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Mycobacterium avium ssp. paratuberculosis detection in animals, food, water and other sources or vehicles of human exposure:

a scoping review of the existing evidence

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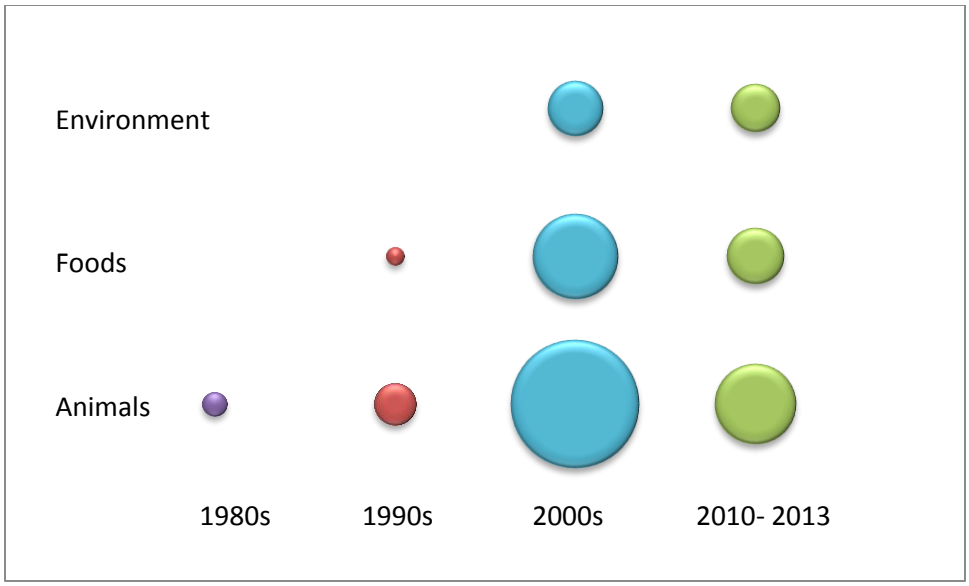

Graphical Abstract: The bubbles in the plot represent the number of studies published on sources of human exposure to *M. paratuberculosis* categories (environment, human food and animal) between 1980 and September 2013.

Key Points (1-5 bullet points, Max= 85 characters including spaces)

- Well-documented *M. paratuberculosis* sources for human exposure include dairy products, meat and drinking water.
- Other potential food sources (e.g. produce and seafood) have not been investigated.
- Cheese and processed meat consumption were identified as risk factors for Crohn's diseases or *M. paratuberculosis* seropositivity in humans.
- Many animal species, both ruminant and non-ruminant, have been shown to be infected with and shed *M. paratuberculosis*.
- The potential role of non-ruminants as reservoirs should be further investigated to confirm the relative contribution to the burden of *M. paratuberculosis*.

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- There is insufficient data on many potential *M. paratuberculosis* sources for humans to develop an exposure assessment model.

Abstract (243, max=400)

Mycobacterium avium ssp. *paratuberculosis* is the etiologic agent of Johne's disease in ruminants and is hypothesized to be an infectious cause of Crohn's disease, as well as some other human diseases. Due to key knowledge gaps, the potential public health impact of *M. paratuberculosis* is unknown. This scoping review aims to identify and characterised the evidence on potential sources and vehicles of *M. paratuberculosis* exposure for humans to better understand how exposure is likely to occur. Evidence from 255 primary research papers is summarized; most examined the prevalence or concentration of *M. paratuberculosis* in animals (farmed domestic, pets and wildlife) (n=148), food for human consumption (62) (milk, dairy, meat, infant formula) or water (drinking and recreational) and the environment (farm, pasture and areas affected by runoff water) (20). The majority of this research has been published since 2000 (Figure- abstract). Nine case-control studies examining risk factors for Crohn's disease highlighted significant associations with the consumption of processed meats and cheese, while direct contact with ruminants, high risk occupations (farmer, veterinarian), milk consumption and water source were factors not associated with the disease and/or *M. paratuberculosis* exposure status. Molecular epidemiology studies demonstrated strain-sharing between species. Produce and seafood were the only previously suggested sources of human exposure for which there was no supporting evidence identified in this scoping review. The results of this review indicate that ruminant populations from around the globe are infected with *M. paratuberculosis* and many non-ruminant species have also been found to carry or be infected with *M. paratuberculosis.* Several potential sources for human exposure to *M. paratuberculosis* were identified; however there remain important gaps in quantitative information on

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the prevalence and concentration of *M. paratuberculosis* in contaminated sources of exposure. This information is critical to understanding the risk of exposure, opportunities for risk mitigation interventions and modelling exposures to distill the importance of various sources of human exposure to *M. paratuberculosis* including direct contact with animals and the environment as well as consumption of contaminated foods and water. Results of this study may be used to prioritize future research and to support evidence-informed decision-making on the *M. paratuberculosis* issue.

Keywords (max=6): Mycobacterium avium ssp. paratuberculosis**,** Crohn's disease, Johne's disease, scoping review, food, water.

Introduction

Mycobacterium avium ssp. *paratuberculosis* belongs to the *Mycobacterium avium* complex that includes 24 species, some of which are pathogenic to humans and animals (Biet et al., 2005). *Mycobacterium paratuberculosis* is the etiologic agent of Johne's disease, a severe production-limiting gastrointestinal disease that affects domestic and wild ruminants worldwide and in advanced cases is characterized by wasting and profuse diarrhea leading to death (Behr and Collins, 2010). The disease is important to ruminant industries for animal health and economic reasons (Waddell et al., 2016). However, the zoonotic potential of *M*. *paratuberculosis* is not fully understood despite 30 years of research. Several studies have identified that *M. paratuberculosis* is more readily isolated from Crohn's disease patients compared to controls, however the role that *M. paratuberculosis* may play in Crohn's disease is unknown (Waddell et al., 2015). While there are many knowledge gaps about exposure and conditions under which disease in humans develops; it is critical to better understand the risk of human exposure to *M. paratuberculosis* from various sources including direct contact with animals, the environment and consumption of potentially contaminated foods and opportunities for risk mitigation interventions.

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Synthesis research methodologies offer transparent and replicable ways to identify, characterise and synthesize the literature (Sargeant J. et al., 2006; Pham et al., 2014; Young et al., 2014). The improved transparency and accountability ensured by synthesis research methodologies is important for evidence-informed policy-making in zoonotic public health, particularly on questions and issues that cut across many disciplines and for which there may be sparse and contradictory evidence (Rajic et al., 2013). Scoping reviews (ScR) are well-suited to assess broad, policy-relevant questions, whereas systematic review and meta-analysis (MA) are better suited to address focused questions (Sargeant J. et al., 2006; Pham et al., 2014; Young et al., 2014). The objective of this ScR was to identify and assess evidence of potential sources and vehicles of human exposure to *M. paratuberculosis*, and identify knowledge gaps. To the best of our knowledge, previous studies have not included all potential sources of *M. paratuberculosis* for humans, nor have they attempted to summarise this evidence for use in decision making.

Methods

Team, question, protocol and definitions

The scoping review research team had expertise in the following area: *M. paratuberculosis*, food safety, risk assessment, epidemiology, library science, and synthesis research (e.g. scoping review, systematic review and meta-analysis). The team defined a broad research question to capture all French and English language primary research; "What is the global evidence evaluating potential sources of human exposure to *Mycobacterium avium ssp. paratuberculosis*?" The sources were hypothesized to contribute to transmission via food, direct contact and the environment. The types of studies examined included surveys of *M. paratuberculosis* in food, animal and environmental sources for humans, studies on the survival of *M. paratuberculosis* under different conditions, and molecular epidemiological evidence of strain-sharing among animal species and humans. Articles on the *M. paratuberculosis* status of wild and domestic animals were identified for prevalence information. Farm level or animal level

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intervention or risk factor studies (Elliott et al., 2014; Rangel et al., 2015) related to *M. paratuberculosis* in ruminant herds and the evaluation of diagnostic tests for *M. paratuberculosis* (Collins, 2011; Gilardoni et al., 2012) have been reviewed elsewhere and were excluded as beyond the scope of this ScR.

An *a priori* developed and pre-tested ScR protocol (Supplementary Material: Appendix 1) included the study question, sub-questions, definitions, procedure for literature search, study inclusion/exclusion criteria and checklists for conducting relevance screening and study characterization of relevant primary research following the principles and steps of ScR methodology (Rajic et al., 2013; Pham et al., 2014; Young et al., 2014). Six animal health, human health and agri-food experts from across Canada were engaged to provide feedback on the scope of this project, input on hypothesized sources of *M. paratuberculosis* and insight into the relevance of the issue within their disciplines. The feedback resulted in the addition of potential sources that expanded the scope to include animal and bulk tank milk results. The search strategy and ScR tools were adjusted based on the feedback to improve clarity and ensure comprehensive results.

Search strategy

The following four search algorithms were implemented on September 20th, 2013 in four databases; PubMed, Scopus (Health Canada) and CAB (Health Canada) and Current Contents (web of science, university of Guelph).

((paratuberculosis) OR (crohn* disease) OR (crohn disease) OR (crohn's disease) OR (inflammatory bowel disease) OR (inflammatory bowel diseases)) AND (milk OR dairy)

Limits year: (2005 –present)

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(paratuberculosis OR (crohn* disease) OR (crohn disease) OR (crohn's disease) OR (inflammatory bowel disease) OR (inflammatory bowel diseases)) AND (yogurt OR cheese* OR (meat OR (beef AND (carcass* OR ground OR cut))))

Limits Year: (1984- present)

(paratuberculosis OR Johne OR Johne's OR Johne's) AND (crohn OR crohn's OR crohn* OR krohn OR krohn's OR krohn*) AND (meat OR beef OR carcass* OR water OR food OR air OR aerosol OR soil OR environment* OR (risk factor) OR exposure OR genetic*)

Limits Year: (1984- present)

paratuberculosis and (wildlife or wild or captive)

Limits Year: (1984- present)

More specific terms were investigated to further refine the algorithms, however with little gain in specificity we preceded with more inclusive search algorithms. The year limit of 1984 was selected because it was the year *M. paratuberculosis* was first isolated in a human (Chiodini et al., 1986). The paratuberculosis and dairy algorithm was implemented from 2005 as a previous SR implemented by this group already included the literature up to 2005 (Waddell et al., 2008), the results of which were transferred into the current review and re-screened using the tools for this ScR. Citations retrieved from all databases were imported into reference management software RefWorks (Copyright 2015, ProQuest LLC) and de-duplicated. Search verification included screening reference lists of seven review articles (Anon, 2010; Grant, 2010; Kaevska and Hruska, 2010; Mor-Mur and Yuste, 2010; Singh et al., 2010a; Over et al., 2011; Carta et al., 2013) and five systematic reviews published on *M. paratuberculosis* (Feller et al., 2007; Abubakar et al., 2008; Eltholth et al., 2009; Okura et al., 2012; Fernandez-Silva et al., 2014).

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Potentially relevant unique citations identified by search verification were added to the ScR at the relevance screening stage and processed through all ScR tools as appropriate.

Abstract and article-level relevance screening and study characterization

Abstract-based screening (Figure 1) was conducted by two reviewers working independently. All potentially relevant primary research in English or French investigating *M. paratuberculosis* contamination of potential sources for humans was identified. Non-primary research studies (e.g. narrative reviews) and primary research studies outside of the study scope were excluded. Reviewer agreement ($\kappa \geq 0.8$) was evaluated using 30 abstracts prior to proceeding with screening. Conflicts were resolved through consensus by reviewers and if this was not possible a third team member was consulted. All citations deemed relevant at the abstract-based screening level were procured as full articles. At the next level, full papers were used to confirm relevance prior to proceeding with study classification.

Classifying Relevant Research

Pertinent characteristics and results were extracted from relevant articles for study classification. These characteristics included source of *M. paratuberculosis*, study design, representativeness, outcome and sampling information. Study utility was evaluated by identifying articles with and without the minimum extractable data. Prevalence, concentration and/or association results were extracted as reported. Studies that failed to isolate or identify *M. paratuberculosis* by culture or PCR (polymerase chain reaction) or through an immune response were flagged as not useful for meta-analytic summary.

Study management and data analysis

Relevance screening and classification of studies were conducted using DistillerSR (Evidence Partners, Ottawa, ON, Canada) a web-based systematic review management software. Dataset management and descriptive analysis were conducted in Microsoft Excel 2010. As this was a scoping review, risk of bias

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evaluation was kept to a minimum (Higgins and Altman, 2008; Higgins and Green, 2011; Pham et al., 2014). We evaluated control groups and sampling frame for each published study, and whether there were data to extract. Representativeness was evaluated based on study design, target population and selection of the sampling frame. Finally, where more than one study measured the same outcome on comparable populations, we performed meta-analyses to summarize the overall results.

Selected random effect meta-analyses (MA) were conducted in STATA 13 (StataCorp 2013. Stata Statistical Software: Release 13. College Station, TX, USA: StataCorp LP), which employs the method of moments weighting procedure (DerSimonian and Laird, 1986). Homogenous meta-analyses with more than 10 lines of data were evaluated for publication bias by Begg's and Egger's tests (Begg and Mazumdar, 1994; Egger et al., 1997). If publication bias was detected, Duval and Tweedie's trim and fill method was used to estimate the potential impact on the estimate and conclusions of the MA (Duval and Tweedie, 2000). For outcomes with prevalence between 10-90% the logit transformation was used. Where data were close to the extremes (<10% or >90%), the logit transformation tends to push the overall estimate towards 50%, so the double arc sine transformation was used to calculate a summary prevalence and confidence interval that better reflects the data (Barendregt et al., 2013).

Results

From 3378 unique citations captured by the literature search and search verification there were 255 references considered relevant to the scoping review with 713 lines of data (Figure 1). The majority of studies examined the prevalence/concentration of *M. paratuberculosis* in animals (n=148) (farmed domestic, pets and wildlife), food for human consumption (62) (milk, dairy, meat, infant formula) and in water and the environment (20). Nine studies examined risk factors for human exposure to *M. paratuberculosis* and the association with Crohn's disease for which *M. paratuberculosis* is hypothesized

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to be an infectious disease candidate. Finally, 13 genotyping studies examined the relatedness of *M. paratuberculosis* isolates from various combinations of domestic animals, dairy products and environmental samples.

M. paratuberculosis in food for human consumption

Food for human consumption was investigated in 76 studies; 62 describing the prevalence of *M paratuberculosis* in raw and pasteurized milk, other dairy products, infant formula, breast milk, raw and pasteurized milk cheese, and meat (Figure 2). Summaries of the prevalence observed in these studies by detection method and product can be found in Table 1. Figure 3 graphically shows the meta-analytic summaries of prevalence for dairy samples from teat milk samples from individual animals, bulk tank milk, raw and pasteurized milk and cheese products as determined by culture and IS900 polymerase chain reaction (PCR) detection methods.

The prevalence on raw meat (mainly beef; one study examined mutton) was similar to commercial dairy products. Several studies demonstrated an increased likelihood of *M. paratuberculosis* detection on meat if the animal was clinically suspected of Johne's disease and/or positive for *M. paratuberculosis* by ELISA, PCR or culture, although the pathogen load in muscle was low, Table 1 (Reddacliff et al., 2010; Pribylova et al., 2011b). Some studies demonstrated a strong correlation between positive test results (culture and PCR) from fecal, mesenteric lymph node and intestinal samples (Pribylova et al., 2011b). One study demonstrated 80% of hamburger samples containing mesenteric lymph nodes from a cow diagnosed with clinical Johne's disease were positive (Mutharia et al., 2010). The effect of cooking or freezing was investigated in a few experiments (Mutharia et al., 2010; Whittington et al., 2010; Saucier and Plamondon, 2011); freezing to -18 or -196^{o}C did not eliminate *M. paratuberculosis* (Mutharia et al., 2010), and cooking to 70^{o}C for 2.5 minutes or 71.1^{o}C for 1.5 minutes gave a 12D reduction in *M. paratuberculosis* (Saucier and Plamondon, 2011).

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One study evaluated several European brands of infant formula by PCR for *M. paratuberculosis* and found a high prevalence; however none of the samples were culture-positive (Hruska et al., 2005). A small case control study identified *M. paratuberculosis* by culture from the breast milk of mothers affected by Crohn's disease, but not from control mothers, demonstrating that *M. paratuberculosis* may be excreted in breast milk, and that this excretion may be associated with Crohn's disease (Naser et al., 2000).

Prevalence in water

Since 2003 several studies examined *M. paratuberculosis* contamination in treated and untreated water. PCR results for drinking water were highly variable with an overall meta-analytic average of 23% (95%CI 6.1, 57.0), however there was only one report of a culture-positive drinking water sample (Aboagye and Rowe, 2011). Contamination of surface water, including lakes and rivers fed by runoff areas and water troughs, was investigated in seven studies that reported an overall prevalence of 12.8% culture-positive and 42.6%- 90% PCR-positive samples in several countries (Table 2). *M. paratuberculosis* can survive for 16-20 weeks in water and 28 to more than 90 weeks in sediment, thus the aquatic environment may be a significant source for both humans and animals (Pickup et al., 2005; Whittington et al., 2005). Based on environmental inoculation experiments, slope of the land and concentration of *M. paratuberculosis* on the soil were strong determinants of the contamination in runoff water (Salgado et al., 2013).

Prevalence in the environment

M. paratuberculosis has been shown to be a resilient organism in studies (n=9) investigating its survival in the environment and levels of contamination in and around ruminant farms (n=21). Seven studies collected non-manure samples in cattle barns with a culture-positive prevalence of 35.5% (95%CI 27.2, 43.9) and five studies collected manure samples with a prevalence of 54.9% (30.7, 79.1) (Table 2). In two of the studies the prevalence in manure was lower in the yard 2.0% (0.0, 7.1) and in the fields 7.6% (0.0, 31.1). Eisenberg (2011) demonstrated that in barns where 81% of dust samples were positive for *M.*

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paratuberculosis, high pressure cold water wash, disinfectant and two week waiting period before repopulation were necessary to reduce *M. paratuberculosis* below detectable levels (Eisenberg et al., 2011). Other studies showed composting was ineffective at eliminating *M. paratuberculosis* (Tkachuk et al., 2013) and *M. paratuberculosis* can survive in a biogas plant for up to 6 months with DNA detectable by PCR for longer (Slana et al., 2011). In experimental challenge trials invertebrates were shown to be competent *M. paratuberculosis* hosts; protozoa (Whan et al., 2006) and nematodes (Lloyd et al., 2001) were shown to take up the organism and provide a host environment for *M. paratuberculosis* to survive and replicate; another study demonstrated persistence and replication within protists over 24 weeks (Mura et al., 2006).

M. paratuberculosis Infection in animals

The majority of studies captured in this review focused on animal-level *M. paratuberculosis* infection in domestic ruminants (cattle, buffalo, sheep, goats, deer), other domestic animals and wildlife. These have been summarized in Figures 1-2 and in Table 3 by species and continent. There was large heterogeneity between studies and between meta-analytic prevalence estimates for apparently healthy animals by isolation method and continent for dairy cattle 4.5-20.7%, other cattle 0.5-29.9%, buffalo 1.0-37.0%, goats 3.8-46.4%, sheep 6.1-50.0%, farmed deer 1.0-39.0%, and in wild animal populations 0-100% (Table 3). Five studies were not included in Table 3 because the study sample was not representative of the target population or the article failed to report results of their survey. Among these, two non-representative surveys of dairy cattle in Iran and Turkey reported a meta-analytic prevalence in dairy cattle of 21% (11.6-32.3), I^2 70.8% for fecal staining (Anzabi et al., 2013; Yildirim and Civelek, 2013). *Mycobacterium ssp. paratuberculosis* was reported in farmed red deer in Ireland, wild red deer in Austria and farmed Tundra Reindeer in Scotland although no prevalence estimates were reported (Power et al., 1993; Glawischnig et al., 2006; Del-Pozo et al., 2013).

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A number of studies examined clinical or suspect Johne's disease ruminants or non-ruminants with apparent gastrointestinal disease. Two studies summarized the apparent prevalence of clinical Johne's disease in their national herd; in England 1995, JD cases per head of cattle 2.0% (132/6738) (Cetinkaya et al., 1998) and in the Czech Republic from 1995-2002 4,000,372 cattle carcasses were inspected and of these 0.026% (n =1039) were diagnosed with Johne's disease (Vecerek et al., 2003). Several other studies were undertaken to assess infection in ruminants with Johne's disease or non-ruminants with gastrointestinal disease, and are summarized in this paragraph. Dogs with gastrointestinal disease were not significantly more likely to be *M. paratuberculosis* positive (OR 7.14, 95%CI 0.39 -132.13) compared to control dogs (Glanemann et al., 2008). Prevalence of *M. paratuberculosis* in suspect Johne's disease dairy cattle by fecal culture was reported to be 50% in Egypt and 41% by culture or 32% by ELISA in Brazil (Salem et al., 2005; Ristow et al., 2007). Meta-analysis of results from buffalo suspected of Johne's disease in Pakistan and India were *M. paratuberculosis* positive in 12% of cases (95%CI 11-15, I^2 0%) by PCR, 5% (2-10, I^2 0%) by ELISA and 7% (0-27, I^2 99%) by visual confirmation (Sivakumar et al., 2006; Khan et al., 2010; Sikandar et al., 2012). Suspected Johne's disease in beef and dairy cattle from Europe and Asia were *M. paratuberculosis* positive in 16.8% of cases (10.9-23.7, I^2 91%) by PCR and culture (Branciari et al., 2008; Kaur et al., 2010; Khan et al., 2010; Munster et al., 2011). Studies of suspect caprine Johne's disease cases from Europe yielded a meta-analysis *M. paratuberculosis* prevalence of 24.6% (14.2-36.8, I^2 55%) by ELISA (Hartnagel, 2000; Stau et al., 2012). The only swine investigation collected lymph nodes with Johne's disease like lesions at slaughter and reported 4/50 were PCR-positive for *M. paratuberculosis* (Miranda et al., 2011). Two studies examining suspected Johne's disease in bison in the USA reported culture-PCR-positives in 66.5% (14.5-100, I^2 95%) of samples (Buergelt and Ginn, 2000; Ellingson et al., 2003). Five studies of suspected Johne's disease cases in deer were conducted; in white tailed deer in the USA, *M. paratuberculosis* culture prevalence was 22.1% (0-67.4, I^2 98%), and in red and fallow deer in Europe the culture prevalence was 23.8% (8.1-

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43.8, I^2 0%) (Marco et al., 2002; Hattel et al., 2004; Glawischnig et al., 2006; Woodbury et al., 2008; Sleeman et al., 2009). Pygmy goats from an infected herd were *M. paratuberculosis* positive by culture in 76.9% of cases and captive elk within a newly infected herd had a 35% mortality rate within the first two years of life due to *M. paratuberculosis* (Manning et al., 1123; 1280; Manning et al., 2003).

Molecular Epidemiology of *M. paratuberculosis*

Thirteen studies were captured that compared strain differences among *M. paratuberculosis* isolates recovered from various species and from different geographic areas. Most of these studies were based on existing isolate collections, and showed a fair amount of homogeneity within *M. paratuberculosis* strains and revealed that *M. paratuberculosis* strains from human infections were less diverse compared to cattle (Wynne et al., 2011). Results were derived from several methods making it difficult to make direct comparisons, including IS1311 restriction fragment length polymorphism (RFLP) analyses (Whittington et al., 2000; Motiwala et al., 2003; Singh et al., 2010b; Okuni et al., 2012; Liapi et al., 2015), IS900 RFLP (de Lisle et al., 1993; Francois et al., 1997; Whittington et al., 2000; Stevenson et al., 2009), pulsed-field gel electrophoresis (PFGE) (Stevenson et al., 2009), amplified fragment length polymorphism (AFLP) (Stevenson et al., 2009), mycobacterial interspersed repetitive unit (MIRU)-variable number tandem repeats (VNTR) locus (Stevenson et al., 2009; Fernandez-Silva et al., 2012) randomly amplified polymorphic DNA (RAPD) (Pillai et al., 2001), multi-locus short sequence repeat sequencing (MLSSR) (Fernandez-Silva et al., 2012), Multiplex PCR of IS900 integration loci (MPIL) (Motiwala et al., 2004), short sequence repeats (SSR) (Ghadiali et al., 2004), and single nucleotide polymorphisms (SNPs) (Wynne et al., 2011). A tally of the type of *M. paratuberculosis* (e.g. cattle, sheep, bison) found by species category is shown in Figure 4. Multiple fingerprinting techniques were used to show the diversity of *M. paratuberculosis* within populations, and it was argued that it is possible to demonstrate strain sharing within and across species by the degree of homogeneity between isolates (Stevenson et al., 2009). These results indicate that while cattle type *M. paratuberculosis* still

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predominates among ruminants (with the exception of sheep) and non-ruminants, there is also increasing evidence on the importance of bison type *M. paratuberculosis* and its ability, like the cattle type, to transfer between host species (Singh et al., 2010b; Sohal et al., 2014; Ahlstrom et al., 2015; Podder et al., 2015).

Risk Factors for developing Crohn's disease or *M. paratuberculosis* seropositivity

Risk factors for developing Crohn's disease or reactivity to *M. paratuberculosis* were evaluated in nine case-control studies, and the observed odds ratios for each examined risk factor are described in Table 4. Risk factors involving many types of foods, direct contact, occupation and environmental factors were inconsistently associated, showing a combination of positive, negative or no significant association with Crohn's disease or *M. paratuberculosis* seropositivity (Table 4). Several studies observed no association among Crohn's disease or *M. paratuberculosis* seropositivity and dairy consumption, living on a farm, having contact with animals or having Johne's disease on the farm, all of which were expected to increase an individual's exposure to *M. paratuberculosis* and risk of disease. Several studies found strong and consistent positive associations with consumption of processed meat and cheese (Van Kruiningen et al., 2005; Maconi et al., 2010; Spehlmann et al., 2012).

Drinking water did not have an association with Crohn's disease or *M. paratuberculosis* seropositivity in four case control studies (Table 4) (Bernstein et al., 2004; Van Kruiningen et al., 2005; Bernstein et al., 2006; Abubakar et al., 2007), but in another study was hypothesized to be the most likely common source for a cluster of Crohn's disease patients who lived in close proximity but did not know each other (Pierce, 2009).

Discussion

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This ScR identified and summarized 255 studies evaluating potential sources of *M. paratuberculosis* considered to be relevant to human exposure, including food intended for human consumption, water, ruminant and other animals, and a variety of environmental sources (e.g. within the farm environment, on pasture, river sediment). We identified published evidence for *M. paratuberculosis* contamination of water and some foods prepared for human consumption (milk, dairy products and meat), but no evidence for other hypothesized foods such as produce and seafood was found.

Most of the research captured in this ScR provided evidence of *M. paratuberculosis* contamination in the form of point prevalence outcomes from surveys of defined target populations/ samples, but few studies provided results estimating the average concentration in contaminated samples. The latter information is needed to develop a quantitative human exposure assessment model that could aid in the interpretation of the relative importance of various sources of *M. paratuberculosis*. Future studies should address these knowledge gaps. *M. paratuberculosis* is an extremely difficult organism to culture and/or identify in various samples (feces, milk, blood, water). Although not a focus of this ScR, variation in laboratory protocols across studies is an important source of heterogeneity. The exclusion of languages other than English and French from the review meant that 17 potentially relevant papers were excluded and although this is unlikely to change the conclusions, it may mean that some areas of the world are under-represented.

A large proportion of the research focused on the ruminant reservoir for *M. paratuberculosis* in different parts of the world. Although the results varied across studies, *M. paratuberculosis* was reported on every continent. Research examining wild and captive ruminant and non-ruminant species also highlighted that *M. paratuberculosis* may be isolated from many species, but the contribution of non-domestic ruminant species to human exposure to *M. paratuberculosis* relative to domestic ruminants has not been closely examined (Mura et al., 2006; Carta et al., 2013). The global evidence reported in

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this ScR shows that although there are some regional differences, *M. paratuberculosis* is a global animal health problem, an agri-food problem and possibly a zoonotic public health issue (Waddell et al., 2015).

Mycobacterium paratuberculosis has been shown to survive in a variety of environmental conditions ranging from several weeks in barn dust samples to almost two years in river sediment (Pickup et al., 2005; Tkachuk et al., 2013). This highlights the plausible link between ruminant excretion of *M. paratuberculosis* and subsequent contamination of the wider environment due to manure management, runoff from manure storage or use of manure as fertilizer (Pribylova et al., 2011a; Salgado et al., 2013). This probably leads to contamination of drinking water as well as produce (e.g. through irrigation) and seafood. Further research is required to examine how hardy *M. paratuberculosis* is in its spore-like state and how long it survives in manure storage and composting to better understand the risk of contaminating other potential environmental sources of *M. paratuberculosis* for humans (Lamont et al., 2012). Control programs for *M. paratuberculosis* will have to consider manure management strategies to manage the burden of *M. paratuberculosis* in the environment. Addressing these upstream sources of *M. paratuberculosis* at the animal and farm level will likely impact the level of contamination for many foods destined for human consumption and identified as possible sources of *M. paratuberculosis* in this ScR.

Studies of risk factors for Crohn's disease or *M. paratuberculosis* infection in humans (n=9) did not report significant association with the consumption of dairy products or contact with ruminants, both of which are considered likely routes of human exposure. There was also no association with water, or produce consumption (Van Kruiningen et al., 2005; Bernstein et al., 2006; Maconi et al., 2010). However, consumption of cheese and fermented meats were strongly associated with developing Crohn's disease in several studies (Van Kruiningen et al., 2005; Maconi et al., 2010; Spehlmann et al., 2012). The retrospective case control surveys offer a very low level of evidence and do not provide

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evidence of a causal relationship with Crohn's disease, but they are excellent studies for hypothesis generation and establishing directions for future research. On the other hand, there is likely to be a long latency period for any *M. paratuberculosis*-related disease in humans, and this poses challenges to the proper classification of food and environmental risk factors. Gathering evidence to address the remaining uncertainties concerning the importance of various exposures and the relationship between exposure and developing Crohn's disease is likely more complex than a simple exposure-dose response relationship and there are other necessary individual and /or environmental factors required to produce Crohn's disease. Research to date has been unable to clarify the necessary or sufficient factors that lead to development of Crohn's disease and there is a lack of evidence to support the assumption that prevention of human exposure to *M. paratuberculosis* will prevent Crohn's disease in some or all of the susceptible population. As an initial step, evaluating how humans are exposed to *M. paratuberculosis* and what role *M. paratuberculosis* plays in human disease are complementary priorities that can contribute to evidence-informed and risk-based evaluation of mitigation strategies.

For most groups of studies measuring the same outcome in this ScR, there was considerable outcome heterogeneity that could be due to variability in any individual or combination of factors including herd-level burden of Johne's disease, region, time and detection limits of isolation methods. Ruminant derived foods for human consumption including milk, dairy products and meat were shown to be sources of *M. paratuberculosis* sporadically at low concentrations. From the beef and sheep meat studies there were two important observations; meat samples that contained tissue from the intestine or mesenteric lymph nodes were at increased risk of *M. paratuberculosis* contamination, and the times and temperature required to kill *M. paratuberculosis* were longer than those required by USDA regulation for the elimination of E. coli O157 (Mutharia et al., 2010; Pribylova et al., 2011b; Saucier and Plamondon, 2011). The studies that identified *M. paratuberculosis* in human breast milk of Crohn's disease patients and commercial infant formula by PCR and recently by culture identifies route of

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exposure for infants (Naser et al., 2000; Hruska et al., 2005; Hruska et al., 2011; Botsaris et al., 2016). Drinking water may be a source of *M. paratuberculosis* for humans as shown in a small number of studies. Future research that investigates *M. paratuberculosis* susceptibility to drinking water treatments is needed to understand the potential risk of exposure through consumption of treated drinking water.

Molecular epidemiology studies can be used to compare isolates of *M. paratuberculosis*. This can aid in our understanding of potential sources and the degree of strain sharing within and between species and whether all or limited types of *M. paratuberculosis* are potentially zoonotic or shared between a variety of animal species. To date the results of these studies mainly indicate which ruminant species usually carry particular types of *M. paratuberculosis* and that there is some strain sharing across these species (Stevenson et al., 2009). Molecular methods have rapidly evolved and as our results show, multiple methods have been used, most recently whole genome sequencing of *M. paratuberculosis* (Singh et al., 2013; Ahlstrom et al., 2015). The multitude of molecular methods used to date has been a barrier to meaningful synthesis of molecular epidemiological results (Ahlstrom, et al., 2015; Muellner, et al., 2015), therefore as data from whole genome sequencing become more widely used in epidemiological investigations, researchers should seek consensus on target markers to aid in the comparability of results across studies. In future, molecular epidemiology studies should also be used to trace sources of human exposure (food, water, and environment) to *M. paratuberculosis* and further investigate the pathogenic characteristics of various *M. paratuberculosis* (Ghadiali et al., 2004).

Conclusion

This scoping review identified evidence for potential sources of human exposure to *M. paratuberculosis.* Food sources such as milk, cheese, other dairy products (both pasteurized and unpasteurized) and raw meat were shown in several studies to be contaminated. However, in case control studies consumption

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of many of these foods was not associated or had contradictory evidence of association with Crohn's disease or *M. paratuberculosis* seropositive status. Similarly, a number of foods (produce, seafood, processed meats) that are possible sources have not been examined and we were unable to rule out any hypothesized source of *M. paratuberculosis* for humans. The evidence supports that people consuming a typical diet are likely exposed at least intermittently to some (quantity uncertain) *M. paratuberculosis* via food and water. Successful Johne's disease controls programs may lead to a decrease in *M. paratuberculosis* load in many of the animal, food and environmental sources identified in this ScR (Waddell et al., 2015). Future research should address knowledge gaps pertaining to the concentration and prevalence of contamination in various sources that should be addressed to provide the necessary data for an exposure assessment model for humans and evidence-informed decision-making on this potential zoonotic public health issue.

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Figures

Figure 1: Flow of citations and articles through the scoping review on sources of human exposure to M. *Paratuberculosis*. *Articles sum to more than the total as some citations contribute to more than one category.

Figure 2: The number of studies captured in the scoping review is tabulated by study design and further tabulated by source of *M. paratuberculosis* under the main headings of animal, food and environment.

Figure 3: Summary of 12 random effect meta-analyses of the prevalence of *M. paratuberculosis* in products from dairy cattle, sheep and goat by culture and IS900 PCR for teat milk from individual cows, bulk tank milk, raw milk pasteurized milk, raw milk cheese, pasteurized milk cheese.

Figure 4: Bubble plot of the type of *M. paratuberculosis* reported in 10 molecular epidemiology studies by species category

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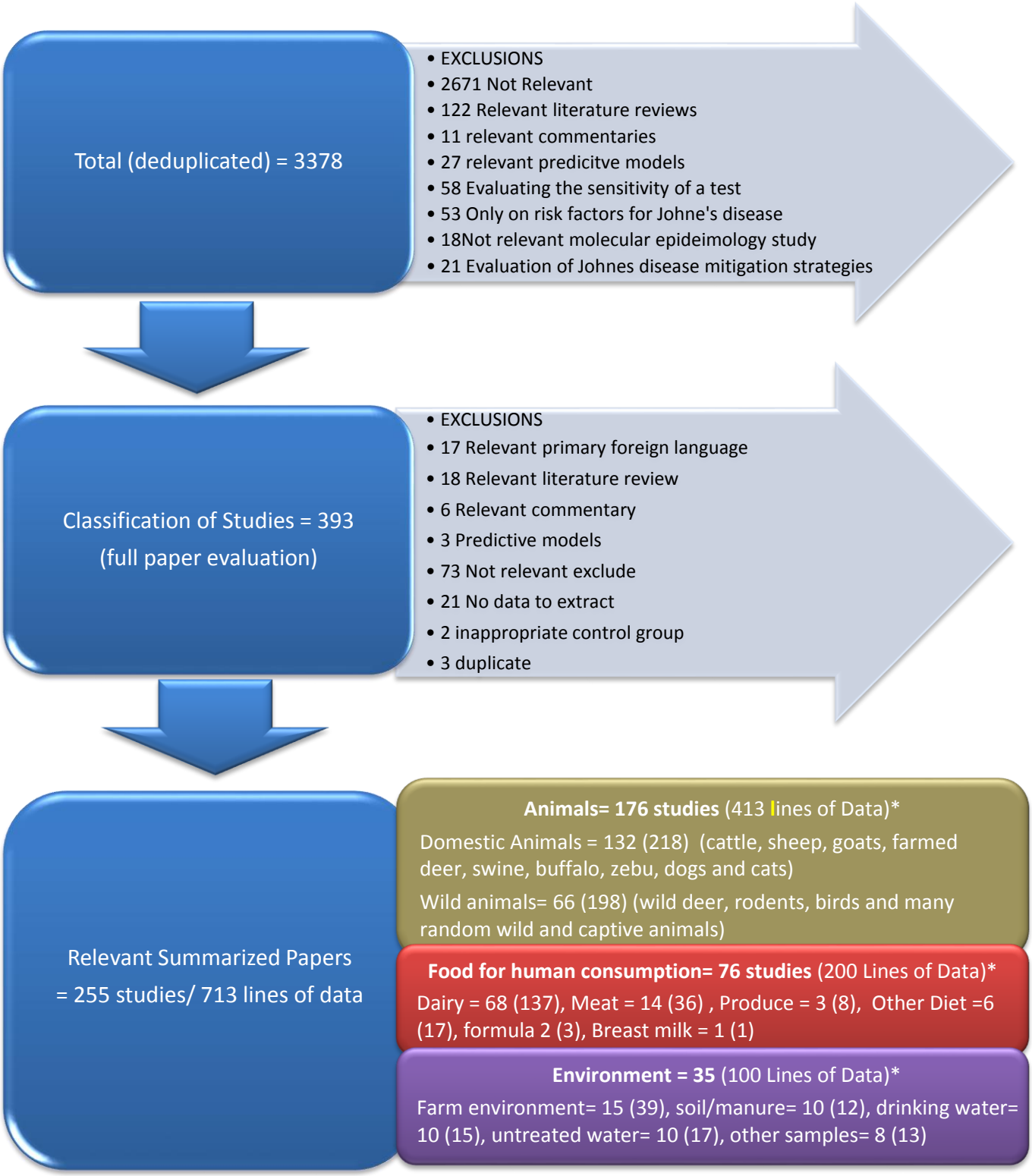

Figure 1

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Total Studies	N
Prevalence	164
Longitudinal prevalence	11
Cross-sectional	30
Case control	9
Cohort	2
Case report	11
Case series	7
Genotyping	13
Controlled trial	1
Challenge trial	8

Animal	N studies	Cattle	Buffalo	Sheep	Goats	Deer	Other Domestic	Wildlife
Prevalence	**117**	56	7	5	9	8	4	44
Longitudinal prevalence	**3**	2	0	0	0	0	0	1
Cross-sectional	**23**	17	1	4	3	1	0	2
Case Control	**3**	0	0	1	0	0	3	1
Case Report/Series	**17**	1	0	1	1	2	1	12
Genotyping	**13**	10	0	11	9	2	2	6

Food	N studies	Raw milk[Ŧ]	Past. milk	Raw cheese	Past. cheese	Other dairy[¥]	meat	Seafood	Produce	Other*
Prevalence	**56**	43	10	3	2	1	4	0	0	2
Longitudinal prevalence	**2**	1	0	0	1	0	0	0	0	0
Cross-sectional	**7**	5	1	0	0	0	2	0	0	1
Case Control	**8**	3	1	1	1	2	7	3	3	6
Genotyping	**1**	0	1	0	0	0	0	0	0	0
Controlled Trial	**1**	0	0	0	0	0	1	0	0	0
Challenge Trial	**1**	0	0	0	0	1	0	0	0	0

Ŧ = raw milk study counts include sampling of teat milk from individual animals, bulk tank milk and retail unpasteurized milk in this table. **Past.** = pasteurized, **¥**= Other dairy studies included yogurt, ice cream, flavoured milk drinks. ***** = formula (2), breast milk (1), risk factor studies that found an association with Crohn's disease and consumption patterns for coffee/ tea consumption, sugars, confections, grains, rice, pasta, oils, fats, soft drinks etc.

Environment	N studies	Treated Water	Untreated Water	Farm environment	Manure	Soil	Other*
Prevalence	**6**	4	2	2	0	1	0
Longitudinal prevalence	**6**	1	3	1	1	3	1
Cross-sectional	**7**	0	3	6	2	3	2
Case Control	**4**	5	0	1	0	0	1
Cohort	**2**	0	0	2	0	0	0
Case Report	**1**	1	0	0	0	0	0
Genotyping	**1**	0	0	1	0	0	4
Challenge Trial	**7**	0	2	1	0	1	4
Quasi-experiment	**1**	0	0	1	0	0	0

* Other includes studies that examined invertebrates (4), plant uptake of *M. paratuberculosis* (2) and farm related risk factors (3).

Figure 2.

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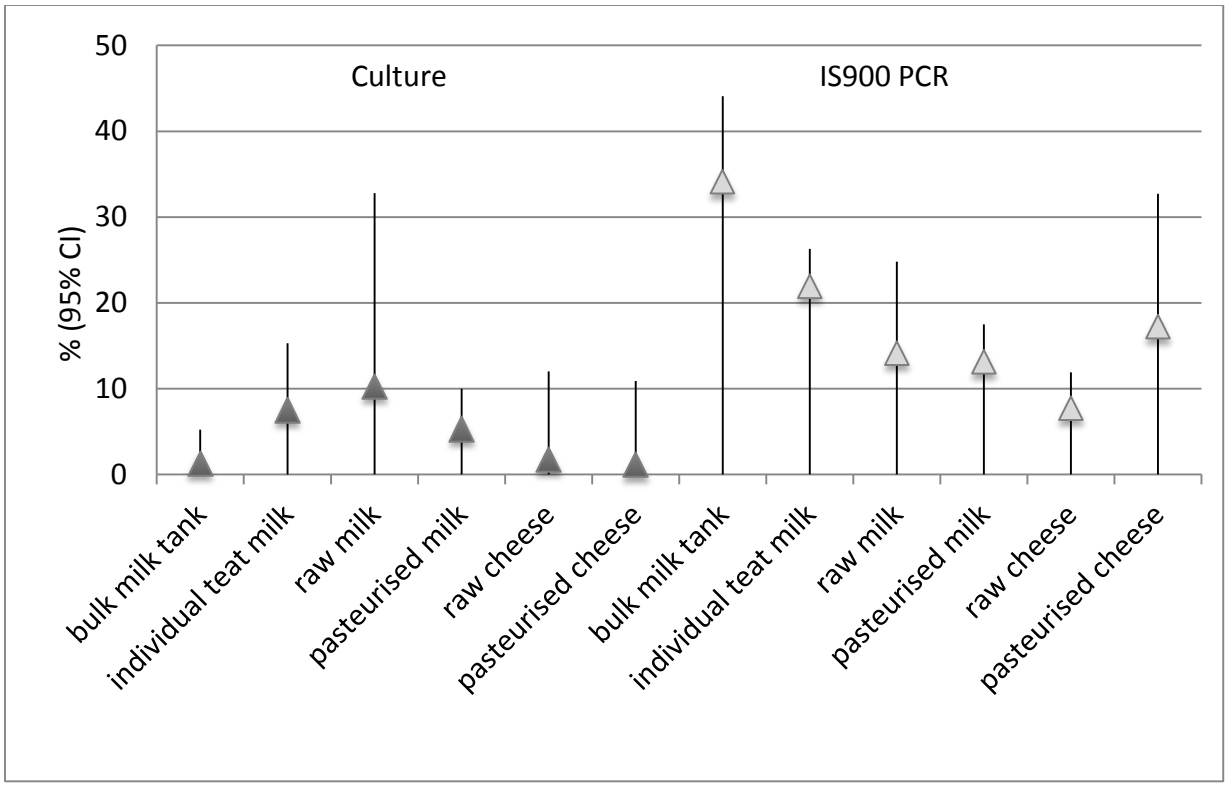

Figure 3

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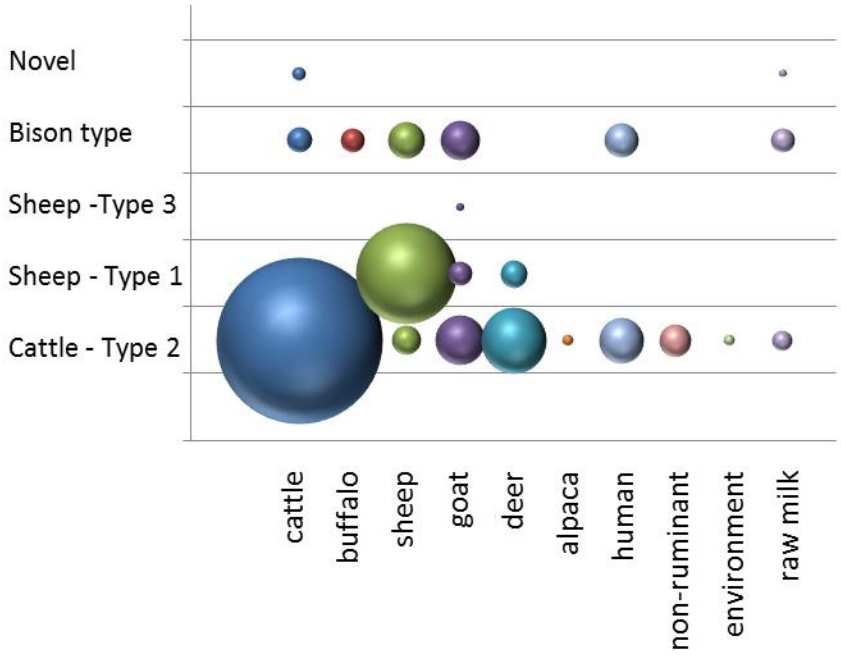

Figure 4

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Tables

Table 1

Table 1, Summary of Findings: the prevalence of *M. paratuberculosis* in milk, dairy products or other foods for human consumption

Population: milk, dairy and other foods for human consumption

Outcome: prevalence or concentration of *Mycobacterium avium* ssp. p*aratuberculosis*

Study Design: prevalence survey, longitudinal prevalence, cross-sectional

Studies grouped by sample the *M. paratuberculosis* detection method	Prevalence (95% CI) from a meta-analysis/ a single study value	Heterogeneity I^2	Number of observations / trials / studies	Comments
Bulk Tank Milk				
Culture	1.3% (0.0, 5.2)[MA+]	83%	1067/13/10	(2001-2010) 9 dairy cattle and 3 sheep and goat.
Dairy Cattle	3.5% (0.2, 9.1)[MA+]	88%	942/9/9	
Sheep and Goats	0%	0%	125/4/3	
ELISA	14.6% (12.9, 16.2)[MA]	99%	1701/2/2	Dairy cattle USA (2010) (Wilson et al., 2010) & Italy (2012) (Bergagna et al., 2012)
PCR – IS900	34.1% (24.1, 44.1)[MA]	98%	2974/16/13	(2001-2010) 12 dairy cattle and 3 sheep and goat.
Dairy Cattle	37.1% (26.3, 47.9)[MA]	89%	2849/12/12	
Sheep and Goats	14.4% (0.0, 54.9)[MA+]	86%	125/4/3	
PCR – F57	6.4% (1.5, 23.5)		220/1/1	Dairy cattle, Cyprus (2009) (Slana et al., 2009)
PCR – ISMav2	5.4% (1.3, 20.0)		423/1/1	Dairy cattle, Germany (2006) (Stratmann et al., 2006)
qPCR	28.6% (8.7, 62.8)		220/1/1	Dairy cattle, Cyprus (2010) (Ridge et al., 2010)
Teat Milk from Individual Animals				
Culture	7.5% (2.1, 15.3)[MA+]	97%	2938/13/11	(1995-2013) 9 dairy cattle, 3 goat and 1 sheep study
Dairy cattle	10.9% (3.4, 21.4)	97%	2550/9/9	(1995-2013) Dairy cattle teat prevalence ranged from 1%- 84% in global studies.
Sheep and Goats	0.7% (0, 12.5)[MA+]	81%	388/4/3	(2003-2010) One sheep and goat study from Cyprus (Botsaris et al., 2010), and goat studies from Norway (Djonne et al., 2003) reported 0% prevalence and India (13%) (Ronald et al., 2009)
ELISA	27.0% (8.4, 45.7)[MA]	96%	1361/4/4	(2000-2012) the prevalence in 3 dairy cattle from Denmark (8.8%) and India (32-58%) and 1 goat study (12%) from Chile.
PCR – IS900	21.9% (17.6, 26.3)[MA]	97%	4791/27/19	(2002-2013) 16 dairy cattle, 2 goat, and 1 sheep study.
Dairy cattle	19.3% (14.8, 23.8)[MA]	95%	3981/20/18	(2002-2013) representing herds in the Americas, Asia and Europe.

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Sheep and Goat	35.7% (19.7, 51.6)	99%	860/5/3	(2003-2009) the Studies from India found a prevalence of 55% in sheep (Selvam et al., 2009) and 27% in goats (Ronald et al., 2009) and 11% in goats from Norway (Djonne et al., 2003).
PCR – F57	14.6% (1.5, 65.5)		72/1/1	Dairy cattle (2008) Czech republic (Slana et al., 2008)
Staining	14.3% (10.5, 18.1)^{MA}	0%	319/3/3	Dairy cattle (2012-2013) in India (Vinodh Kumar and Gunaseelan, 2012),Iran (Anzabi et al., 2013) and Turkey (Yildirim and Civelek, 2013)
Raw Milk				
Culture	10.2% (0.0, 32.8)$^{MA+}$	93%	320/3/3	Prevalence studies from the United Kingdom (2002) (Grant et al., 2002a; Grant et al., 2002b) showed 0.8-6.7% and from India (2010) (Shankar et al., 2010) 44%.
PCR-IS900	14.1% (2.5, 24.8)^{MA}	95%	517/5/5	(2002-2012) Studies from the United Kingdom (Grant et al., 2002a; Grant et al., 2002b) Germany (Dzieciol et al., 2010), Italy (Giacometti et al., 2012) and India (Shankar et al., 2010)
PCR- F57	12.5% (3.1, 38.6)		16/1/1	Switzerland (2005) (Tasara and Stephan, 2005)
Pasteurized Milk				
Culture	5.3% (1.9, 10.0)$^{MA+}$	91%	2091/9/7	(2002-2012) Prevalence from the United Kingdom (2-7%), Czech republic (0.7-1.6%), USA (2.8%), Argentina (2.9%), Chile (2.7%) and India (56 – 72%).
PCR-IS900	13.1% (8.7, 17.5)^{MA}	79%	1792/8/7	(1996-2012) Prevalence from the United Kingdom (7-21%), Italy (4.5%), Canada (15.5%), and India (10-39%).
Staining	4.5% (0.2, 56.2)		10/1/1	India (2012) (Vinodh Kumar and Gunaseelan, 2012)
Pasteurized Milk Cheese				
Culture	1.1% (0.0, 10.9)$^{MA+}$	58%	143/5/3	(2005-2010) Variety of cheese from soft to hard. Czech Republic (0-4.3%), USA (1%), Scotland (67%).
PCR – IS900	17.2% (1.8, 32.7)^{MA}	89%	182/5/2	(2005-2006) A variety of cheese from Czech republic (3-20%), Greece (50%) and USA (5%)
Raw Milk Cheese				
Culture	1.7% (0, 12.0)$^{MA+}$	85%	258/4/3	(2007 – 2010) Variety of cheeses sampled Switzerland (0%), Scotland (0-36%), Cyprus (0%)
PCR- IS900	7.7 % (4.3, 11.9)$^{MA+}$	88%	200/2/1	(2010) Cyprus (Botsaris et al., 2010)
PCR- F57	4.5% (2.0, 9.7)		133/1/1	(2007) Switzerland (Stephan et al.,

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				2007)
Meat				
Culture – raw beef and sheep meat	3.3% (0.5,6.2)[MA]	95%	813/6/4	(2007-2011) Reported prevalence from Spain (12.8%) (Alonso-Hearn et al., 2009), USA (0%) (Jaravata et al., 2007) , Denmark (0.4%) (Okura et al., 2011) and Australia (4.5% in healthy sheep – 59% in clinical Johne's animals with concentrations ranging from 0.88-1.77 log10 *M. paratuberculosis* per gram of tissue.) (Reddacliff et al., 2010)
PCR – IS900	25.5% (5.7, 50.8)[MA]	96%	1057/9/3	(2008-2011) Reported prevalence from Canada (36.5%) (Meadus et al., 2008) beef carcass swabs, Denmark (4%) (Okura et al., 2011), and Czech Republic (16.7 – 50%) (Klanicova et al., 2011) raw beef samples mainly. Pork, chicken, lamb and cooked and fermented meats also sampled in Czech Republic.
PCR- F57	8.9% (6.7, 11.8)		482/1/1	Canada (2008) beef carcass swabs. (Meadus et al., 2008)
Infant Formula				
PCR- IS900	49% (35.7, 62.5)		51/1/1	(2005) Czech republic, 10 brands from 7 EU countries (Hruska et al., 2005)
PCR – F57	35.3% (23.5, 49.2)		51/1/1	(2005) Czech republic, 10 brands from 7 EU countries (Hruska et al., 2005). Concentration of *M. paratuberculosis* range: 48 – 32.5 x 10^3 per gram of dried milk (Hruska et al., 2011)
Breast Milk				
Culture	OR 55 (0.83, 3650)		7/1/1	(2000) Small case control study that cultured *M. paratuberculosis* from human breast milk of Crohn's disease patients (2/2), but not controls (0/5) (Naser et al., 2000)

CI: Confidence interval, MA: meta-analysis, MA+: A double arc sine transformation was used instead of the standard logit transformation. OR= odds ratio.
PCR: polymerase chain reaction, I^2: measure of heterogeneity

Footnote: The prevalence of *M. paratuberculosis* in foods was investigated in 62 studies by culture, PCR, ELISA or hybridization to identify *M. paratuberculosis* in samples (milk, cheese, other dairy, meat, infant formula and breast milk). 56/62 studies provided useable data and are included in the summaries and meta-analyses presented in this table. All 8 excluded studies were from teat milk from individual animal surveys where 3 sampled only Johne's disease positive cattle, not a representative sample (2), sample not reported (1), results (1) or total sample (1) not reported. Most studies were surveys or cross-sectional studies, one was a case control and there was a lot of unexplained heterogeneity across studies. Study estimates vary by sample, time, and location and there was not enough data globally to draw conclusions on trends or burden in particular areas. Thus the prevalence values presented here indicate the current findings; however, future research will likely

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alter the estimates from this summary of findings table.

Table 2

Table 2, Summary of Findings: the prevalence of *M. paratuberculosis* in water, soil and environment
Population: water, soil and environmental samples
Outcome: prevalence or concentration of *Mycobacterium avium* ssp. p*aratuberculosis*
Study Design: prevalence survey, longitudinal prevalence, cross-sectional

Studies grouped by sample then *M. paratuberculosis* detection method	Prevalence (95% CI) from a meta-analysis/ a single study value	Hetero-geneity I^2	Number of observations / trials / studies	Comments
Drinking Water				
Culture	2.3% (0.0, 66.8)		43/1/1	(2011) N. Ireland, clean water samples at a water treatment plant.
PCR – IS900	35.7% (21.5, 49.8)[MA]	98%	534/8/5	(2006-2012) One study from the USA reported high PCR results in Texas (76-88%), but not in a country wide survey (0%) (Beumer et al., 2010). Prevalence in South Wales (2%) (Pickup et al., 2006), Italy (3%)(Pistone et al., 2012), N. Ireland (47%)(Aboagye and Rowe, 2011), Germany and Spain (2%) (Villarreal et al., 2010)
Untreated Water				
Culture	8.7% (2.5, 17.0)[MA+]	64%	350/6/5	(2003-2010) Prevalence in water troughs in Slovakia (2%) and USA (17%). In runoff water in Australia (17%) and USA (38%) and in river water in UK (8-13%).
PCR – IS900	42.5% (25.5, 60.4)[MA]	88%	297/5/2	(2005-2011) Prevalence in river and lake water 29-69%, 23% in sewage and 56% at the water treatment plant in the UK and Northern Ireland (Pickup et al., 2006; Aboagye and Rowe, 2011)
Lake sediment				
PCR- IS900	90% (53.3, 98.6)		10/1/1	South Wales, UK (2006) (Pickup et al., 2006) samples from lake sediment on farms.
Barn Samples				
Culture	35.5% (27.2, 43.9)[MA]	99%	2584/25/7	(2004-2012) A variety of samples within the barn from cattle farms in the USA, the Netherlands and Slovakia .
PCR- IS900	36.4% (23.6, 51.4)		44/1/1	Dust samples, USA (2010) (Eisenberg et al., 2010)
Field samples				
Culture	7.6% (0.0, 31.1)[MA+]	96%	387/3/2	(2003, 2010) Pasture prevalence from Australia (9.5-20%) (Whittington et al.,

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				2003) and Slovakia (0.4%) (Pavlik et al., 2010)
PCR-IS900	69.1% (58.6, 79.6)[MA]	0%	72/4/1	One study from Czech republic (2011) examining the *M. paratuberculosis* uptake of plants in a naturally contaminated field. (Pribylova et al., 2011a)
Farm - manure				
Culture	54.9% (30.7, 79.1)[MA]	98%	846/5/5	(2004-2011) USA environmental samples on cattle farms mainly focused on manure and manure storage.
Yard				
Culture	2.0% (0.0, 7.1)[MA+]	0%	97/2/2	(2003-2011) yard samples from a sheep farm in Australia (0%) (Whittington et al., 2003) and a cattle farm in the Netherlands (4.4%) (Eisenberg et al., 2012)

CI: Confidence interval, MA: meta-analysis, MA+: A double arc sine transformation was used instead of the standard logit transformation. OR= odds ratio.
PCR: polymerase chain reaction, I^2: measure of heterogeneity

Footnote: The prevalence of *M. paratuberculosis* in water was investigated in 11 studies using culture and PCR, 10 of which provided useable data and are included in the summaries and meta-analyses presented in this table. One excluded study did not use a *M. paratuberculosis* specific PCR. There were few studies that have investigated *M. paratuberculosis* in water and those captured in this review are not representative of their countries or of the global burden. The heterogeneity was high between studies and reasons for this have not been explained. Future research will likely change the estimates in this summary of findings table.

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Table 3: Individual animal prevalence of *M. paratuberculosis* in domestic and wild animal populations from meta-analyses or individual study results organised by continent.

Number of observations/trials/studies (% trials with zero prevalence)[a]
Meta-analysis prevalence (%) estimates (95% CI) [b]
Heterogeneity rating / Risk of selection bias (low, medium or high)[c]

	North America	Europe	Australasia	South America	Asia/ Middle East	Africa
Cattle						
Dairy Cattle - Culture	16145/8/8 (0%) 6.6% (4.6-9.0)[f] High / Low	1735/1/1 (0%) 6.4% (5.3-7.6)[f] na /Low	N/A	N/A	1022/4/4 (0%) 11.3% (3.2-23.4)[f] High / High	N/A
Dairy Cattle – PCR	328/2/1 (0%) 4.7% (3.2-6.5) [s,f] High / Low	404/4/1 (0%) 4.0% (1.3-7.8) [f,t] Med / High	N/A	N/A	750/4/3 (0%) 21.5% (11.5-33.5)[f] High / High	N/A
Dairy Cattle- ELISA	67858/14/12 (0%) 5.1% (3.9-6.4)[s] High / Low	25817/9/9 (0%) 7.5% (4.2- 11.7)[s] High / Low	N/A	715/2/2 (0%) 5.3% (3.8-7.1)[s] High / Low	2014/3/3 (0%) 5.9% (4.9-7.0)[s] Low/ Low	N/A
Beef Cattle – ELISA	12287/5/5 (0%) 2.1% (1.2-3.4)[s] High / Low	6576/3/3 (0%) 1.7% (0.4-4.0)[s] High / Low	11515/1/1 (0%) 0.6% (0.5-0.7)[s] Na/ Low	N/A	1646/2/2 (0%) 0.8% (0.4-1.3)[s] Low / Low	N/A
Mixed Cattle – culture/ PCR	N/A	756/1/1 (0%) 0.5% (0.1-1.2) Na / Low	N/A	N/A	96/1/1 (100%) - Na / High	N/A
Mixed Cattle – ELISA - Bovine type target	N/A	138780/5/5 (0%) 2.8% (1.5-4.6)[s] High / Low	22612/1/1 (0%) 0.9% (0.8-1.0)[s] Na / Low	N/A	927/2/2 (0%) 3.2% (2.1-4.5)[s] High / Low	943/1/1 (0%) 3.7% (2.6-5.0)[s] Na/ Low
Mixed Cattle – ELISA Bison type target	N/A	N/A	N/A	N/A	452/1/1 (0%) 29.9% (25.7-34.2)[s] Na / Low	N/A
Other Large Ruminants –ELISA / immune reaction – Bovine type target	N/A	Buffalo 1350/1/1 (0%) 2.7% (1.9-3.7)[s] Na / Low	N/A	N/A	Buffalo 711/3/3 (33%) 1.1% (0.0-5.3)[s] High / Med	N/A
Other Large Ruminants –ELISA - Bison type target	N/A	N/A	N/A	N/A	Buffalo 1140/2/2 (0%) 31.1% (28.5-33.9)[s] High / Med	N/A

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Other Large Ruminants – culture/PCR	N/A	N/A	N/A	Zebu 160/1/1 (0%) 1.3% (0.3-4.9)[f] Na/ Low	Buffalo 75/2/1 (0%) 35.9% (25.1-46.8)[t] Low/ High	N/A
Goat						
Goat - Culture	N/A	220/1/1 (0%) 5.5% (2.8-8.9)[f] Na / Low	N/A	602/3/3 (0%) 9.2% (6.9-11.5)[f] Low / Low	101/2/2 (0%) 46.5% (36.8-56.2)[f] Low / High	N/A
Goat – PCR	N/A		N/A		30/1/1 (0%) 60.0% (41.1-77.0)[f] Na/ High	N/A
Goat – ELISA	N/A	12076/2/2 (0%) 3.0% (2.7-3.3)[s] High / Low	N/A	41/1/1 (0%) 22% (12-37)[s] Na / high	953/3/3 (0%) 30.3% (9.9-50.6)[s] High / Med.	12/1/1 (0%) 8.3% (0.0-32.0) Na/ High
Sheep						
Sheep – Culture	N/A	180/1/1 (0%) 6.1% (3.0-10.1)[f, t] Na / Low	26/2/2 (0%) 50% (27.2-72.8)[t, f] Low / High	N/A	N/A	N/A
Sheep – PCR	N/A	N/A	N/A	211/1/1 (0%) 9.5% (5.9-13.8)[s] Na/ Low	N/A	N/A
Sheep – ELISA / immunoassay	N/A	4740/5/5 (0%) 7.9% (3.7-13.3)[s] High / Low	N/A	211/1/1 (0%) 7.6% (4.4-11.6)[s] Na / Low	320/1/1 (0%) 18.1% (14.1-22.5)[s] Na/ Low	N/A
Farmed Deer						
Farmed Deer[d] – culture	205/2/2 (0%) 3.6% (1.3-6.8)[t] High/Low	2814/6/2 (0%) 14.8% (4.4-29.8)[f] High / Low	251/1/1 (0%) 39.0% (33.2-45.2)[f] Na/ Low	N/A	N/A	N/A
Farmed Deer [d] - ELISA	341/2/2 (0%) 3.5% (1.6-5.5)[s] Na/ High	670/4/4 (0%) 9.6%(2.7-16.6) [s] High / Low	N/A	N/A	N/A	N/A
Wild Deer						
Wild Deer [d] – Culture	1487/5/5 (0%) 2.2% (0.6-4.6)[t] High/ med	3995/10/4 (20%) 4.4% (1.5-8.5)[t,f] Low / High	N/A	N/A	N/A	N/A
Wild Deer [d] - PCR	170/1/1 (0%) 1.2% (0.3-4.2)[t]	114/2/2 (0%) 9.9% (4.7-16.4)[t]	N/A	N/A	N/A	N/A

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	Na/ low	High/ High				
Wild Deer [d]- ELISA	1381/3/3 (0%) 2.2% (1.5-3.1)[s] Low/low	2390/9/6 (22%) 6.7% (0.9-16.4)[s] High/ High	N/A	N/A	N/A	N/A
Other wild ruminants[W]						
Wild Antelope- (Boselaphus tragocamelus) culture	N/A	N/A	N/A	N/A	42/1/1 (0%) 23.8% [f] na / High	N/A
Wild Bison – PCR / ELISA	385/1/1 (0%) 3.11%[f] Na/low	62/2/1 (0%) 8.3% (0-24.5)[s] High/High	N/A	N/A	N/A	N/A
Mouflon (Ovis aries musimon)- culture / ELISA	N/A	798/2/2 (0%) 2.3%[t,f] / 1.0%[s] N/a / Low	N/A	N/A	N/A	
wild guanacos (lama guanicoe) - culture	N/A	N/A	N/A	501/1/1 (0%) 4.2%[f] Na/ low	N/A	N/A
Wild rocky mountain bighorn sheep – culture/ PCR	69/2/1 (50%) 0.0% /4.35%[f,t] Na/ low	N/A	N/A	N/A	N/A	N/A
Wild- Captive Zoo Herds[z]	N/A	74/4/1 (0%) 10.5% (3.0-21.3)[s] Low/Low	N/A	N/a	N/A	N/A
Wild Animals[W]						
Wild Armadillo – culture	23/1/1 17.4%[t] Na / High	N/A	N/A	N/A	N/A	N/A
Badger - culture	5/2/2 (50%) 22.7% (0-77)[t] High/High	N/A	N/A	N/A	N/A	N/A
Wild brown bear (Ursus arctos) – culture	N/A	20/1/1 (0%) 10%[t] Na / Low	N/A	N/A	N/A	N/A
Wild Boar- culture/PCR	N/A	851/2/2 (0%) 0.0% (0-0.4)[f,t]	N/A	N/A	N/A	N/A

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		Med/ Low				
Wild Brushtail possum – culture	N/A	N/A	73/1/1 (0%) 25%[t] Na / high	N/A	N/A	N/A
wild coyotes (Canis latrans)	63/1/1 (0%) 23.8%[t] Na / Low	N/A	N/A	N/A	N/A	N/A
Feral cats (felis familiaris) – culture	30/3/3 (0%) 31.3% (6.9-61.9)[t] High/ high	N/A	23/1/1 (0%) 17.4%[t] Na/ high	N/A	N/A	N/A
Wild ferret – culture	N/A	N/A	44/1/1 (0%) 6.8%[t] Na/ high	N/A	N/A	N/A
Wild fox- culture and ELISA	73/1/1 (0%) 39.7% [t] Na/ high	343/3/3 (33%) 21.0% (0.0-79.2)[f,t] High/ high	N/A	N/A	N/A	N/A
Wild Hares – culture	N/A	36/2/2 (50%) 0.0% (0.0-6.3)[f,t] Med/High	81/2/1 (50%) 3.2%(0.0-9.9)[t] Low/High	380/2/1 (0%) 12.6%[t], 4.21%[f] NA/ Low	N/A	N/A
Wild hedgehog – culture	N/A	N/A	55/2/1 (0%) 36.2% (23.5-49.7)[t] Low/ high	N/A	N/A	N/A
Wild Mice - culture	9/1/1 (0%) 11.1%[t] Na/high	149/1/1 1.3%[t] Na / high	N/A	N/A	N/A	N/A
Wild opossums (Didelphis virginiana)- culture	62/3/3 (33%) 3.9% (0.0-8.9) Low / High	N/A	N/A	N/A	N/A	N/A
Wild rabbits	64/2/2 (50%) 0.3% (0.0-5.2)[t,f] Low/ High	1267/7/5 (14%) 19.0% (6.3-36.2)[t,f] High/ Low	142/3/3 (33%) 15.5% (5.0-29.0)[t,f] Low/High	N/A	N/A	N/A
Wild racoons – culture	115/4/4 (25%) 17.5% (0.0-36.7)[t] High/ high	N/A	N/A	N/A	N/A	N/A
Wild rat - culture	45/2/1 (0%) 2.7% (0.0-7.4)[t] Med/ Med	90/2/2 (0%) 42.7% (0.0-6.0)[t] Med/Low	4/1/1 (100%) - N/a / High	N/A	N/A	N/A

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Wild rhesus macaques – culture	N/A	N/A	N/A	N/A	25/1/1 (0%) 8.0%[f] Na/ high	N/A
Wild skunks (Mephetis mephetis) – culture	15/2/2 (0%) 17.9% (0.8-35.0)[t] high/ high	N/A	N/A	N/A	N/A	N/A
Wild southeastern shrew- culture	4/1/1 (0%) 25% [t] Na/ high	N/A	N/A	N/A	N/A	N/A
Wild Stoat - culture	37/1/1 (0%) 45.6%[t] Na/Low	N/A	5/1/1 (100%) - Na/ High	N/A	N/A	N/A
Wild weasel - culture	N/A	5/2/2 (50%) 30.0% (0.0-68.0)[f,t] High/High	N/A	N/A	N/A	N/A
Wild voles- culture	N/A	39/1/1 (0%) 5.1%[t] Na/ high	N/A	N/A	N/A	N/A
Wild birds						
Wild black-backed gull – culture	N/A	N/A	5/1/1 (0%) 20%[t] Na / high	N/A	N/A	N/A
Wild Jackdaw- culture	N/A	38/1/1 (0%) 8% [t] n/a/ High	N/A	N/A	N/A	N/A
Wild house sparrow – culture	60/1/1 (0%) 1.67%[t] Na/ high	44/1/1 (0%) 2.27%[t] Na/ high	N/A	N/A	N/A	N/A
Wild magpie - culture	N/A	10/1/1 (0%) 10%[t] Na / high	4/1/1 (100%) - Na / high	N/A	N/A	N/A
Wild Starling – culture	104/2/2 (50%) 6.1% (0.00-31.7)[t, f] High/ High	N/A	2/1/1 (100%) - Na/ high	N/A	N/A	N/A
Wild waterfowl - PCR	N/A	73/1/1 (0%) 11% [f] n/a / Low	N/A	N/A	N/A	N/A

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N/A = No data available. Med. = medium. [f] feces, [t] tissue, [s] blood/ serum, [d] Deer species sampled in Kovecna (2006) included Sika and fallow deer; in Boadella (2010) Iberian roe deer and the rest of the data is based on Red deer. [W] *M. paratuberculosis* was not cultured in Australasian Harrier (n=3) (Nugent et al., 2011), bezoar (Capra aegagrus) (27) (Kopecna et al., 2006), chamois (Rupicapra rupicapra) (134) (Kopecna et al., 2006), hawk (1) (Florou et al., 2008), owl (1) (Florou et al., 2008), Spur-winged plover (1) (Nugent et al., 2011), wolf (1) (Florou et al., 2008), duck (1) (Whittington et al., 2003). *M. paratuberculosis* was cultured in all samples for Eurasian Otter (n=2) (Matos et al., 2013), Common snipe (1) (Corn et al., 2005), Tapir (1), Okapi (1), Gayal (1), Blesbok (3), Banteng (1), Barbary Sheep (3) (Vansnick et al., 2005). [z] Eland (Taurotragus oryx), Nilgai (Boselaphus tragocamelus), Pudu (Pudu pudu), Yak (Poëphagus mutus grunniens).

[a] Observations/trials/studies: The observations are the total number of samples for all studies included in the summarized category. The number of studies is the number of articles captured. In some cases, articles report data on multiple prevalence trials or sampling frames. While the observations for each trial are independent by time and sample, they are part of a larger study where the methods and investigators are the same. Thus, there is not full independence in these observations and we note this by acknowledging there are multiple trials within a study.

[b] Indicates an average prevalence estimate (and 95% confidence interval) from a random-effects meta-analysis. Meta-analysis estimates were calculated only at least one trial found a positive sample. For those with an overall prevalence <10% or >90% we used a double arc sine transformation and for prevalence 10-90% we used the standard logit transformation.

[c] I^2 is a measure of the degree of heterogeneity between trials combined in the meta-analysis. Heterogeneity rating definitions: low = I^2 0-30%; medium = 31-60%; high = >60%. Selection bias rating definitions: high = 0-30% of trials used a representative sample; medium = 31-60% of trials used a representative sample; low = >60% of trials used a representative sample. Studies that conducted random or systematic sampling were considered representative.

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Table 4:

Table 4, Summary of Findings: The association between being Crohn's disease positive or M. *paratuberculosis* seropositive^ and food, water and environmental risk factors reported in 9 studies.
Outcome: Odds Ratio (95% Confidence interval): N/trials/studies
Study Design: case-control

Risk Factor	Protective Association	No Association	Risky Association	Reference
Meat		NS: 532/2/2	1.19 (1.06, 1.34): 218/1/1	(Sakamoto et al., 2005; Abubakar et al., 2007)
Processed Meat			7.8 (1.61, 37.89): 185/1/1 7.9 (2.15, 38.12): 104/1/1	(Maconi et al., 2010; Spehlmann et al., 2012)
red meat		NS: 185/1/1 1.34 (0.72, 2.51): 217/1/1		(Van Kruiningen et al., 2005; Maconi et al., 2010)
white meat (chicken)		NS: 185/1/1 1.42 (0.92, 2.16): 797/1/1		(Bernstein et al., 2006; Maconi et al., 2010)
Pork			2.48 (1.4, 4.4): 797/1/1 2.52 (1.06, 6): 217/1/1	(Van Kruiningen et al., 2005; Bernstein et al., 2006)
Eggs		NS: 532/2/2		(Sakamoto et al., 2005; Maconi et al., 2010)
Dairy		0.95 (0.85, 1.06): 218/1/1 0.5 (0.24, 1.05)*: 347/1/1 4.62 (0.52, 218): 5361/1/1		(Sakamoto et al., 2005; Jones et al., 2006; Abubakar et al., 2007)
Unpasteurized Milk	0.67 (0.49, 0.91): 797/1/1	0.55 (0.07, 4.11): 5361/1/1	2.24 (1.1, 4.58): 217/1/1	(Van Kruiningen et al., 2005; Bernstein et al., 2006; Jones et al., 2006)
Pasteurized Milk	0.86 (0.77,0.96): 218/1/1	NS: 185/1/1		(Abubakar et al., 2007; Maconi et al., 2010)
Cheese			3.7 (1.14, 12.01)*:185/1/1 6.54 (1.94, 22): 217/1/1	(Van Kruiningen et al., 2005;

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				Maconi et al., 2010)
Fish	0.18 (0.05, 0.67)*:185/1/1	1 (0.9, 1.12): 218/1/1 NS: 347/1/1	2.41 (1.18, 4.89)* 347/1/1	(Sakamoto et al., 2005; Abubakar et al., 2007; Maconi et al., 2010)}
Tuna	0.25 (0.08, 0.77)*:185/1/1			(Maconi et al., 2010)
Vegetables	0.21 (0.05, 0.78)*:185/1/1		2.19 (1.14, 4.22)~:347/1/1	(Sakamoto et al., 2005; Maconi et al., 2010)
Potatoes	0.24 (0.06, 0.91)*:185/1/1	NS: 347/1/1		(Sakamoto et al., 2005; Maconi et al., 2010)
Mushrooms		NS: 347/1/1		(Sakamoto et al., 2005)
Fruit	0.78 (0.7, 0.87): 218/1/1	NS: 532/2/2		(Sakamoto et al., 2005; Abubakar et al., 2007; Maconi et al., 2010)
Bread		NS: 532/2/2		(Sakamoto et al., 2005; Maconi et al., 2010)
Grains	0.51 (0.27. 1) - bran 0.38 (0.21, 0.7)- oat 0.2 (0.1, 0.38) - rye 217/1/1	NS: 185/1/1		(Van Kruiningen et al., 2005; Maconi et al., 2010)
Rice		NS: 532/2/2		(Sakamoto et al., 2005; Maconi et al., 2010)
Pasta		NS: 532/2/2		(Sakamoto et al., 2005; Maconi et al., 2010)
Nuts and Seeds		NS: 347/1/1		(Sakamoto et al., 2005)
Oil		NS: 185/1/1		(Maconi et al., 2010)

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Butter/ margarine		NS: 185/1/1		(Maconi et al., 2010)
Fats			2.64 (1.29, 5.39)*:347/1/1	(Sakamoto et al., 2005)
Sugar			2.12 (1.08, 4.17)*:347/1/1	(Sakamoto et al., 2005)
sweets/ confections		NS: 185/1/1	2.83 (1.38, 5.83): 347/1/1	(Sakamoto et al., 2005; Maconi et al., 2010)
soft drink consumption		0.68 (0.44, 1.04): 797/1/1		(Bernstein et al., 2006)
Coffee/Tea consumption		NS: 5361/1/1		(Jones et al., 2006)
Filtered Water	0.45 (0.27, 0.76): 218/1/1			(Abubakar et al., 2007)
Public Water supply	0.34 (0.18. 0.66): 217/1/1	1.35 (0.39, 4.72): 218/1/1 0.93 (0.69, 1.26)^: 967/1/1		(Bernstein et al., 2004; Van Kruiningen et al., 2005; Abubakar et al., 2007)
Private Water		0.77 (0.56, 1.06): 797/1/1 NS: 217/1/1		(Van Kruiningen et al., 2005; Bernstein et al., 2006)
Pet cat as a child	0.68 (0.5, 0.92): 797/1/1			(Bernstein et al., 2006)
Pet cat	0.13 (0.06, 0.29): 217/1/1			(Van Kruiningen et al., 2005)
Pet dog	0.49 (0.27, 0.92): 217/1/1			(Van Kruiningen et al., 2005)
Pet bird	0.45 (0.22, 0.93): 217/1/1			(Van Kruiningen et al., 2005)
Relative with CD or IBD		CD 2.8 (0.5, 14.8): 1526/1/1 IBD 1.01 (0.74, 1.37):967/1/1		(Qual et al., 2010)
Occupation: veterinarian		NS: 1526/1/1		(Qual et al., 2010)
living on a farm		0.69 (0.44, 1.07): 797/1/1 NS: 1526/1/1 1.52 (0.18, 70.1): 5361/1/1 0.68 (0.43, 1.06)^ - poultry 0.92 (0.65, 1.31)^ - cattle 0.97 (.62-1.51)^ - pig 967/1/1		(Bernstein et al., 2004; Bernstein et al., 2006; Jones et al., 2006; Qual et al., 2010)
visiting a farm		1.02 (0.68, 1.54): 218/1/1 3.81 (0.2, 224.6): 5361/1/1		(Jones et al., 2006; Abubakar et

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				al., 2007)
Contact with farm animals		1.28 (0.74, 2.21): 218/1/1 2.5 (0.3, 20): 1526/1/1 0.64 (0.11, 4.39): 5361/1/1		(Jones et al., 2006; Abubakar et al., 2007; Qual et al., 2010)
JD on the farm		1.63 (0.15, 9.95): 5361/1/1		(Jones et al., 2006)
Animal density in water catchment		NS: 218/1/1		(Abubakar et al., 2007)
JD in water catchment		NS: 218/1/1		(Abubakar et al., 2007)

CD: Crohn's disease, JD: Johne's disease, NS: not significant, OR odds ratio, CI: confidence interval, * high calorie consumer, ~ low calorie consumer, ^ odds of being *M. paratuberculosis* ELISA positive.