.,* 2002).

The ELISA is the most widely used tests for detecting an antibody response to MAP infection. Several commercial ELISA kits for PTB diagnosis are currently available and multiple studies have compared their performance (Sonawane and Tripathi, 2013; Donat *et al.,* 2014; Nielsen and Toft, 2014; Lavers *et al.,* 2014; 2015). The main advantages of ELISAs are that they are inexpensive, rapid and easy to perform (1-2 hours) and provide quantitative results (Constanzo *et al.,* 2012). A major disadvantage of ELISA is its low sensitivity (Se) in subclinical animals (7-15%; Gilardoni *et al.,* 2012). It must be also considered that ELISA results should be interpreted given the different objectives for screening (e.g., identification of infected animals, identification of the most likely to shed mycobacteria; OIE, 2011; Nielsen *et al.,* 2002; Nielsen and Toft, 2012).

Cultivation of MAP from feces and tissues is the most reliable method of detecting infected animals (Nielsen and Toft, 2009; Fecteau and Whitlock, 2010). Usually, its Se is 30-70% and its specificity (Sp) 98% if the isolates obtained are confirmed to be MAP by molecular methods such as PCR (Whittington *et al.,* 2011; Gilardoni *et al.,* 2012). Although fecal culture (FC) has many limitations, such as a long incubation period, high costs, risk of contamination with other mycobacteria or fungi, and time required to report the results, it is still the most commonly used reference test for the detection of MAP (Whittington, 2010).

Detection of MAP genes by PCR has shown advantages (rapidity, identification of agent, lack of contamination) and disadvantages (moderate sensitivity, high cost, special equipment, and skilled personnel required (Collins, 1996). However, due to recent developments, PCR has been suggested for herd screening (Collins *et al.,* 2006; Anonymous, 2010b), and it has been recently discussed as a possible new reference test for PTB (Stevenson, 2010b). The PCR´s Se is 70-97% and its Sp 95% (Gilardoni *et al.,* 2012) and, in contrast to a culture-based diagnostic, it is rapid and no additional tests are required to confirm the identity of the organism detected (Collins, 1996).

The PPD antigens used in the *in vivo* IFN-γ test and in the IJT are crudely steam-sterilized mycobacterial culture extracts containing many cross-reacting antigens with other related bacteria (Stabel and Whitlock, 2001). Unapparent infected animals do not manifest weight loss or diarrhea but may have an altered immune response with increased IFN-γ production by T cells sensitized to specific antigens and/or increased antibody response to MAP (Nielsen, 2010; Gilardoni *et al.,* 2012). The IFN-γ (*in vivo/in vitro*) Se is around 41% and its Sp 10% (Gilardoni *et al.,* 2012).

The lack of a perfect *ante-mortem* reference test is a significant obstacle for PTB diagnostic test evaluation (McKenna *et al.,* 2006). Complicating diagnostic test evaluations is the high degree of variability among animals in their response to MAP infections, some may produce antibodies (ELISA-positive) years before consistently shedding MAP in feces (culture or PCR-positive), while others will be fecal shedders of MAP long before becoming ELISA-positive (Kalis *et al.,* 2002; McKenna *et al.,* 2006; Nielsen, 2010). Current diagnostic tests cannot,

with a single application, discriminate between MAP-infected and uninfected animals at any age with 100% accuracy, highlighting the need for improved tests (Nielsen and Toft, 2009).

Dalziel (1913) described the clinical and pathologic similarities between PTB in cattle and Crohn´s disease (CD) in humans, which are both chronic inflammatory bowel diseases. This report initiated the controversy about the etiological role of MAP in CD and implied a potential zoonotic behavior for MAP (Uzoigwe *et al.,* 2007; Sechi and Dow, 2015). In agreement with this, MAP has been detected in the tissues of CD patients (Di Sabatino *et al.,* 2011; Tuci *et al.,* 2011; Wagner *et al.,* 2013; Dalton *et al.,* 2014). The source, the route of infection, the persistence mechanisms, and the consequences of MAP infection in humans are unknown (Uzoigwe *et al.,* 2007; Lowe *et al.,* 2008). Therefore, an association between CD and PTB has been shown, but a causal relationship remains to be demonstrated (Liverani *et al.,* 2014; Sechi and Dow, 2015).

The MAP-infected animals, whether clinically normal or showing signs of disease, can shed live bacteria in both feces and milk. If these animals are farmed for food production, the safety of foods derived from them becomes important because of its impact on public health (Sweeney *et al.,* 2012; Atreya *et al.,* 2014; Liverani *et al.,* 2014).

Both MAP infections and clinical cases of JD have been reported from all continents that have ruminant populations in any degree of husbandry (Barkema *et al.,* 2010), and countries acquire the infection by animal importation. Multiple studies on the determination of the within-herd and between-herd prevalence of MAP infections around the world have been carried out (Nielsen and Toft, 2009; Salem *et al.,* 2013; Fernández-Silva *et al.,* 2014). Intensive farming systems, acid soils, low dietary intake, stress related to transport, lactation and parturition, and immunosuppression by agents such as bovine viral diarrhea virus (BVDV) are reported as risk factors worldwide (Lepper *et al.,* 1989; Johnson-Ifearulundu and Kaneene, 1998; Wells and Wagner, 2000; Dieguez *et al.,* 2008; Ansari-Lari *et al.,* 2009; Tiwari *et al.,* 2009; Pithua *et al.,* 2013; Benavides *et al*., 2016; Correa-Valencia *et al.,* 2016).

The apparent prevalence among cattle appears to be at least 0 and 24% in several European countries (Nielsen and Toft, 2009). Between-herd prevalence estimates appear to be >50% (Clarke, 1997; Nielsen and Toft, 2009). Wells and Wagner (2000) reported an apparent 3.4 and 21.6% at the animal and herd-level, respectively, using ELISA to test US dairy cattle herds in 1996. According to Manning and Collins (2010) and Nielsen and Toft (2009), over 60% of dairy cattle herds in Europe and >50% in North America are infected, respectively. According to Fernández-Silva *et al.* (2014), prevalence studies in Latin American and Caribbean countries revealed an overall prevalence of 16.9 and 75.8% in cattle at the animal and herd levels, respectively. In the same report the prevalence was 16% in sheep at the animal level, and 4.3 and 3.7% in goats at the animal and flock levels, respectively. The prevalence reported in small ruminants in several other countries is 73.7% in sheep in Italy (Attili *et al.,* 2011), 46.7% in sheep in Portugal (Coelho *et al.,* 2007), and 52% in sheep and 50% in goats in Cyprus (Liapi *et al.,* 2011). The prevalence of infection tends to increase in countries that do not have control programs (Salem *et al.,* 2013; Fernández-Silva *et al.,* 2014).

The JD causes important economic losses in infected flocks and herds (Nielsen and Toft, 2009) and produces a 6-19% decrease in the production of meat, milk, or both (Djønne, 2010; Kostoulas *et al.,* 2006; Marce *et al*., 2009). Ovine and caprine PTB causes losses related to death, early culling, and reduced milk production (Arsenault, 2001). Control of PTB in farm ruminants by testing, culling, and herd/flock management helps limiting the economic impact of the disease and are used in control programs in the USA, Australia, and Europe (Bakker, 2010; Kennedy and Citter, 2010; Whitlock, 2010; Khol and Baumgartner, 2012). The lack of a fully functional immune system renders neonatal ruminants more susceptible to MAP infection than adult animals. Thus, control programs are primarily focused on limiting opportunities for MAP contamination of colostrum, milk, water and feed by hygienic programs (Tiwari *et al.,* 2009; Whitlock, 2010; Khol and Baumgartner, 2012).

The aim of the present review was to summarize original studies and abstracts on MAP carried out in Colombia since 1924, as well as to highlight

the strengths, weaknesses, and future research opportunities for PTB research in the country with emphasis on diagnostic and epidemiology.

Materials and Methods

The review of MAP/PTB original investigations in Colombia was carried out by searching all available reports published in scientific and informative journals, as well as in theses or degree works. Searching was done using electronic databases (i.e. Scielo, Medline/Pubmed, and Virtual Health Library), national libraries, institutional repositories, and the Internet. Because the aim of the review was to summarize only original studies, publications not considered original by the three authors of this report were excluded through consensus and not further analyzed. The main characteristics (year of publication, province of report, species, diagnostic test used, study design, and results) of selected MAP original studies were extracted and analyzed.

Results

The review process produced 20 original studies and abstracts on MAP carried out in Colombia (Table 1). These studies refer to PTB and MAP detection. No studies in Colombia attempted detection of MAP in food or humans. One publication by Albornoz (1949) comparing bovine PTB with human leprosy was not available. Its significance as an original study could not be evaluated, therefore it was not considered in this review.

Thirteen publications not considered original studies were not further analyzed or discussed in this review, but they are of great value for the national knowledge base about MAP. These reviews, case reports, case series reports, and editorials demonstrate the national academic concern about MAP and its impacts in Colombia (García, 1957; Góngora and Villamil, 1999; Calderón and Góngora, 2008; Zapata *et al.,* 2008; Villalobos *et al.,* 2008; Anonymous, 2010a; de Waard, 2010; Peña *et al.,* 2011; Ramírez *et al*, 2011; Ramírez and Maldonado, 2013a; 2013b; Fernández *et al.,* 2014; Correa *et al.,* 2015).

The existence of MAP in Colombia was first documented in 1924 by the Cuban veterinarian Ildefonso Pérez Vigueras in cattle with PTB (reported by Plata, 1931 according to Vega, 1947). This documentation was the first confirmation of PTB in the country and occurred in the municipality (primary political division of provinces in Colombia) of Usme (Cundinamarca) in imported cattle.

Most studies on MAP or PTB (60%, 12/20) were carried out during the present decade (2010-2020). No more than two studies on MAP or PTB in Colombia were published in previous decades. Most studies were carried out in the provinces of Antioquia (60%; 12/2) and Cundinamarca (30%; 6/20), Caldas and Tolima (5%; 1/20), as well as in Nariño (5%; 1/20). The original studies concerning MAP in Colombia reported the results from cattle, sheep, goats, and buffaloes. Studies on cattle were the most common (80%; 16/20) compared to sheep and goats (15%; 3/20), and buffaloes (5%; 1/20). Other relevant species in the country (wild mammals or humans) were not found or cited in any original study reviewed.

The most common diagnostic test used to investigate MAP in Colombia is ELISA (36.1%; 13/36), followed by microscopy on ZN-stained samples (on feces, rectal mucosa scrapings, or tissues; 19.4%; 7/36), PCR (13.9%; 5/36), IJT (with bovine and/or avian-PPD; 11.1%; 5/36), culture (from feces or tissues, and individual or pooled; 8.3%; 2/36), CF (complement fixation; 5.6%; 2/36), IF (indirect immuno-fluorescence; 2.8%; 1/36), and CIE (counter immuno-electrophoresis; 2.8%; 1/36).

The studies reviewed included cross-sectional, diagnostic test comparisons, risk factor analyses, and clinical trials (on treatments). Thus far, no cohort or case and control studies have been published in Colombia.

Discussion

This review summarizes for the first time the original studies on MAP carried out in Colombia since 1924. In recent years, MAP presence and distribution in the country, especially in farmed animals and humans, have been reviewed (Góngora and Villamil,

Table 1. Summary of published original studies on *Mycobacterium avium* subsp. *paratuberculosis* in Colombia, 1924-2016.

Year of publication	Province of report	Species	Diagnostic test	Study design	Summary of results	Reference
1947	Cundinamarca	Bovine	IJT- avian PPD; ZN	30 animals with different ZN-fecal staining results (6 negative, 8 suspicious, and 16 positive) were inoculated with PPD. Body temperature was taken three times before PPD inoculation. Next day results were determined and temperature was measured every 2 h	6 animals negative to the ZN were also negative by IJT; 12.5% (1/8) of the animals were suspicious and 37.5% (6/16) were positive by ZN and by IJT, respectively	Vega
1954	Cundinamarca	Bovine	IJT - avian PPD; ZN	9 medical cases were reviewed looking for PTB. Administration of isonicotimilhidrazina (orally) and cortisone (intramuscular) in the treatment of AFB-related diseases was performed	AFB were confirmed in all the animals which were also negative to IJT; body temperature and weight after treatment improved in 40% (4/9) of the cases	Huber
1978	Cundinamarca	Bovine	ZN; CF; IF	2 groups of adult cattle (>2 years of age) were sampled for serum and feces. Sixty-seven Holstein and Normando clinically normal animals, and 65 animals clinically compatible with PTB were tested twice with 6 months of difference	3.51% (7/199) were positive to ZN; 2.02% (4/199) serums were positive to CF; 5.52% (11/199) serums reacted positively to IF	Isaza
1983	Cundinamarca	Ovine	CF; ZN	Blood and fecal samples were taken from 480 adult sheep	11.25% (54/480) of the serums were positive to CF; 5.62 % (27/480) of the fecal samples were positive to ZN. A necropsy was performed and PTB was confirmed	Mogollón *et al.*
1984	Cundinamarca	Bovine	ZN and HE-staining; CIE	94 older than 3 years Holstein, Normando, and cross-breed cows and bulls were sampled. The groups were designated according to presence of diarrhea compatible with PTB: females with diarrhea (n = 52) and without diarrhea (n = 18), males with diarrhea (n = 3), and without diarrhea (n = 21)	11.70% (11/94) of the animals were positive to ZN and HE tissue staining; 6.38% (6/94) of the animals were positive to ZN in rectal mucosa scrapings; 9.57% (9/94) were positive to CIE in serum samples	Góngora and Perea
1999	Caldas and Tolima	Bovine	ELISA	177 Normando animals from 3 farms were serum sampled	Seroprevalence for each farm was 3.4% (2/59), 1.7% (1/59), and, 0% (0/59)	Patiño and Estrada
2001	Antioquia	Bovine	IJT - avian and bovine PPD	3 farms from 3 municipalities were sampled: San Pedro de los Milagros (n = 77), Gómez Plata (n = 76), and Barbosa (n = 78). The study population was 176 animals over 1 year of age	11% (19/176) of animals were positive to bovine PPD, all of them from San Pedro de los Milagros, while 27.8% (49/176) were suspicious; 2% (1/49) of suspicious was positive to avian PPD, and 2% (1/49) was suspicious; four clinical animals were confirmed by necropsy	Ramírez *et al.*

Year of publication	Province of report	Species	Diagnostic test	Study design	Summary of results	Reference
2009	Cundinamarca	Ovine	ZN; IJT- bovine PPD; ELISA	250 female sheep (Black face, Cheviot, Corriedale, Hampshire, Merino rambouillet, Romney marsh, Mora, creole, and cross-breeds) aged between 1-9 years old were sampled	4% (10/250) of fecal samples were positive to ZN; animals between 2-6 years old presented AFB in fecal samples, whereas animals older than 8 years were all suspicious; 4.9% (16/250) were positive to the IJT, and 1.1% (3/250) were suspicious; 0.8% (2/250) were positive to ELISA. Animals that resulted suspicious and positive to intradermal test were confirmed by ZN in fecal samples: 62.5% (10/16) were positive to both (ZN and IJT), 18.8% (3/16) were negative to both, and other 18.8% (3/16) were positive to the IJT only	Mancipe *et al.*
2010	Antioquia	Bovine	ZN; FC; IS*900* q-PCR	15 Holstein and BON x Holstein cows in a herd enzootic for PTB were sampled. Average age of sampled cows was 6.7 years. Fecal samples were individually taken from clinical healthy cows and cows with diarrhea	56% (9/15) were positive to ZN to FC, whereas 20% (3/15) that were positive by PCR applied to positive FC	Zapata *et al.*
2011a	Antioquia	Bovine	Non-absorbed indirect ELISA (A); Pre-absorbed indirect ELISA (B); IS*900* nested-PCR; F5/ISMav2 q-PCR; FC	14 dairy herds of 9 districts were fecal and serum sampled. Only 1 herd had presented sporadic clinical cases compatible with PTB confirmed by PCR and histopathology	10% (31/315), 87% (268/315), and 2.6% (8/315) of samples were positive, negative, and doubtful, respectively, to ELISA A; 70% (10/14) of herds were considered positive when having at least one ELISA A-seropositive animal; 5.1% (2/39) positive and doubtful samples in ELISA A were also positive with ELISA B, 94% (37/39) were negative, and none was doubtful; 19% (6/31) positive animals with ELISA A were positive to nested-PCR. One positive animal to q-PCR were also positive to nested-PCR; 19 and 6.5% of the ELISA A-positive animals were positive to nested PCR and q-PCR, respectively. The FC was negative in all samples	Fernández *et al.*
2011b	Antioquia	Bovine	Pre-absorbed indirect ELISA (C); FC; F57/ ISMav2 PCR; IS*900* nested-PCR	5 herds previously tested by the authors, referring to those that resulted ELISA and PCR positive but FC negative for MAP, and one additional herd not previously tested were included in the study. The herds participated with 384 cows (>2 years of age). Serum samples (n = 329) and fecal samples (n = 386) were taken from all animals in every herd. Slurry samples of one herd (n = 3) and tissue samples (n = 2) were also taken	1.8% (6/329) results were positive to ELISA C, 97.5% (321/329) were negative, and 0.6% (2/329) was doubtful, as well as positive results in 40% (2/5) of the herds. The FC and nested and q-PCR supported that 1/36 herds was positive to culture; ELISA C results were confirmed by FC in only one symptomatic animal of one herd; eight MAP isolates were recovered	Fernández *et al.*
2013	Antioquia	Bovine	IS*900* q-PCR	48 cows with compatible signs of PTB were euthanized. Lymph nodes were cultured and DNA from macrophages was extracted	Macrophages from four cows were infected by MAP; 8.51% of the cultures were positive and confirmed by q-PCR	Del Río *et al.*

Year of publication	Province of report	Species	Diagnostic test	Study design	Summary of results	Reference
2015	Antioquia	Ovine and caprine	Pre-absorbed indirect ELISA	Blood samples from all animals in a farm in Barbosa (>2 years of age) (n = 53 goats and 6 sheep) were obtained	ELISA results were all negative	Hernández *et al.*
2015	Antioquia	Bovine	Pre-absorbed indirect ELISA	Blood samples from all animals in a farm at San Pedro de los Milagros, (>2 years of age) (n = 83) were obtained	17% (14/83) of animals were ELISA positive; statistical association between ELISA results and breed was found	Jaramillo *et al.*
2015a	Antioquia	Bovine	Pre-absorbed indirect ELISA	Blood samples from all animals in a farm in Gómez Plata, (>2 years of age) (n = 50) were obtained	4% (2/50) of the animals were ELISA positive	Tuberquia *et al.*
2015b	Antioquia	Buffalo	Pre-absorbed indirect ELISA	Blood samples from all animals in a farm in Gómez Plata, (>2 years of age) (n = 21) were obtained	ELISA results were all negative	Tuberquia *et al.*
2016	Nariño	Bovine	Pre-absorbed indirect ELISA	Blood samples were obtained from 958 cows (>2 years of age) in 16 dairy farms	94% (15/16) of the farms with at least one positive animal and 8% (77/958) of the cows were ELISA positive; statistical association between ELISA results and BCS was found	Benavides *et al.*
2016	Antioquia	Bovine	Pre-absorbed indirect ELISA	Risk factors assessment, related to seropositive results from screening 696 randomly selected bovines in 28 dairy herds located in 12 districts of San Pedro de los Milagros was done	3.6% (1/28) and 2% (14/696) of the herds and animals were ELISA positive, respectively. Days in milk between 100 and 200 days and over 200 days, and daily milk production between 20 to 40 L/cow and over 40 L/cow, were associated with MAP seropositivity with OR of 4.42, 3.45, 2.53, and 20.38, respectively	Correa *et al.*
2016	Antioquia	Bovine	Pre-absorbed indirect ELISA	Blood samples from all animals in a farm in the municipality of Caucasia (>2 years of age) (n = 151) were obtained	33.8% (51/151) of the animals were ELISA positive	Vélez *et al.*
2016	Antioquia	Bovine	Pre-absorbed indirect ELISA	Risk factors assessment was performed. Blood samples were obtained from 19-25 cows, (>2 years of age) randomly selected in 14 dairy herds located in 9 districts of Belmira and San Pedro de los Milagros	10.09% (31/307) of the animals and 70% (10/14) of the herds were ELISA positive; OR for PTB seropositivity increased 20% in cows with >1 parity; OR was 0.74 times lower in herds feeding calves with pooled colostrum from several cows, compared to herds feeding calves with colostrum from their own dams	Fernández *et al.**

IJT: Intradermal Johnin test; PPD: Purified protein derivate; ZN: Ziehl-Neelsen; PTB: Paratuberculosis; AFB: Acid fast bacteria; CF: Complement fixation; IF: Indirect immuno-fluorescence; HE: Hematoxylin and eosin; CIE: Counter immuno-electrophoresis; ELISA: Enzyme-linked immunosorbent assay; FC: Fecal culture; BON: Blanco orejinegro; PCR: Polymerase chain reaction; q-PCR: Quantitative, real-time polymerase chain reaction; ELISA (A): Svanovir Para-TB Ab ELISA Kit (Svanova Biotech AB, Uppsala, Sweden); ELISA (B): ELISA paratuberculosis antibody verification (Institut Pourquier, Montpellier, France); ELISA (C): ID Screen Paratuberculosis Indirect (IDVET, Montpellier, France); MAP: *Mycobacterium avium* subsp. *paratuberculosis*; BCS: Body condition score; OR: Odds ratios. *Refers to an analysis of the information from the study done in 2011a by Fernandez *et al.*

1999; Calderón and Góngora, 2008; Zapata *et al.,* 2008; Fernández *et al.*, 2014). However, no review of the original studies has been undertaken. According to several anecdotal reports, opinions about the national -not even regional- distribution of MAP or PTB in cattle and small ruminants are not homogeneously defined or conclusive. Some academics and producers consider MAP (especially PTB) as a significant problem, while others claim the absence or very low prevalence of MAP in farmed animals.

The number of publications reporting original studies on MAP, especially PTB, in recent years is relatively low compared to other countries in Latin America (Fernández *et al.,* 2014), but is higher than expected for Colombian conditions. This finding suggests a growing interest about MAP research in the country, as well as an increasing preoccupation about this microorganism and its negative effects on animal health, animal production, and its zoonotic potential (public health impact) from academic and productive perspectives.

Although PTB is a notifiable disease in Colombia (ICA, 2015), it is not of major concern to animal health authorities and its control is a responsibility of the farmer (Anonymous, 2010a; Fedegán, 2010; Fernández *et al.,* 2014). This could explain the low number of initiatives for the research, prevention, and control in animals, as well as for the detection of the microorganism in food, the environment, and humans. In South America, only one country-wide PTB review has been published (Yamasaki *et al.,* 2013). According to this review, 35 studies have been carried out in Brazil since its first report in 1915. These studies were carried out in cattle, sheep, goats, and buffaloes and using the same diagnostic tests that have been used in Colombia according to the present report.

The locations of most studies do not follow a clear trend, but could be related to the high concentrations of cattle in some of the provinces (i. e. Antioquia and Cundinamarca; ICA, 2016), or to the interests of academics, scientists, or cattle producers. Since the first report in 1924, Cundinamarca has been a province with common reports of PTB (Vega, 1947; Huber, 1954; Isaza, 1978; Mogollón *et al.,* 1983; Góngora and Perea, 1984; Mancipe *et al.,* 2009). This could be explained by the long tradition of the Facultad de Medicina Veterinaria of the Universidad Nacional de Colombia in Bogotá, the oldest veterinary school in the country, where the first studies in the early 20th century were carried out, most of them being degree works. More recently, Antioquia province has been publishing the majority of original studies, all of them from academics at Universidad de Antioquia and Universidad CES.

As expected, studies on cattle were the most common, most likely due to the size of the population in the country and to the production systems related to milk and meat. In contrast, studies on sheep are less common in the country probably due to its smaller population (ICA, 2016).

The common use of ELISA, ZN-staining, IJT using bovine and/or avian-PPD is not surprising given their relatively low cost and availability of materials, qualified personnel, and infrastructure for these tests (Collins, 1996). However, the use of culture and PCR is becoming more common and could be related to the recent development of diagnostic capacities in universities compared to national laboratories, and to the expansion of the reagents and equipment supplies for such diagnoses in the country.

The absence of cohort and case-control studies is common in animal health research in Colombia. These high-profile observational studies, as well as experimental approaches, are more complex, laborious, demanding and expensive, given the microbiological and pathophysiological characteristics of MAP. Nevertheless, the current MAP situation in Colombia demands additional observational studies in addition to surveys and case reports to enhance our comprehension of the epidemiological situation and to assess the true zoonotic threat.

Definitively, Colombia needs to cover some knowledge gaps to get to a true understanding of the disease. It is necessary to define the exact status of the disease through well-designed prevalence/incidence studies, considering that no whole national data is available. In this regard, just some local estimates are currently available (Patiño and Estrada, 1999; Ramírez *et al.,* 2001; Fernández *et al.,* 2011a; 2011b; Benavides *et al.,* 2016; Correa *et al.,* 2016).

Harmonization of diagnostic methods, considering the epidemiologic and biological behavior of MAP under local agro-ecological, productive, and cultural conditions are also needed. In addition, laboratory infrastructure —mainly developed for foot-and-mouth disease control— should cover other entities with relevance for public health and international trade such as PTB (Calderón and Góngora, 2008), improving their testing capacity and the access to diagnostic reagents.

It is also necessary to improve farmer training highlighting the importance of disease control, not only for PTB, but also other diseases that cause economic losses and are considered of sanitary risk.

Only one previous study reported molecular characterization of strains isolated in Colombia (Fernández *et al.,* 2011b), being this insufficient to consider the definition of "indigenous strains" and the ulterior design of vaccines. It would be necessary to conduct studies on wider regions, considering infection-assessment on cattle and other-than-cattle susceptible populations (even local wildlife) to generate prophylactic strategies according to Colombian MAP molecular and epidemiological diversity.

The relationship between MAP and CD has been not discussed in the country, except for some sporadic reviews (Góngora and Villamil, 1999; Calderón and Góngora, 2008; de Waard, 2010). The zoonotic potential of MAP has been debated for almost a century because of similarities between JD in cattle and CD in humans. Nevertheless, evidence of MAP zoonotic potential has not been proven, but should not be ignored (Patel and Shah, 2011). The CD has been known in Colombia since the 1950s and the incidence and prevalence rates are increasing (estimated point prevalence of 77,000 CD cases), but no national consolidated information about the disease is available (Calderón and Góngora, 2008). According to some researchers, efforts should be made to correlate these two diseases in areas with high prevalence or incidence of both.

In general, progress has been made on MAP diagnosis and epidemiology as reported in the studies covered in this review. However, unanswered questions remain, offering many research opportunities.

Conclusion

In Colombia 20 original studies about MAP have been carried out so far in four different animal species, mainly using ELISA, and predominantly in Antioquia and Cundinamarca provinces. In general, the results reported by the original studies included in this review are still insufficient to accurately reflect the epidemiologic situation about MAP or its economic and public health impact in Colombia. Although the existence of MAP in Colombia has been confirmed for almost a century, the small number of studies, as well as several flaws in the published studies, limits the evidence about the magnitude of MAP circulation in animals, humans, the environment, and food in Colombia.

It is imperative to improve the laboratory diagnostic capabilities for MAP in the near future and increase the number of studies dealing with the microbiologic, immunologic, epidemiologic, and economic aspects of MAP in several domestic and wild animal species. Determination of at least regional prevalence in domestic animal populations is of high priority. It is advisable to initiate studies on the detection of MAP in humans, the environment, and in food for human consumption.

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Conflicts of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

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