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A One Health Approach to the Suspected Zoonosis of *Mycobacterium avium* subspecies *paratuberculosis*: A systematic review of reviews

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Abstract

A One Health Approach to the Suspected Zoonosis of *Mycobacterium avium* subspecies *paratuberculosis*: A systematic review of reviews

By Victoria Novak

Mycobacterium avium subspecies *paratuberculosis* (MAP) has long been suspected as a zoonotic pathogen because of the similarity between Johne's disease, a chronic diarrheal wasting disease in animals caused by MAP, and Crohn's disease, a chronic inflammatory bowel disease in humans with unknown etiology. Studies in humans investigating MAP in Crohn's have found an association with presence of the bacteria, but pathogenicity of MAP in humans has never been conclusively proven. A new approach is required to determine if MAP is a zoonotic pathogen in humans. The present umbrella review of systematic reviews and meta-analyses evaluated health impacts, risk factors, and prevalence of MAP in domestic and wild animals. This systematic evaluation of the review articles is the first step in determining how the animal literature can guide future research evaluating the possible role of MAP in human illness. This review of reviews identified several new mechanisms and risk factors for MAP that have not been considered in human MAP research to date. Further research and a One Health approach to synthesize data across disciplines, including human, animal, and environmental health, are needed to determine if MAP is zoonotic and elucidate its impact on public health.

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Table of Contents

Chapter 1

Introduction and Rationale

Mycobacterium avium subspecies *paratuberculosis* (MAP) has long been discussed as a possible zoonotic pathogen. In animals, MAP causes a chronic diarrheal wasting disorder, called Johne's disease, that is endemic in multiple in countries (Uzoigwe, Khaitsa, & Gibbs, 2007). For over a century, it has been suspected that MAP may be responsible for Crohn's disease, an inflammatory bowel disease in humans, with clinical presentation of intestinal lesions that is similar to those of Johne's disease. A number of human case-control studies demonstrated an association between the presence of MAP and Crohn's disease but causation has not been conclusively proven (L. A. Waddell, Rajic, Stark, & Mc, 2015).

MAP is prevalent worldwide in both wild and domestic animals, but it's prevalence in human populations is unknown (Stevenson, 2015). Several studies have proven that MAP can persist in the environment, including soil and water, for several months serving as a source of infection for animals and perhaps humans (Pickup et al., 2005; Rhodes et al., 2014). The bacteria can also be found in certain foods, including some meats, milk, and unpasteurized cheeses (Atreya et al., 2014; L. Waddell, Rajic, Stark, & McEwen, 2016). Thus, exposure to MAP may occur through a variety of routes indicating a significant impact on public health if it is determined to be pathogenic in humans.

Johne's is primarily a disease of ruminant animals, particularly cattle, goats, and deer, but can cause disease in several non-ruminant species including rabbits, dogs, and non-human primates (Uzoigwe et al., 2007). Animals are typically infected while they are very young via the fecal-oral route; however, clinical symptoms, tend to develop at 3-4 years of age (Davis, 2015). While Johne's disease can be controlled with antibiotics, the treatment does not eradicate MAP from infected animals. This is a serious issue as it is prohibitively expensive to provide antibiotics for the entirety of the animal's life (M. Feller et al., 2007). Several vaccines exist for MAP in animals, but while vaccines can help prevent clinical development of Johne's, they do not prevent infection and shedding of bacteria can still occur spreading infection to other animals (Juste, 2012; Park & Yoo, 2016).

Crohn's disease is a human chronic inflammatory bowel disease with no known cure or etiology and is estimated to affect 1-2 million people worldwide(L. A. Waddell et al., 2015). Crohn's disease usually manifests between the ages of 15-30, but can occur at any age. Symptoms often include bouts of bloody diarrhea, cramping, abdominal pain, fever, weight loss and bloating (Uzoigwe et al., 2007). The chronic inflammation leads to thickening of the intestinal walls and narrowing of the lumen, as well as distinctive lesions formed by granulated tissue (Bannantine & Bermudez, 2013). There is no standardized treatment for Crohn's disease and the specific etiology has not been identified. Typically, symptoms are managed with various immunosuppression drugs and occasionally, surgery is required to remove damaged sections of the bowel (Packey & Sartor, 2009).

The similarities between Johne's and Crohn's were first noted in 1913 by Dalziel, but it wasn't until 1984 that MAP was first isolated from the intestinal lesions of a Crohn's patient (Chiodini, Van Kruiningen, Merkal, Thayer, & Coutu, 1984; Dalziel, 1989). Despite the demonstrated presence of MAP in Crohn's patients, the causal link between MAP and Crohn's remained unproven. In addition to Crohn's, several studies have found an association between MAP and other human conditions including multiple sclerosis, HIV infection, sarcoidosis, diabetes mellitus type 1 and type 2, and Hashimoto's thyroiditis (L. A. Waddell et al., 2015). The complexity and variety of the microbiome, and the realization that many organisms are still unidentified, indicate that the presence of a bacterium alone is not sufficient to prove disease causation (Lowe, Yansouni, & Behr, 2008).

Problem Statement

A recent systematic review by Waddell in 2015, included 128 studies evaluating the association between MAP and Crohn's disease. In addition, there are several systematic reviews attempting to characterize the MAP potential for zoonosis. Results continue to be inconclusive despite the increasing number of primary studies and systematic reviews on the topic. Because of the nature of the bacteria and the disease, Koch's postulates (used to prove causation of an infectious disease) cannot be fulfilled. Likewise, Hill's epidemiologic criteria cannot be used because of the incubation period of the disease, the potential role of genetic susceptibility, and complexity of the microbiome. With few options available in human research to demonstrate pathogenicity of the disease, a new approach is required before a conclusion can be made regarding the health impact of MAP and the importance of control measures in protecting human health.

One Health is a concept advocating the interconnectedness of human, animal, and environmental health and the need for cross-sectoral collaboration to address health challenges. MAP has plagued the animal industry for decades and generated substantial effort to understand and control the disease. MAP research in the animal industry may present an untapped resource in an effort to understand the health impact of this bacterium in humans. An assessment of the current landscape of literature regarding MAP pathogenesis in animals is necessary before conclusions can be drawn regarding its use in conjunction with human research evaluating the pathogenesis of MAP in humans.

Purpose Statement

The purpose of this thesis is to systematically evaluate the current state of literature of MAP pathogenesis in animals. By understanding the current literature base in animals, we can begin to inform future research trying to characterize the impact of MAP on human health and its potential contribution to Crohn’s disease. This thesis will investigate the current knowledge regarding the impact of MAP on animal health, risk factors for colonization, infection, the development of disease, and prevalence of MAP in animal populations.

Research Question

In evaluating the current knowledge about pathogenesis of MAP in animals, 3 research questions will be specifically addressed.

- In what ways does MAP impact the health and well-being of animals?
- What risk factors are associated with development of MAP infection or disease in animals?
- What factors impact prevalence of MAP in various animal populations?

Significance Statement

By characterizing the known information about pathogenesis of MAP in animals, we can explore new approaches towards evaluating the potential of this bacterium to act as a zoonotic pathogen. In a disease with an elusive etiology and suspected zoonotic origin, a One Health approach may generate necessary evidence or identify new research questions about the role of MAP in human disease.

Chapter 2

Literature Review

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a globally endemic bacteria that can be found in a wide range of species (Uzoigwe et al., 2007). A slow-growing, acid-fast bacteria, it is an obligate pathogen, however, the thick cell wall can allow it to persist in the environment and remain viable for several months (Atreya et al., 2014). MAP is resistant to heat and can sometimes withstand the process of pasteurization, but it is susceptible to prolonged desiccation and freezing (Lund, Gould, & Rampling, 2002). The two major strains of MAP are sheep (S) type and cattle © type. MAP that is isolated from humans is generally type C (Stevenson, 2015).

MAP is one of four subspecies of *Mycobacterium avium*. *Mycobacterium avium* is known for its role in the Mycobacterium avium complex (MAC), which is an opportunistic respiratory infection in immunocompromised patients (Biet, Boschiroli, Thorel, & Guilloteau, 2005). The other three subspecies of *Mycobacterium avium* are *avium*, *silvaticum*, and *hominissuis*. *Mycobacterium avium* subspecies *hominissuis* commonly infects pigs, but it is the most common subspecies of Mycobacterium avium found in people, where it can cause a respiratory infection. *Mycobacterium avium* subspecies *avium* is the cause of avian tuberculosis and primarily resides in wild bird populations, although it can occasionally spread to humans. *Mycobacterium avium* subspecies *silvaticum* primarily infects wood pigeons and is rarely found in other species (Tran & Han, 2014).

MAP used to be extremely difficult to diagnose because of its similarity to other mycobacteria. Historically, bacterial culture was considered the gold standard for specificity but because of the slow-growing nature of the bacteria, culture is not a sensitive or efficient means of diagnosis (Atreya et al., 2014). In 1989, the IS900 insertion sequence was discovered; this is a highly-repeated sequence throughout the MAP genome that distinguishes it from other mycobacteria. Since then, IS900 detection via PCR has become the gold standard for MAP identification and differentiation (Autschbach et al., 2005). However, PCR can only be used to detect presence of the bacteria in tissue or fecal samples. Tissue samples are difficult to acquire because they require intestinal biopsies. Fecal samples can be unreliable because intermittent shedding and low bacterial load can occur when the infection is in the sub-clinical or silent stage (Davis, 2015). MAP infection can also be confirmed using an ELISA assay. However, ELISA is not a sensitive test until later in clinical disease during the humoral immune response when antibodies are developed against the bacteria (Gilardoni, Paolicchi, & Mundo, 2012; Stabel, 2000).

Known MAP mechanisms of action are similar to other known mycobacterial pathogens, including *Mycobacterium tuberculosis*. Initial infection is controlled by the host for a period that can last several years before unknown triggers, such as stress or weakened immune capacity, initiate clinical development of the disease (Davis & Madsen-Bouterse, 2012). During initial infection, MAP is known to target M cells in Peyer's Patches in the intestine. These cells lack brush-border microvilli and the digestive enzymes and mucous that protect other intestinal cells, making them easy points of entrance for foreign cells to pass through, including pathogens like *Salmonella* (Stabel, 2000). MAP is carried through the M cells by the process of transcytosis where it is then picked up by roaming macrophages (Pravda, 2011). MAP can persist in macrophages and resist clearance by the immune system. One proposed mechanism of MAP action is dysregulation of the cytokine response that triggers T cells to clear the bacteria from the macrophage (Coussens, 2004; Stabel, 2000). Initial MAP infection is heavily regulated by the cell-mediated rather than humoral immune response. In fact, humoral immune response is thought to have no effect on MAP and only develops when the infection is advanced to clinical disease (Stabel, 2000). For this reason, testing for the presence of antibodies is not a useful means of detecting early MAP infection in animals. MAP is also capable of causing systemic infection and moving beyond the intestinal tract into blood and even human breast milk (Bannantine et al., 2014; Barta, Csipo, Mekkel, Zeher, & Majoros, 2004; Bernstein, Blanchard, Rawsthorne, & Collins, 2004).

The possible association between MAP and Crohn's was first noted in 1913 by Dalziel, but in recent years several other diseases including multiple sclerosis, HIV, sarcoidosis, diabetes mellitus and thyroiditis have been linked to MAP infection (Cossu et al., 2011; Frau et al., 2013; Masala, Cossu, Palermo, & Sechi, 2014; Masala, Cossu, Piccinini, et al., 2014; Songini, Mannu, Targhetta, & Bruno, 2016; L. A. Waddell et al., 2015). It has even been suggested that MAP may play a role in the etiology of autism, as it is thought that autism could be triggered by gastrointestinal auto-immune disorders (Samsam, Ahangari, & Naser, 2014). All of these conditions are possible sequelae of immune dysregulation that could theoretically be triggered by MAP or other infections.

The etiology of Crohn's disease is unknown but it is generally concluded that genetic, environmental, and infectious agents interact to trigger disease development (Carriere, Darfeuille-Michaud, & Nguyen, 2014). The major symptom is chronic inflammation of the intestine which can lead to severe abdominal pain, constipation, and diarrhea as well as some systemic symptoms such as lethargy, headaches, and muscle aches (Scanu et al., 2007). Treatment usually includes lifelong medication with immunosuppressants and occasional surgeries to relieve severe bowel obstructions or intestinal bleeding (Pravda, 2011). MAP is not the only microorganism suspected in Crohn's etiology and several other pathogens have been considered as a trigger including *Listeria monocytogenes* and *Yersinia enterocolitica* (Carriere et al., 2014; Martin Feller et al., 2010). Whether Crohn's is the result of infection by a singular organism or several different organisms needs to be considered. Crohn's disease is known to have altered gut microbiome and has been associated with an increased presence of harmful pathogens such as adhesive invasive *E. coli* (AIEC) and a decrease in beneficial organisms as well as a decline in the microbiome diversity overall (Nazareth et al., 2015). Treatment with antibiotics shows some improvement in active Crohn's symptoms but is not curative, and anti-mycobacterials do not necessarily prove more effective (Nitzan, Elias, Peretz, & Saliba, 2016).

Much of the human data on MAP originate from case-control studies in hospitals and samples are usually collected from patients undergoing colonoscopies (Autschbach et al., 2005; Timms, Daskalopoulos, Mitchell, & Neilan, 2016; L. A. Waddell et al., 2015; Zamani et al., 2017). Cases are patients with Crohn's or ulcerative colitis and controls are typically patients undergoing colonoscopies for non-inflammatory bowel conditions such as colon cancer. Large variations are seen in detected prevalence, at times ranging from 0% to 100% in both cases and controls (Autschbach et al., 2005; Pravda, 2011; Uzoigwe et al., 2007). One study found 47% prevalence of MAP among healthy controls sampled from the general population (Juste et al., 2008). Another study surveyed dairy cattle producers for Crohn's disease and did not find any evidence of the association with bovine paratuberculosis (Jones, Farver, Beaman, Cetinkaya, & Morgan, 2006). However, producers also are not at higher risk for *E. coli* 0157:H7 despite increased exposure and a clear understanding of bacterial pathogenesis (Lowe et al., 2008). A recent meta-analysis evaluating the association between MAP and Crohn's in 108 studies calculated an odds ratio in the range of 4.26-8.44. The authors also found associations with type 1 diabetes mellitus (range: 2.91-9.95) and multiple sclerosis (range: 6.5-7.99) but concluded that due to persisting knowledge gaps, the current evidence is still insufficient to determine the public health impact of MAP (L. A. Waddell et al., 2015).

Chapter 3

Methods

The idea of an umbrella review was developed for a topic in which several systematic reviews have previously been conducted. Umbrella reviews constitute a systematic search and analysis of published systematic reviews and meta-analysis on a topic instead of performing a redundant systematic review of the original studies (Bellou et al. 2016). The protocol and principles of an umbrella review developed by Bellou and Belbasis were applied in this communication.

PubMed was systematically searched from inception to March 7, 2017 for all systematic reviews and meta-analyses regarding *Mycobacterium avium subsp. paratuberculosis* (MAP) in animals. The broad search terms were (paratuberculosis AND (systematic review OR meta-analysis)). The titles of articles identified using electronic search were reviewed to identify systematic reviews specific to animals. Abstracts and Methods sections of each article selected during the evaluation of titles were then examined to identify candidates for the full-text review. Articles were classified as systematic reviews if they reported search terms and eligibility criteria. Full text of articles was then reviewed to eligible publications. The following inclusion criteria were used for the final review of reviews:

- Animals, either livestock or wildlife species, were the targeted population of the review
- Mycobacterium avium subspecies paratuberculosis was the primary pathogen being evaluated
- A systematic procedure for reviewing the literature or a meta-analysis was used in the study
- The study addressed at least one of the following three research areas related to MAP

    - health impact
    - risk factors
    - prevalence

Exclusion criteria were:

- Humans or human diseases were the focus of the review
- Mycobacterium avium subspecies paratuberculosis was not the primary pathogen evaluated
- Narrative reviews, primary studies, or opinion papers
- The study addressed topics beyond the scope of this review (e.g. mouse models, MAP microbiology, diagnostic procedures, or vaccine evaluation)

The extracted reviews were grouped into three categories based on which research question they addressed. All reviews were evaluated for quality using a data extraction table designed for the purposes of this study. The quality of the review papers was assessed by determining if systematic search procedures were used with inclusion/exclusion criteria, if a systematic quality assessment of the individual papers included was conducted, and if a systematic evaluation of reasons for disagreement and heterogeneity was included in the results. Reviews that met all of the quality criteria were given more weight and consideration than reviews that did not meet these criteria.

For all reviews, further information was extracted pertaining to the number of papers that were evaluated, location of the publishing authors, and the target population of the review. To address the three central research questions, additional information extracted from the relevant reviews included health characteristics of animals infected with MAP, risk factors for MAP introduction or development of Johne’s disease, and prevalence of MAP in animals. Conclusions of the reviews were extracted verbatim from the abstracts or, if the abstract provided no conclusions, from the text of the review.

Submission to IRB was not required for this study because human subject research was not conducted.

Results

A total of 289 records were identified in the PubMed search. Of those, 117 included relevant titles specific to MAP in animals. Of the 117 records, 19 studies met the inclusion criteria based of further evaluation of abstracts (See Figure 1). Upon full-text review, 15 were included in the final analysis (See Appendix 1). Nine of the eligible articles were systematic reviews and additional six included a meta-analysis. Seven of the reviews met all three quality assessment criteria. All reviews used systematic inclusion/exclusion criteria but eight reviews either did not assess quality of the included studies or discussed possible methodological limitations non-systematically. Reviews that did not report systematic analysis of heterogeneity in results either did not evaluate heterogeneity or differences in the included studies were speculated by the authors non-systematically.

All but three of the reviews reported how many studies were included in the analysis (McKenna, Keefe, Tiwari, VanLeeuwen, & Barkema, 2006; Tiwari, VanLeeuwen, McKenna, Keefe, & Barkema, 2006; Whittington & Windsor, 2009). The minimum number of studies included in a review was two and the maximum was 179, although most articles included between 20 and 40 studies. The type of studies in each review depended on the objective of the publication, but observational studies were the most commonly cited.

The included reviews were conducted in eight different countries but predominantly in Canada (n=4), the United Kingdom (n=3), and Australia (n=3). Only three of the reviews restricted their searches to their own region, this being Canada (McKenna et al., 2006; Tiwari et al., 2006) and Latin America (Fernandez-Silva, Correa-Valencia, & Ramirez, 2014). All other reviews did not restrict their search to one region. All reviews were published in the last 20 years; the oldest reviews were published in 2006 and the most recent reviews included were published in 2015.

Cattle particularly dairy cattle were the focus of most studies. Several reviews specified focus on cattle but did not specify whether information for beef cattle was included or excluded in contrast to dairy cattle. Four reviews looked at farmed animals regardless of species, focusing on cattle, goats, and sheep (Begg & Whittington, 2008; Elliott, Hough, Avery, Maltin, & Campbell, 2015; Fernandez-Silva et al., 2014; Nielsen & Toft, 2009). Three studies considered MAP in wildlife (Carta, Alvarez, Perez de la Lastra, & Gortazar, 2013; Elliott et al., 2015; Rangel et al., 2015).

The two studies by McKenna and Tiwari were published as a two-part review of Johne’s disease in Canada (McKenna et al., 2006; Tiwari et al., 2006). Both were broad overviews of the MAP literature and were large enough in scale to answer two of this study’s research questions.

Health Impact of MAP

Four reviews addressed the impact of MAP on animal health. Two of the articles were meta-analyses. Only one of the meta-analyses met all three quality criteria and reported the number of studies included.

The article by McAloon and colleagues was rated the highest on all quality criteria and included a meta-analysis evaluating the impact of MAP infection on milk yield (McAloon et al., 2016). A total of 33 studies were included in the final analysis and were pooled based on case definition of Johne’s and lactation number. Both longitudinal and cross-sectional studies were included if they met the data quality criteria necessary for the meta-analysis. The study found that milk yield decreased with MAP infection, but there was significant heterogeneity in results. The researchers reported that greater milk reductions were present when fecal detection rather than ELISA testing was used to diagnose MAP infection and that study design accounted for some of the heterogeneity. Tere was a decreasing trend in milk yield with increasing lactation number, but due to significant heterogeneity and few studies reporting lactation number, an overall pooled analysis was not justified. The authors attempted to evaluate the impact of MAP on milk fat and protein yield in milk but there was too much heterogeneity to justify pooled estimates. The authors reported no discernable evidence of publication bias.

Another meta-analysis evaluated vertical transmission of MAP in animals (Whittington & Windsor, 2009). The paper only met two of the quality criteria because there was no formal assessment of heterogeneity, and the authors only examined possible differences non-systematically in the Discussion section. The article reviewed pathogenesis and extra-intestinal spread of MAP, experimental infection of the bovine reproductive tract, natural in utero transmission of MAP, in utero infection of fetuses in species other than cattle, sequelae of fetal infection of MAP, and incidence of calf infection in utero. The meta-analysis concluded that about 9% of fetuses become infected if the cow was sub-clinically infected and 39% of fetuses become infected if the cow was showing clinical symptoms of Johne's disease.

Part 1 of the Canadian study by Tiwari et al systematically reviewed clinical symptoms and pathophysiology of MAP in cattle (Tiwari et al., 2006). Data was reported qualitatively and neither quality assessment nor heterogeneity evaluation was reported for the included studies. Tiwari and colleagues described four clinical stages of paratuberculosis infection: silent infection, subclinical infection, clinical infection, and advanced clinical infection. Silent infection is undetectable and found in young cattle usually less than two years old. Clinical infection is usually found between the ages of two and ten years. All other stages are dependent on other factors including the individual's immune response and ability to control the infection. MAP pathophysiology explains these transitions due to an initial cell-mediated immune response and a delayed humoral immune response that reflects whether the infection is controlled in the animal or not.

Part 2 of the Canadian study summarized the impact of MAP on cattle productivity (McKenna et al., 2006). Some of the reported effects such as premature culling and reduced slaughter weight served as proxy indicators for overall poor health and low productivity, as reported by farm management. Other effects included lower milk yield, lower milk fat, and lower protein content of the milk, but there was disagreement on the magnitude of the effect. Two other reported health effects were reduced fertility and increased incidence of mastitis although the evidence was inconsistent.

Risk Factors

Eight reviews were evaluated for risk factors of which only three met all three quality criteria. One study met two of the quality criteria and four studies only met one of the criteria. Only three of the studies evaluating risk factors included meta-analyses. All reviews were focused on cattle or livestock.

Rangel and coauthors evaluated risk factors for introducing MAP into dairy herds. While all three quality criteria were met, the results were presented qualitatively due to the variability across primary studies (Rangel et al., 2015). The most important risk factor noted in the review was introduction of new cattle into herds. The authors acknowledge that there are very few MAP-free certified herds from which to draw replacement cattle and that it is impractical to test all cattle before introduction because of the low sensitivity of diagnostic tests for sub-clinical cases. They do report that the number of cattle introduced did not appear to have an impact on MAP infection. Infected wildlife was also identified as a potential risk factor for MAP introduction into dairy herds; however, few studies exist that quantify the risk or specifically target active transmission. The authors concluded that introducing infected livestock posed greater risk than exposure to wildlife.

The Doré et al study evaluated within-herd risk factors for transmitting MAP to newborn calves (Doré et al., 2012). The review met all three quality criteria but a meta-analysis was not performed due to variability in reporting of primary data. The authors evaluated neonatal environment, exposure to colostrum and milk, group-housing, and exposure to adult cattle feces as risk factors for MAP infection. The primary risk factors identified were exposure to adult cattle feces and neonatal environment. The authors reported that data from high quality studies assessing MAP infection from milk or colostrum did not show an association.

Mitchell et al conducted a meta-analysis on the effect of experimental dose and age on the fecal shedding of MAP by cattle (Mitchell, Medley, Collins, & Schukken, 2012). The study met all three quality criteria. The authors used time to event risk analysis and were able to provide a robust picture of disease progression in long-term experimental infection studies. They indicate that early MAP shedding is a threat not often considered in disease transmission studies. However, they conclude that young calves that become infected and go through an early shedding phase do pose a risk to other uninfected calves. Few studies have been done on the risk of MAP in relation to the animal's age as it is widely held that older cattle are not as at risk for infection.

Begg and Whittington used a systematic review to screen experimental model studies but only provided qualitative assessment of study quality and reasons for heterogeneity (Begg & Whittington, 2008). The study surveyed data regarding experimental models of Johne's infection across various species. The authors concluded that the optimal experimental model varies depending on the needs of the study. They assessed the results of various infections and concluded that animal species, breed, and age all have an impact on infection and disease manifestation. They also conclude that strain of MAP, route of experimentally administered infection, and dose also have an effect on the immune response and disease progression.

Minozzi et al conducted a genome-wide association study (GWAS) and included a meta-analysis using datasets from two different herds of Holstein cattle (Minozzi et al., 2012). The eligible studies included genetic data from 1190 cattle but were not searched systematically or evaluated for quality. The meta-analysis identified chromosomes 1, 12, and 15 as being associated with paratuberculosis infection. The gene identified by SNPs on chromosome 12 was an ATP-binding cassette associated with multi-drug resistance. The region identified on chromosome 15 is near four different genes: *LDLRAD3*, *PAMR1*, *CACNA1B*, and *COMMD9*. Five genes were identified on chromosome 1: *SSRG*, *SCL33A1*, *LDLRAD3*, *KCNAB1*, *GMPS*. These genes cover several functions including receptors, channels, and proteins for muscle regeneration and guanine monophosphate synthesis. Several other locations were identified in each study, but those were not confirmed in the overall analysis and the results differed depending on whether cattle were classified as MAP positive with ELISA or tissue culture.

Windsor and Whittington examined the effect of age on susceptibility to Johne's disease but only evaluated quality and heterogeneity in a subjective fashion(Windsor & Whittington, 2010). Eight studies were included in a meta-analysis. The conclusion was that calves less than six months of age are at a higher risk of contracting MAP and developing Johne's disease.

Elliot et al reviewed the impact of environmental risk factors on incidence of Johne's disease at various livestock farms. Included studies were searched systematically but were not assessed for quality or heterogeneity (Elliott et al., 2015). As MAP is spread via fecal contamination, various environmental factors can lead to persistence of the bacteria in the environment for several months depending on weather conditions and substrate. It is postulated that MAP may be capable of adopting a dormant state. While presence of MAP is confirmed in wildlife, transmission would only occur when the animals have the opportunity to share the same pasture or water sources. It is reported that MAP may be able to survive by interacting with amoeba and other soil invertebrates.

The previously cited Canadian a study (McKenna et al., 2006) identified several risk factors including age, milk exposure, and transmission across the placenta, although this is reportedly rare and is more common in cattle displaying clinical signs. Breed and genetics appeared to be only minor factors. Other risk factors that were identified as having an impact were exposure dose of MAP bacteria and temperature and soil acidity in the environment.

Prevalence

Five systematic reviews investigated prevalence of MAP. Three reviews assessed regional prevalence in Canada, Europe, and Latin America while the other two considered prevalence in wildlife and in milk tanks on farms.

Okura and co-authors evaluated the occurrence of MAP in milk on dairy farms (Okura, Toft, & Nielsen, 2012). The milk was for human consumption and both individual tanks and bulk tanks were tested. The methods of assessing MAP prevalence of bulk milk tanks are similar to testing of pooled fecal samples. Both quality and heterogeneity were systematically assessed for all 45 studies included in the review and a meta-analysis was performed. The authors reported considerable variation in data due to poor reporting in the primary studies. They were unable to quantify the amount of MAP in samples due to limitations of data collection, and they only reported an apparent prevalence for cows that were deemed positive by PCR and MAP culture. The MAP prevalence estimates (95% confidence intervals [CI]) were 0.1 (0.04-0.2) in bulk tanks and 0.2 (0.1-0.3) in individual milk samples. No risk factors that may have affected prevalence within a herd were identified other than the irregularity of diagnostic procedures used in the primary studies.

The Fernandez-Silva et al study evaluated prevalence of MAP in the Caribbean and Latin America (Fernandez-Silva et al., 2014). The review met all quality criteria but a meta-analysis was not performed. The herd level prevalence was estimated as 76.8% with a 95% CI from 50 to 100% and the individual cattle prevalence was estimated at 17% (95% CI: 13-21%). Only the individual level prevalence was calculated for sheep at 16% (8-24). The levels for individual and herd level for goats were 4% (2-7) and 4% (0.1-7%), respectively. The authors theorized that farm size, breed, and use of animal were possible reasons for varying prevalence across the region.

Nielsen and Toft conducted a review of MAP prevalence throughout Europe (Nielsen & Toft, 2009). The review met all three quality criteria but a meta-analysis was not performed due to the variability in primary study reporting. Estimates for cattle were around 20% though they were as low as 3-5% in some countries. For cattle, the herd level prevalence could only be estimated as above 50%. Within-herd prevalence was not calculated for sheep and goats but herd level prevalence was estimated as above 20% based on 2 data points from Switzerland and Spain. No risk factors or theories for differences in prevalence were described aside from variations in diagnostic technologies and sampling strategies used in the primary studies.

The study by Carta et al evaluated prevalence of MAP in wildlife overall and did not restrict their search to a particular region (Carta et al., 2013). Quality and heterogeneity were not assessed systematically. The authors pointed out the poor quality of primary studies; but estimated a prevalence of around 2%. They indicated that excretion of MAP was reported to be lower for wildlife than for domestic animals. They also observed higher prevalence of MAP in wildlife in areas where prevalence was higher in domestic animals.

The Canadian review by Tiwari and colleagues reported a range of prevalence estimates across the country Canada depending on location (Tiwari et al., 2006). The review reported estimates similar to those from Belgium (0.8%) and the United States (17%). At the herd level, reported prevalence as 17% similar to the US prevalence overall and 44% as reported in the State of Michigan. The authors cite provincial differences and varying farm risk management practices as possible explanations for the observed differences in MAP prevalence, but also conclude that evolving diagnostic technologies make historical comparisons difficult.

Chapter 4

Discussion

A One Health approach advocates multi-sectoral partnerships and collaborations across disciplines. The question whether *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is zoonotic pathogen is an example of research that would benefit from this trans-disciplinary approach. The animal industry has been conducting research on MAP and coping with the consequences of its pathogenesis for the last century. The wealth of knowledge available regarding the health impacts, risk factors, and prevalence of MAP in animals can serve as a reference for human health experts attempting to understand the impact of MAP on human health. To the best of the author’s knowledge, this systematic review represents the first attempt to synthesize animal literature on MAP with the intention of applying it to the context of human medicine.

Only a few systematic reviews focus on actual impact of MAP on health characteristics in animals. The two higher quality studies that sought to assess the impact of MAP on a health characteristic used milk yield and vertical transmission as the endpoints of interest. Reduced milk yield due to MAP infection is a frequently reported problem but it was concluded that the primary studies were not of sufficient quality or quantity to justify this conclusion. An important point, of possible relevance to human health is that intestinal infection with MAP can become systematic depending on the stage of the disease. MAP bacteria have been isolated from human breast milk as well, indicating that while the bacteria usually target the intestines, it is capable of moving through animal and human organs and systems in similar ways (Bannantine et al., 2014).

The finding by Whittington and Windsor that MAP bacteria can be transmitted vertically in utero may also have human health implications. While authors were cautious in interpreting their results, as the fecal contamination is common in farm settings, they did conclude that vertical transmission is possible. The review further indicated that offspring born to a MAP infected mother were more likely to develop disease and to develop it earlier than calves that were born to uninfected cows. Reduced fertility was also reported as a symptom of MAP infection but the evidence was not of sufficient quality to justify this conclusion.

Assessing risk factors for MAP in the current literature poses some problem because the outcomes varied across studies. The two highest quality studies concluded that the introduction of infected animals and exposure to feces are the greatest risk factors for MAP infection. These findings are important for producers trying to control the infection on their farms but from a One Health perspective, they are of limited value except to reaffirm that exposure to infected individuals and fecal-contamination are the important routes of transmission. The findings that MAP dose and age at exposure act as a risk factors may also have relevance to human health. The two factors are inter-related, as indicated by the observation that cattle that are exposed at an older age can still be susceptible but require exposure to higher doses of MAP. It was consistently reported that calves younger than 6 months were at higher risk for becoming infected with MAP and the risk decreased with increasing age. One review included in the discussion that the fundamental structure and composition of the intestinal walls changes during the first few months of life, at first allowing larger molecules like antibodies to pass through the intestines. As the immune system further develops and diet advances, the composition of the intestines change and become more defensive, disallowing the passage of larger molecules. This transition is also found in human beings (Pravda, 2011). If MAP infection is more likely to occur in this neonatal phase, then humans may also be more susceptible to MAP exposure in the first few months of life. This finding indicates that risk factors for possible MAP-related diseases (e.g. Crohn’s) may need to be examined in early life. An important direction in MAP research is evaluation of genetic risk factors. One meta-analysis identified several genes that may play a role in the development of infection in animals. Two of these genes in particular were reported as also being associated with Crohn’s in humans.

Studies that evaluated prevalence of MAP indicate that this bacterium is widespread. Higher prevalence in animals has implications for public health because it may indicate probability of transmission (Jones et al., 2006).

This analysis is not sufficient to determine if MAP is capable of human infection or is the causative agent in Crohn's. However, it does synthesize evidence that MAP may be capable of impacting human health. This study is only the first step in analyzing the animal literature and supports the argument that animal literature needs to be considered more frequently in the human medical sphere. These risk factors that are known in the animal industry need to be considered in future human studies when evaluating the impact and risk factors of MAP.

While the primary data were not examined this paper, this analysis was appropriate for getting an overview of important research directions and best evidence of MAP in animals. The important limitation in this study is lack of agreed-upon methodology of conducting reviews of reviews. Existing guidelines such as PRISMA and AMSTAR were developed for reviews and meta-analyses of primary studies but they are not designed to guide umbrella reviews such as this. While the criteria developed for this analysis helped assess the quality of the reviews, they did not allow assessment of the underlying data

It is important to keep in mind that the quality of a review depends on the quality of the underlying literature. Several of the reviews included in this communication fell short of fulfilling the quality criteria listed in this communication. Moreover several reviews indicated the relatively poor quality of many original studies comprising the extant body of literature. These limitations notwithstanding, the review of reviews may offer ideas for future studies in humans. For example in order to understand the health impact of MAP in humans it is important to determine the population prevalence of MAP carriage in the general population. Most human studies so far have been limited to case-control studies sampled from hospitals. Other important research directions may include studies in non-ruminant animals, as their digestive tracts are more similar to those of humans. While it is well known that non-ruminant animals are capable of carrying MAP, the data have not been evaluated systematically. Additional next steps in MAP research may include assessment of the microbiology literature. Combining data from animal, human and microbiology studies, with the use of methods from epidemiology, ecology, and informatics will fulfill the vision of the One Health approach, and may lead to the breakthroughs in MAP research.

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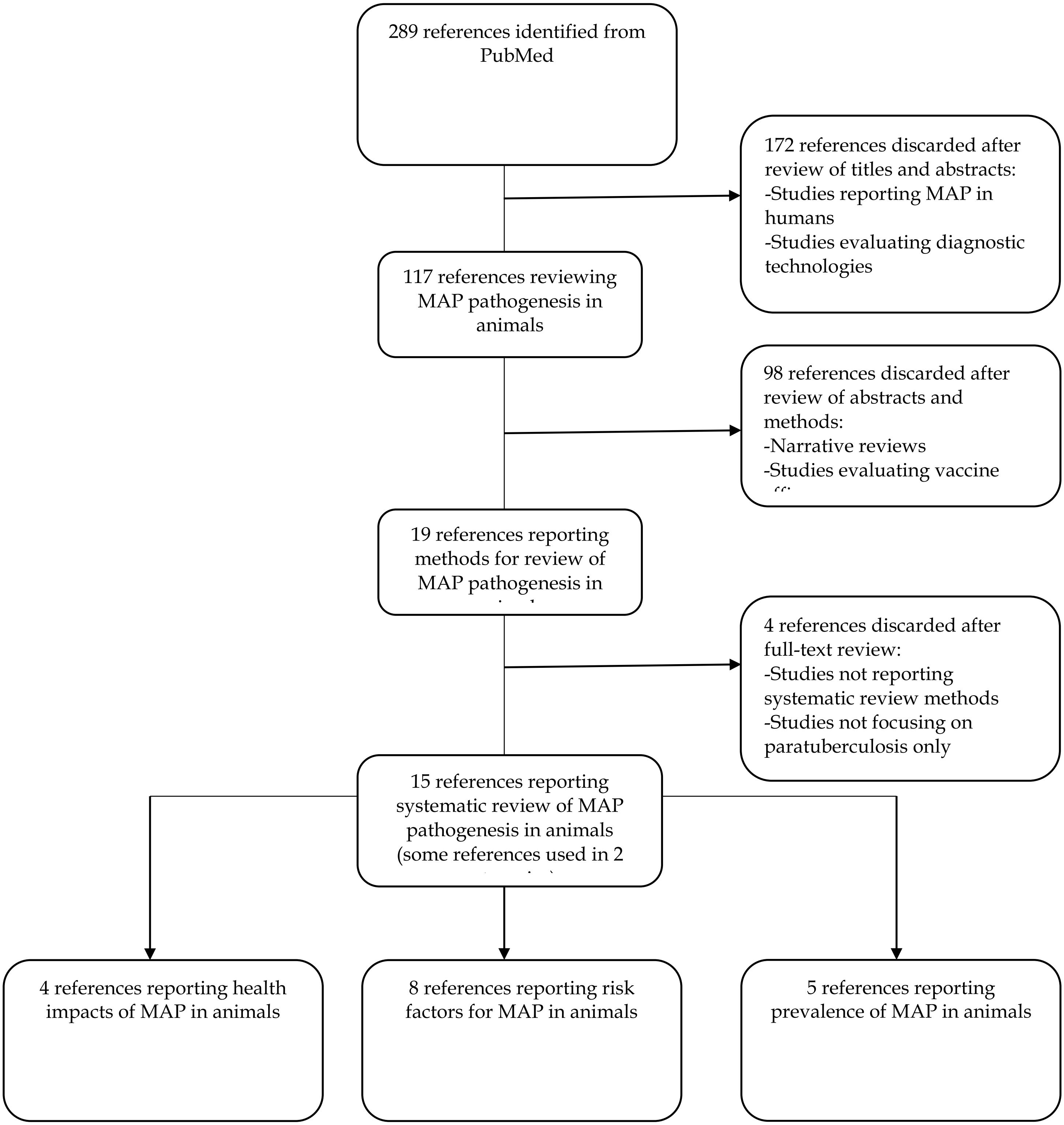
Figures

Figure 1: MAP PRISMA Flowchart



Tables

Table 1: Systematic Review Data Extraction for Health Impact Studies

Publication	Author's Location	Population	# of Studies Included	Systematic search & retrieval of articles	Structured evaluation of data quality	Systematic assessment of heterogeneity	Meta-Analysis Performed	Overall conclusions	Risk Factors
Whittington & Windsor, 2007	Australia	Cattle, no distinction between dairy or beef	Not Reported	Yes	Yes	No	Yes	About 9% of fetuses from sub clinically infected cows and 39% from clinically affected cows were infected with Mptb. These are underestimates for methodological reasons. The estimated incidence of calf infection derived via the in utero route depends on within-herd prevalence and thee ratio of sub-clinical to clinical cases among the infected cows. Assuming 80:20 for the latter, estimates of incidence were in the range of 0.44-1.2 infected calves per 100 cows per annum in herds with within-herd prevalence of 5% and 3.5-9.3 calves in herds with 40% prevalence. These estimates were not markedly sensitive to the value chosen for the proportion of clinical cases.	• In utero transmission of MAP • Prevalence of fetal infection with MAP • Incidence of fetal infection with MAP
McAloon et al., 2015	United Kingdom	Dairy cows	33	Yes	Yes	Yes	Yes	A reduction in milk yield, corrected for lactation number and herd of origin of 1.87 kg/d, equivalent to 5.9% of yield, was associated with fecal culture or PCR positivity in individual cows.	• Milk yield
McKenna et al., 2006	Canada	Dairy cows in Canada	Not Reported	Yes	No	No	No	In cattle positive by enzyme-linked immunosorbant assay, there is a 2.4 times increase in the risk of their being culled, and their lactational 305-day milk production is decreased by at least 370kg. Reduced slaughter value and premature culling account for losses of CDN$1330 per year per infected	• Thickened intestinal mucosa • Malabsorptive diarrhea • Subsequent decreased intestinal absorption of nutrients • Milk yield • Milk fat and protein yield in milk

								50-cow herd. Research has failed to show a consistent association between Mycobacterium avium subsp. paratuberculosis test status and reduced fertility or risk of clinical or subclinical mastitis.	• Slaughter weight at culling • Premature culling • Decreased fertility • Increased incidence of mastitis
Tiwari et al., 2006	Canada	Dairy cows in Canada	Not Reported	Yes	No	No	No	The subclinical form of this disease results in progressive weight loss, reduced milk production, lower slaughter value, and premature culling, with possible impacts on fertility and udder health. Eventually infection can lead to the clinical form that manifests as chronic diarrhea, emaciation, debilitation, and eventual death.	• Pathophysiology • Clinical effects and stages

Table 2: Systematic Review Data Extraction for Risk Factors Studies

Publication	Author's Location	Population	# of Studies Included	Systematic search & retrieval of articles	Structured evaluation of data quality	Systematic assessment of heterogeneity	Meta-Analysis Performed	Overall conclusions	Risk Factors
Mitchell et al., 2012	United States & United Kingdom	Cattle, no distinction between dairy or beef	16	Yes	Yes	Yes	Yes	Results from parametric time-to-event models indicated that challenging older calves or using multiple-exposure experimental systems resulted in a smaller proportion and shorter duration of early shedding as well as slower transition to late shedding from latent compartments. Calves exposed naturally showed variable infection progression rates, not dissimilar to other infection routes.	• Age • Dose effect
Windsor & Whittington, 2010	Australia	Cattle, no distinction between dairy or beef	8	Yes	No	No	Yes	Approximately 75% of claves <6months of age, 50% of those aged between 6 and 12 months, and just less than 20% of cattle >12 months old developed lesions indicative of BJD infection when exposed to any of the tested routes of *Mptb* infection. No direct evidence was found to support the commonly held view that calf removal from the dam for a maximum period of 12h is preferable to 24h. However the studies did show that if exposure to infection occurs at birth, then the risk of infection progressing to BJD is high, particularly in a highly contaminated environment or if the dam is infected.	• Age
Begg & Whittington, 2008	Australia	Cattle, sheep, goats, deer, mice, pigs, and others	73	Yes	No	Yes	No	The factors that appeared to influence the outcome of experimental infections with Mycobacterium avium subsp. paratuberculosis (*Mptb*) were the species, breed and age of subject used for the infection, the route of infection, and the strain, dose, and number of doses of *Mptb* used to inoculate the subjects.	• Species • Breed • Age • Route of infection • Strain • dose

								The analyses identified several loci (P<5 e-05) associated with ParaTB, defined by positive ELISA and presence of bacteria in tissue compared to ELISA and tissue negative animals, on chromosomes 1, 12, and 15 and one unassigned SNP. These results confirmed associations on chromosome 12 and the unassigned SNP with ParaTB which had been found in the Italian population alone. Furthermore, several additional genomic regions were found associated with ParaTB when ELSIA and tissue positive animals were compared with tissue negative samples. These loci wer on chromosomes 1,6, 7, 13, 16, 21, 23, and 25 (P<5 e-05). The results clearly indicate the importance of the phenotype definition when seeking to identify markers associated with different disease responses.	• genes
Minozzi et al., 2012	United States & Italy	Holstein cattle from Lodi province in Italy and New York, Pennsylvania, and Vermont, U.S.	2	No	No	Yes	Yes		
McKenna et al., 2006	Canada	Dairy cows in Canada	Not Reported	Yes	No	No	No	Host level factors include age and level of exposure, along with source of exposure, such as manure, colostrum, or milk. Agent factors involve the dose of infectious agent and strains of bacteria. Environmental management factors influence the persistence of the bacteria and the level of contamination in the environment.	• Dose and age • Poor nutrition, stress, & immunosuppression may precipitate clinical disease • In utero and direct transmission through milk • Genetics and breed • Substrate, temperature, pH
Rangel et al., 2015	Canada	Cattle, no distinction between dairy or beef	17	Yes	Yes	Yes	No	The review indicated that purchase/introduction of animals was an important risk factor and that the importance of wildlife or other domestic species as a mechanism for transmission into a cattle herd was not measurable.	• Introducing cattle • Exposure to wildlife
Dore et al., 2012	Canada	Cattle, no distinction between dairy or beef	23	Yes	Yes	Yes	No	The contact of calves with adult cow feces is the most important risk factor in MAP transmission. The 5 categories of risk factors are linked to one another.	• Neonatal exposure • Colostrum exposure • Milk exposure • Group-housing • Contact with adult cow feces

Elliot et al., 2015	United Kingdom	Livestock farms	179	Yes	No	No	No	An inclusive approach to disease management that takes into account the persistence and transport of the causative organism in on-farm soils and waters, land use and management, dispersal by domestic and non-domestic host species, as well as general animal husbandry is required on those farms where more traditional approaches to disease management have failed to reduce disease prevalence.	• Persistence in the environment • Dormancy and sporulation • Wildlife vectors • Microorganism interactions
Mitchell et al., 2012	United States & United Kingdom	Cattle, no distinction between dairy or beef	16	Yes	Yes	Yes	Yes	Results from parametric time-to-event models indicated that challenging older calves or using multiple-exposure experimental systems resulted in a smaller proportion and shorter duration of early shedding as well as slower transition to late shedding from latent compartments. Calves exposed naturally showed variable infection progression rates, not dissimilar to other infection routes.	• Age • Dose effect

Table 3: Systematic Review Data Extraction for Prevalence Studies

Publication	Author's Location	Population	# of Studies Included	Systematic search & retrieval of articles	Structured evaluation of data quality	Systematic assessment of heterogeneity	Meta-Analysis Performed	Overall conclusions	Risk Factors
Okura, Toft, Nielsen, 2012	Denmark	Milk for human consumption	45	Yes	Yes	Yes	Yes	The apparent prevalence of MAP in BTM and IM on farm were summarized in relation to strata defined by the test used to identify MAP and the infection status of the herds/animals. There was considerable inconsistency in the reporting, resulting in missing information potentially explaining the dispersion in the estimated AP. The overall AP and 95% confidence intervals based on PCR and culture of MAP were summarized to 0.10 (0.04-0.22) in BTM and 0.20 (0.12-0.32) in IM. Quantifying the MAP load in test-positive milk samples was not possible because very few articles provided quantitative information on individual samples.	• None reported
Nielsen & Toft, 2009	Denmark	Farmed animals in Europe	34	Yes	Yes	Yes	No	The true prevalence among cattle appeared to be approximately 20% and was at least 3-5% in several countries. Between-herd prevalence guesstimates appeared to be >50%. No countries had published sufficient information to claim freedom from MAP or just a near-zero prevalence of MAP infections. No within-flock prevalence estimates were available for goats and sheep. The between-flock prevalence guesstimates were >20%, based only on estimates from Switzerland and Spain.	• None reported
Carta et al., 2013	Spain	Free-living and captive wildlife	66	Yes	No	No	No	The mean MAP prevalence reported in wildlife was 2.41% (95% confidence interval 1.76-3.06). Although MAP should be considered an important disease in farmed cervids, its impact	• Wildlife could introduce MAP into unexposed populations • Lower excretion in wildlife than in

								on free-ranging species is questionable.	domestic animals • Wildlife prevalence higher in areas with high domestic prevalence
Fernandez-Silva et al., 2014	Colombia	Cattle, sheep, and goats in Latin America and the Caribbean	24	Yes	Yes	Yes	No	Studies in Latin American and Caribbean countries revealed an overall prevalence of 16.9 (95% CI (confidence interval) 13.2-20.5) and 75.8% (95% CI 50.1-101.5) in cattle at the animal and herd levels respectively; the prevalence was 16% (95% CI 7.9-24.1) in sheep at the animal level and 4.3% (95% CI 1.9-6.8) and 3.7% (95% CI 0.1-7.4) in goats at the animal and flock levels, respectively. In general, prevalence results reported by the studies were insufficient to accurately determine the prevalence of paratuberculosis in farmed animals in Latin America and the Caribbean.	• Farm size • Animal breed • Provincial differences • Risk management practices • Viability of excreted MAP
Tiwari et al., 2006	Canada	Dairy cows in Canada	Not Reported	Yes	No	Yes	No	Seroprevalences at the animal level in Canada are similar to those in other countries, ranging from 0.8% in Belgium to 17.1% in the USA. At the herd level, the proportion of herds with 2 or more seropositive cows in Canada was also similar to that in other studies, ranging from 17% for the 20 tested states in the USA to 44% in Michigan, USA. The significant advances in the quality of the diagnostic tests used to detect MAP make it difficult to determine if the prevalence of MAP infection is increasing.	• None reported
Okura, Toft, Nielsen, 2012	Denmark	Milk for human consumption	45	Yes	Yes	Yes	Yes	The apparent prevalence of MAP in BTM and IM on farm were summarized in relation to strata defined by the test used to identify	• Wildlife could introduce MAP into unexposed populations • Lower excretion in

	MAP and the infection status of the herds/animals. There was considerable inconsistency in the reporting, resulting in missing information potentially explaining the dispersion in the estimated AP. The overall AP and 95% confidence intervals based on PCR and culture of MAP were summarized to 0.10 (0.04-0.22) in BTM and 0.20 (0.12-0.32) in IM. Quantifying the MAP load in test-positive milk samples was not possible because very few articles provided quantitative information on individual samples.	wildlife than in domestic animals • Wildlife prevalence higher in areas with high domestic prevalence

Table 4: Studies Excluded After Full Text Review

Study	Location	Target Population	Topic	Reason for Exclusion
(Mitchell et al., 2015)	Israel	Cattle, no distinction made between dairy and beef	Fecal shedding of MAP by naturally and experimentally infected cows	Only 3 studies were included and they were not systematically identified
(Bastida & Juste, 2011)	Spain	livestock	Paratuberculosis control with vaccination	Focus on MAP vaccine efficacy not risk factors
(Miller, Farnsworth, & Malmberg, 2013)	USA	Livestock and wildlife	Diseases at the livestock-wildlife interface	Methods for the systematic review were not reported; MAP was mentioned but not the primary focus
(Geraghty, Graham, Mullowney, & More, 2014)	United Kingdom	Cattle, no distinction made between dairy and beef	Johne's control activities	Methods for the systematic review were not reported; MAP risk factors were not reported