Université de Montréal

Facteurs de risque associés au statut de troupeau positif à *Mycobacterium avium* ssp. *paratuberculosis*

par María Puerto Parada

Département de sciences cliniques
Faculté de médecine vétérinaire

Mémoire présenté à la Faculté de médecine vétérinaire
en vue de l'obtention du grade de Maîtrise ès sciences (M. Sc.)
en sciences vétérinaires option sciences cliniques

Décembre 2017

Résumé

La paratuberculose (PTB) est une maladie entérique chronique, contagieuse et incurable qui affecte les ruminants et est causée par *Mycobacterium avium* ssp *paratuberculosis* (MAP). Les pertes économiques et l'association entre le MAP et la maladie de Crohn maintiennent un intérêt pour la paratuberculose. Les animaux s'infectent à un jeune âge, principalement par la voie féco-orale. Après une longue période d'incubation (jusqu'à plusieurs années), les vaches débutent l'excrétion fécale de MAP en absence de signes cliniques, perpétuant ainsi l'infection dans le troupeau. Les pratiques de gestion qui limitent l'exposition aux matières fécales contenant le MAP des animaux susceptibles sont plus efficaces pour réduire la prévalence que la simple élimination des animaux positifs. Les objectifs de ce mémoire sont : 1) Examiner et résumer de façon critique la littérature scientifique disponible sur les pratiques de gestion (mesurées à l'aide d'un questionnaire d'analyse de risque) associées au statut du troupeau pour MAP, et 2) identifier l'association entre les pratiques de gestion utilisées et le statut du troupeau pour MAP (déterminé à l'aide de culture bactériologique d'échantillons environnementaux) dans les troupeaux laitiers du Québec.

Pour le premier objectif, une revue globale de la littérature a été réalisée. Nous avons inclus des études qui ont évalué les facteurs de risque de PTB en utilisant un questionnaire d'analyse de risque (QAR) et mesuré l'association entre les facteurs de risque et le statut du troupeau pour MAP. Pour le deuxième objectif, une étude cas-témoins a été conçue. Un total de 26 troupeaux où MAP a été isolé d'au moins 1 échantillon environnemental et 91 troupeaux témoins (aucun cas clinique de paratuberculose et négatifs lors de 2 prélèvements environnementaux annuels consécutifs) ont été sélectionnés. Une régression logistique multivariée a été utilisée pour évaluer l'association entre les facteurs de risque sélectionnés et le statut du troupeau pour MAP.

En tout, 21 études transversales, 5 études cas-témoins et 3 études longitudinales répondaient aux critères d'inclusion. La taille du troupeau était significativement associée à un statut de troupeau positif à MAP dans 12 (dont 4 avec faible risque de biais (RB)) sur 18, l'introduction de nouveaux animaux était significativement associée à un statut de

troupeau positif à MAP dans 10 (dont 4 avec faible RB) sur 24 études, l'histoire de PTB était significativement associée à un statut de troupeau positif à MAP dans 6 (dont 4 à faible RB) sur 13, et la gestion du colostrum et du lait était significativement associée à un statut de troupeau positif à MAP dans 5 (aucun à faible RB) sur 18. Dans les troupeaux laitiers du Québec la taille du troupeau (OR = 1,17; IC à 95%: 1,02-1,33) et la proportion de vaches achetées par année au cours des 5 dernières années (OR = 5,44 IC à 95%: 1,23-23,98) étaient significativement associées à un statut de troupeau MAP positif.

Les résultats de ce mémoire fournissent une grande compilation des informations disponibles sur les facteurs de risque associés au statut du troupeau pour MAP et évalués à l'aide d'un QAR. Certains facteurs de risque sont apparemment plus consistants d'une étude à l'autre. Cependant, les résultats doivent être interprétés à la lumière de la qualité et du risque de biais de chaque étude. Les pratiques de gestion visant à empêcher l'introduction de nouveaux animaux dans le troupeau et à réduire le contact des veaux nouveau-nés avec les animaux adultes ou leurs excréments sont des éléments clés pour minimiser l'introduction et la transmission du MAP dans un troupeau. Ces éléments devraient être priorisés dans les programmes de contrôle.

Mots-clés : *Mycobacterium avium* ssp. *paratuberculosis*, troupeaux laitiers, facteurs de risque, pratiques de gestion, culture de prélèvements de l'environnement, statut des troupeaux.

Abstract

Paratuberculosis is a chronic and contagious enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Control of paratuberculosis is justified given the associated economic losses and the potential role of MAP in Crohn's disease in humans. Cattle usually become infected at a young age, primarily by the fecal-oral route. After a long incubation period (up to several years), infected cows may start shedding MAP without showing clinical signs, thus perpetuating MAP infections on the farm. Management procedures that limit exposure of susceptible animals to MAP are more effective at reducing disease prevalence than simply testing and culling MAP infected cows. Any management practices that expose (directly or indirectly) susceptible animals to fecal material from MAP shedders can be considered a risk factor for infection. The objectives of this master's thesis are: 1) critically review the available scientific literature that evaluates the association between management practices (measured by a risk assessment questionnaire (RAQ)) and MAP herd status, et 2) identify the association between management practices and MAP herd status (determined using bacteriological culture of environmental samples) of dairy herds in Québec, Canada.

A systematic review was performed to answer the first objective. We included studies that assessed PTB risk factors using a RAQ and measured the association between risks factors and MAP herd status. For the second objective, a case-control study was designed. A total of 26 case herds in which MAP had been isolated from at least 1 environmental sample in each herd and 91 control herds (no clinical cases of paratuberculosis and negative on 2 consecutive yearly environmental samplings) were selected. Multivariable logistic regression was used to evaluate the association between selected risk factors and MAP herd status.

Twenty-one cross-sectional, 5 case control and 3 longitudinal studies met the inclusion criteria. Herd size was significantly associated with MAP herd status in 12 (4 with low RoB) out of 18 studies, introduction of new animals was significantly associated with MAP herd status in 10 (4 with low RoB) out of 24, history of PTB was significantly associated with MAP herd status in 6 (4 with low RoB) out of 13, and management of colostrum and milk were significantly associated with MAP herd status in 5 (none with

low RoB) out of 18. For Québec dairy herds, herd size (OR=1.17; 95% CI: 1.02-1.33) and proportion of cows purchased per year in the last 5 years (OR=5.44; 95% CI: 1.23-23.98) were significantly associated with a positive MAP herd status.

The results of this master's thesis provide a large compilation of available information about risk factors associated with MAP herd status evaluated using a RAQ. Some risk factors are apparently more consistent across studies. However, results should be interpreted in the light of the quality and risk of bias of each study. Management practices aiming to prevent the introduction of new animals into the herd and to reduce the contact of newborn calves with adult animals or their feces are key elements to minimize MAP introduction and transmission into a herd. These elements should be prioritized in control programs.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*, dairy herds, risk factors, management practices, environmental sampling, herd status.

Table des matières

Liste des tableaux

Liste des figures

Liste des sigles et abréviations

ADN: Acide désoxyribonucléique

BIC: Bayesian Information Criterion

CA: Calving Area

CI: Confidence Interval

CPE: Culture de prélèvements de l'environnement

ELISA: Enzyme-linked immunosorbent assay

GRADE: Grading of Recommendations, Assessment and Evaluation

IAP: International Association for Paratuberculosis

ICP: International Colloquium in Paratuberculosis

IC: Intervalle de confiance

IFN-γ: Interféron gamma

IgG: Immunoglobulines G

IL: Interleukine

JD : Johne's disease

LÉAQ: Laboratoire d'épidémiosurveillance animale du Québec

MAP: *Mycobacterium avium* ssp*. paratuberculosis*

MAPAQ: Ministère de l'Agriculture, de Pêcheries et de l'Alimentation

NR: Not reported

OR: Odds Ratio

PCR: Réaction en chaîne par polymérase

PTB: Paratuberculose

PVQPCP: Programme volontaire de prévention et contrôle de la paratuberculose du Québec

QVPPCP: Québec Voluntary Paratuberculosis Prevention and Control Program

RB : risque de biais

RAQ: Risk Assessment Questionnaire

RoB: Risk of Bias

S/P: Sample to positive ratio

UFC: Unité formatrice de colonie

USDA: United States Department of Agriculture

PPV: Positive predictive value

QAR: Questionnaire d'analyse de risque

NPV: Negative predictive value

... que ta volonté soit faite sur la terre comme au ciel...

Remerciements

Tout d'abord, je tiens à remercier mon directeur, le Dr Gilles Fecteau, pour avoir été un exemple à suivre tant dans mon processus d'apprentissage que de formation. Merci de m'avoir donné l'opportunité de travailler avec vous et de m'avoir confié le développement de ce projet. J'ai appris plus que ce que j'aurais imaginé. Je suis fière de mon évolution sous votre encadrement et très contente de continuer! Je n'ai pas de mots pour vous remercier.

J'aimerais également remercier les Drs Julie Paré et Daniel Jean, membres de mon comité conseil, pour leur supervision et leurs avis.

Merci aussi à chaque membre du groupe de recherche sur la paratuberculose de l'Université de Montréal pour leur collaboration et pour m'avoir mise à l'aise ainsi qu'aux collaborateurs dans mon projet : Dr Juan Carlos Arango Sabogal, Dr Vincent Wellemans, Dr Jean-Philippe Roy, Dre Olivia Labrecque, Dre Geneviève Côté, Dr Sébastien Buczinski, Dre Julie-Hélène Fairbrother, Dre Elizabeth Doré et Dre Nathalie Bissonnette.

Je remercie Dr David Francoz d'avoir accepté la présidence du jury et Dr Simon Dufour d'avoir accepté d'être membre du jury.

Merci également aux organismes subventionnaires : le Programme de Soutien à l'Innovation en Agroalimentaire, le Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, le regroupement Op+lait et COLFUTURO.

Je remercie énormément Christine Blondin, Diane Rodier, Isabelle Codo, Geneviève Michon, Annie Lavertu et Annie Bernier pour l'aide constante tout au long de ces deux années.

Enfin, je remercie mes parents Enrique et Marta et mes sœurs Elena, Ana et Lucia pour le support constant étant proche ou loin.

Grand Merci!

Introduction

La paratuberculose est une maladie entérique contagieuse chronique, et incurable des ruminants. Elle est causée par la mycobactérie *Mycobacterium avium* ssp. *paratuberculosis* (MAP) (Manning and Collins, 2010b). La paratuberculose est responsable de pertes économiques associées à la diminution de la production laitière (Hendrick et al., 2005; Lombard et al., 2005), l'augmentation du taux de réforme (Tiwari et al., 2008) et à la diminution du poids de la carcasse à l'abattoir (Kudahl and Nielsen, 2009). Le MAP a été associé avec la maladie de Crohn chez l'humain, mais le lien de causalité n'est pas démontré (Waddell et al., 2015).

Les jeunes individus sont plus susceptibles (Windsor and Whittington, 2010). Ils s'infectent dans les premiers mois de vie (Sweeney, 1996). La voie féco-orale est la principale voie d'infection (Manning and Collins, 2010a), mais la transmission via le colostrum et le lait (Streeter et al., 1995), et *in utero* (Whittington and Windsor, 2009) sont aussi possibles. Dans les premiers mois, les animaux infectés sont asymptomatiques, et habituellement, l'excrétion fécale et la production d'anticorps ne sont pas détectables (Sweeney, 2011). Ce n'est qu'après une longue période d'incubation (jusqu'à plusieurs mois), que les vaches débutent l'excrétion fécale de MAP. Toujours en absence de signes cliniques, ces individus deviennent une source importante de contamination et perpétuent ainsi l'infection dans le troupeau (Manning and Collins, 2010a; Sweeney, 2011). Quelques animaux développent la forme clinique de la maladie, connue sous le nom de Johne, qui se caractérise par une diarrhée intermittente et une perte de poids malgré un appétit normal (Fecteau and Whitlock, 2010). La plupart des animaux sont réformés avant l'apparition des signes cliniques pour des raisons autres que la maladie de Johne (Whitlock and Buergelt, 1996).

Toute pratique de gestion d'élevage qui, directement ou indirectement, permet l'exposition des animaux susceptibles au fèces des animaux excréteurs, pourrait être considérée comme un facteur de risque pour l'infection (McKenna et al., 2006). Identifier les facteurs de risque à partir d'un questionnaire d'analyse de risque est l'une des stratégies des programmes de contrôle (Sweeney et al., 2012). Le contrôle de ces facteurs de risque est plus efficace pour

réduire la prévalence d'un troupeau que la simple élimination des animaux positifs (Garry, 2011).

Ce mémoire de maîtrise vise à répondre à deux objectifs. Le premier est d'identifier, évaluer et résumer les facteurs de risque potentiels associés à un statut de troupeau positif à MAP. Pour cela les études disponibles qui ont mesuré les pratiques de gestion à l'aide d'un questionnaire d'évaluation des risques seront examinés de manière critique et systématique. Le deuxième est d'identifier quelles sont les pratiques de gestion associées à un statut positif à MAP (déterminé à l'aide de la culture bactériologique des prélèvements de l'environnement) dans les troupeaux laitiers du Québec. Pour répondre aux objectifs, une revue systématique de la littérature et une étude cas-témoins ont été réalisés.

1. Revue de littérature

1.1 Définition

La paratuberculose est une entérite chronique, contagieuse et incurable des ruminants, causée par *Mycobacterium avium* ssp. *paratuberculosis* (MAP) (Manning and Collins, 2010b). Distribuée mondialement, la paratuberculose est responsable d'importantes pertes économiques (Tiwari et al., 2005) en plus d'être associée à la maladie de Crohn (Waddell et al., 2015). La forme clinique de la maladie est connue sous le nom de maladie de Johne.

1.2 Histoire

Le premier rapport de la maladie a été fait en 1895 en Allemagne par les docteurs Johne et Fortingham qui utilise le terme « entérite pseudotuberculose » (Manning and Collins, 2010b). Puis, en 1912, de façon fortuite, Frederick William Twort a isolé pour la première fois l'agent causal et l'a nommé '*Mycobacterium enteriditis chronicae pseudotuberculosae bovis*, Johne (Manning and Collins, 2010b). La paratuberculose s'est propagé globalement parmi les ruminants domestiques, émergeant comme l'une des maladies infectieuses des ruminants les plus communes et coûteuses. Depuis ce temps, la paratuberculose a été étudiée mondialement. Depuis 1983 et 1988, par l'initiative du Dr Richard Merkal, le colloque international sur la paratuberculose (ICP) et l'association international sur la paratuberculose (IAP) ont vu le jour. L'IAP et l'ICP contribuent de manière significative à l'échange d'idées concernant cette maladie depuis plus de 35 ans (Manning and Collins, 2010b).

1.3 Importance

Les efforts de recherche sur la paratuberculose sont justifiés par 2 aspects principaux : l'économie et la santé publique.

1.3.1 Pertes économiques

La paratuberculose entraîne des pertes économiques importantes pour l'industrie laitière (Tiwari et al., 2008). Ces pertes ne sont pas seulement associées aux vaches réformées ayant des signes cliniques de la maladie. L'infection subclinique est responsable des plus grandes pertes économiques. Une diminution de la production laitière (Goodell et al., 2000; Hendrick et al., 2005; Lombard et al., 2005), une augmentation du taux de réforme (Goodell et al., 2000; Hendrick et al., 2005; Lombard et al., 2005), une diminution de la fertilité (Johnson-Ifearulundu et al., 2000) et une diminution du poids de la carcasse à l'abattage (Kudahl and Nielsen, 2009), sont les raisons expliquant la réduction de la rentabilité des animaux atteints.

1.3.2 Association avec la maladie de Crohn

La maladie de Crohn est une maladie humaine inflammatoire à médiation immunitaire du tractus gastro-intestinal, dont l'étiologie demeure incertaine (Ranasinghe and Hsu, 2017). En 1913, Dalziel a lancé l'hypothèse que MAP pouvait être présent chez les patients humains souffrant de maladie inflammatoire des intestins (IBD : inflamatory bowel disease). Plus récemment, vers la fin du XXe siècle, la publication de Chiodini et al. associant le MAP à la maladie de Crohn a suscité beaucoup d'intérêt (Chiodini, 1989). Plusieurs études ont trouvé une association entre MAP et la maladie de Crohn (Waddell et al., 2015). Cependant, le lien de causalité et les preuves qui expliquent le rôle de MAP dans la maladie de Crohn sont encore insuffisantes pour conclure à un effet causal (Waddell et al., 2015).

1.4 L'agent pathogène : *Mycobacterium avium* ssp. *paratuberculosis*

Le MAP est une mycobactérie aérobique à croissance lente, dépendante de la mycobactine. Bien que les mycobactéries soient cytochimiquement Gram-positives, le contenu élevé en lipides et acide mycolique de leur paroi cellulaire empêche l'absorption des colorants utilisés dans la coloration de Gram. Avec la coloration de Ziehl-Neelsen, les lipides se lient à la carbol-fuchsine, qui n'est pas enlevée avec le décolorant acido-alcool,

tournant alors rouge, raison pour laquelle on les appelle bacilles acido-acoolo-résistants (Quinn et al., 2011).

Il y a deux souches de MAP qui se différencient par leurs caractéristiques de croissance, leurs préférences d'hôte et leur pathogénicité (Tableau 1.1) : la souche S (de « sheep » en anglais) et la souche C (de « cattle » en anglais), aussi désignées comme type I et type II respectivement (Stevenson, 2010). Le type III et le type Bison ont aussi été décrits. Le séquençage complet du génome de MAP confirme la classification des souches en deux groupes : le type S (incluant le type III) et le type C (incluant le type Bison) (Stevenson, 2010, 2015).

Tableau 1.1 Caractéristiques des différentes souches de *Mycobacterium avium* ssp. *paratuberculosis.*

Caractéristique	**Souche S**	**Souche C**
	Type I, Type III	Type II, « bison »
Facilité d'isolement	Difficile	Moins difficile
Temps d'incubation	2-12 mois	1-4 mois
Préférence d'hôte	Ovins et caprins (principalement)	Large gamme d'hôtes (ruminants et non-ruminants)

Reproduit avec la permission de Dr Juan Carlos Arango-Sabogal.

1.5 Épidémiologie et pathogénie

1.5.1 Transmission

La principale voie de transmission du MAP est la voie féco-orale. Les animaux plus jeunes sont exposés aux fèces des animaux excréteurs (habituellement des adultes) (Manning and Collins, 2010a; Sweeney, 2011). Toutefois, le MAP peut survivre dans l'environnement grâce à sa paroi riche en lipides (Rowe and Grant, 2006). Alors, le contact avec l'environnement contaminé comme des aires de vêlage ou des équipements et le pis des vaches, peut aussi entraîner l'ingestion du MAP (Sweeney, 2011). Les vaches présentant des signes cliniques ou non, peuvent excréter le MAP dans le lait ou le colostrum

(Taylor et al., 1981; Sweeney et al., 1992b; Streeter et al., 1995; Giese and Ahrens, 2000), une autre mode de transmission. La possibilité de la transmission *in utero* doit aussi être considérée (Sweeney et al., 1992c; Whittington and Windsor, 2009).

1.5.2 Susceptibilité

1.5.2.1 Âge

Les jeunes individus sont plus susceptibles à l'infection avec MAP (Mortier et al., 2015). Différentes théories ont été proposées pour expliquer la susceptibilité liée à l'âge. La grande perméabilité de l'intestin des veaux permet la pénétration de macromolécules comme le MAP (Sweeney, 1996). Aussi, la flore mature d'un rumen fonctionnel chez les animaux plus âgés peut aider à diminuer la quantité de MAP qui atteint l'intestin (Windsor and Whittington, 2010). Finalement, il a été proposé que l'exposition répétée au MAP chez les animaux adultes pourrait conférer une résistance à l'organisme (Delgado et al., 2013).

1.5.2.2 Dose

La probabilité d'infection dépend aussi de la dose (Sweeney, 1996; Delgado et al., 2013). Aussi, les animaux exposés à une dose de MAP plus élevée ($5x10^9$ UFC) développent des lésions plus sévères que les animaux exposés à une dose de MAP plus faible ($5x10^7$ UFC) (Mortier et al., 2013).

1.5.3 Infection

Après l'ingestion, le processus de la maladie commence par l'absorption initiale de MAP par les cellules phagocytaires intestinales. Par la suite, il y a translocation à travers la muqueuse intestinale (Fecteau and Whitlock, 2010), principalement dans l'iléum et par la voie des cellules M dans les plaques de Peyer (Momotani et al., 1988). Le MAP est alors phagocyté par les macrophages (Sweeney, 2011), où il survit en évitant la maturation et l'acidification de la vacuole phagocytaire (pas de formation du phagolysosome) (Hostetter et al., 2003). Les macrophages peuvent migrer aux nœuds lymphatiques mésentériques (Fecteau and Whitlock, 2010). La réponse inflammatoire produite par les antigènes de MAP dans la sous-muqueuse intestinale et les nœuds lymphatiques attire plus de

macrophages et de lymphocytes, la formation de granulomes est ainsi amorcée (Sweeney, 2011). Le MAP peut être contenu dans les macrophages et demeurer confiné (en dormance) ainsi pendant des années (Sweeney, 2011) sans activation de la réponse humorale (pas d'anticorps produits) et sans être éliminé (pas d'excrétion dans les fèces). C'est cette caractéristique qui donne la nature progressive et chronique de la paratuberculose (Sweeney, 2011). C'est le premier de 4 stades (Figure 1) qui décrivent l'évolution de la maladie. On l'appelle le stade silencieux (Fecteau and Whitlock, 2010).

1.5.4 Stade I. « Silencieux » : Veaux et jeunes animaux

Ce stade peux durer 2 ans ou plus (Sweeney, 2011). Les animaux ne montrent pas de signes cliniques (Tiwari et al., 2006). Ils peuvent excréter le MAP dans les fèces, mais en quantité minime et non détectable par les méthodes diagnostiques disponibles (Tiwari et al., 2006; Fecteau and Whitlock, 2010; Sweeney, 2011). Les anticorps sériques sont habituellement absents (Sweeney, 2011). L'infection peut être confirmée en démontrant la présence de MAP dans les tissus, comme l'iléon ou les nœuds lymphatiques mésentériques, par culture ou par PCR, ou par la démonstration de la présence de micro-granulomes à l'aide d'histopathologie (Tiwari et al., 2006; Sweeney, 2011).

1.5.5 Stade II. « Infecté asymptomatique excréteur ».

Pour des raisons peu connues, la réponse immunitaire à médiation cellulaire décline avec le temps. Une réponse humorale s'amorce (Coussens et al., 2010; Sweeney, 2011). Un changement de la réponse type-Th1 (Interféron Gamma: activation des macrophages) vers une réponse type-Th-2 (IL-4, IL-10: production d'anticorps) se produit (Stabel, 2000; Koets et al., 2015).

Toujours dans les macrophages, le MAP continue à se multiplier et le macrophage ne le détruit pas (Sweeney, 2011). Les concentrations de MAP dans la muqueuse intestinale atteignent éventuellement des niveaux critiques et le MAP est alors excrété et détectable dans les fèces (Fecteau and Whitlock, 2010; Koets et al., 2015). Les concentrations excrétées sont alors suffisantes pour être détectées par les méthodes diagnostiques comme la culture bactériologique et la PCR (Fecteau and Whitlock, 2010).

Aussi, le MAP migre vers d'autres tissues dont l'utérus, la glande mammaire, rendant possible l'infection *in utero* et l'excrétion dans le lait ou le colostrum (Sweeney et al., 1992b, c; Coussens et al., 2010; Sweeney, 2011).

Les animaux au stade II ne montrent pas de signes cliniques, mais peuvent être détectés pas des méthodes diagnostiques. Ces animaux peuvent avoir une réponse humorale efficace (production des anticorps) et peuvent excréter le MAP dans les fèces (Fecteau and Whitlock, 2010). Habituellement, l'excrétion fécale se produit avant qu'une réponse humorale ne soit détectable (Tiwari et al., 2006; Sweeney, 2011) La progression de la maladie varie selon l'âge et la dose lors de l'exposition initiale, la fréquence d'exposition et certains facteurs génétiques et nutritionnels (Fecteau and Whitlock, 2010). Il peut y avoir un effet négatif subtil sur la production (Sweeney, 2011). Les animaux peuvent rester au stade II (subclinique) sans jamais progresser ni montrer des signes cliniques (Sweeney, 2011).

1.5.5.1 Patrons d'excrétion fécale

Les vaches peuvent excréter le MAP de façon continue ou intermittente (Merkal et al., 1968). Basée sur la culture en tubes de milieu solide, l'excrétion de MAP peut être faible (<10 UFC/tube), modérée (10-50 UFC/tube) ou forte (>50 UFC/tube) (Crossley et al., 2005). Certaines vaches auront une libération intermittente et faible de MAP et une absence de réponse immunitaire humorale (Schukken et al., 2015). D'autres démontreront une excrétion continue et croissante ainsi qu'une réponse immunitaire humorale croissante et clairement détectable (Schukken et al., 2015). Parmi les vaches infectées de façon naturelle, seulement 7% deviennent de fortes excrétrices (Mitchell et al., 2015). Ces vaches conserveront un patron de forte excrétion (Mitchell et al., 2015). Par contre, les vaches avec un patron d'excrétion intermittent ont une faible probabilité de devenir de fortes excrétrices (Mitchell et al., 2015). Ces vaches alternent entre des résultats positifs (excrétion habituellement faible) et négatifs (non-excrétion) à la culture fécale (Schukken et al., 2015).

1.5.6 Stade III. « Maladie clinique »

La prolifération et la croissance des granulomes produisent une entérite granulomateuse diffuse et, par conséquent, une altération de la paroi intestinale et une réduction de la capacité d'absorption (Fecteau and Whitlock, 2010; Sweeney, 2011).

Après une longue période d'incubation (Tiwari et al., 2006) qui dure habituellement 2 ans (Sweeney, 2011), le premier signe clinique est la perte de poids malgré un appétit normal, accompagnée de diarrhée chronique parfois intermittente qui ne répond pas aux traitements habituels (Tiwari et al., 2006; Fecteau and Whitlock, 2010; Sweeney, 2011).

Les animaux à ce stade, excrètent le MAP dans les fèces et ont des anticorps détectables (Tiwari et al., 2006; Fecteau and Whitlock, 2010). La plupart de ces animaux ne demeurent pas au sein du troupeau, et seront réformés rapidement car leur production est décevante (Tiwari et al., 2006; Fecteau and Whitlock, 2010; Sweeney, 2011).

1.5.7 Stade IV. « Maladie clinique avancée »

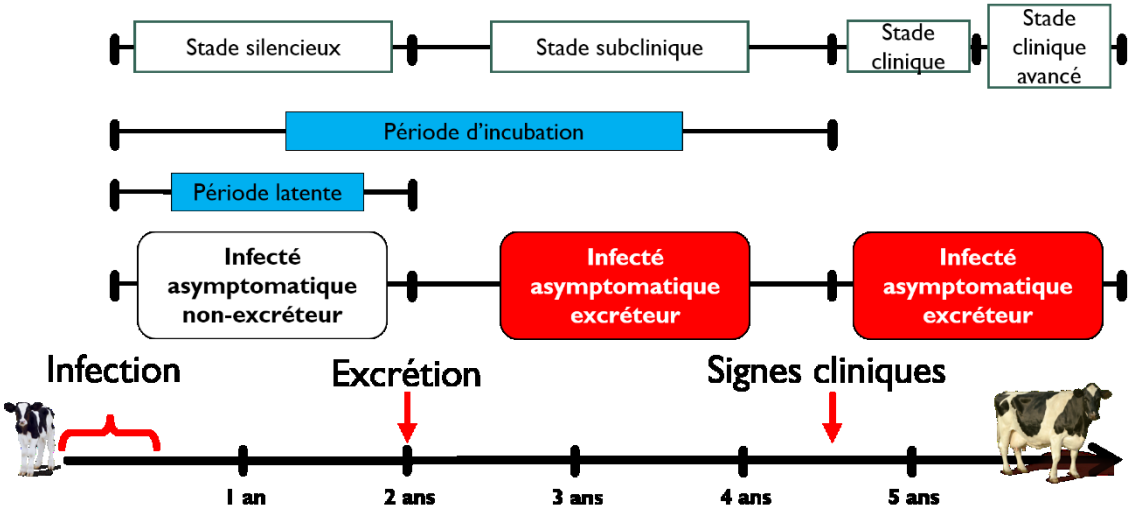
Les animaux à ce stade sont émaciés, faibles, présentent une diarrhée chronique et profuse et de l'œdème inter-mandibulaire (Tiwari et al., 2006; Fecteau and Whitlock, 2010). Si les vaches ne sont pas reformées, elles peuvent mourir de déshydratation et de cachexie (Fecteau and Whitlock, 2010).

1.5.8 Effet iceberg

La plupart des vaches n'arrivent jamais au stade plus avancé de la maladie (Tiwari et al., 2006) parce que les animaux sont reformés dès que leur production devient décevante (Tiwari et al., 2005; Sweeney, 2011) ou pour d'autres raisons que la paratuberculose (Tiwari et al., 2006).

La longue période latente, la longe période d'incubation, et le caractère chronique de cette maladie ont comme résultat que l'on parle d'un effet iceberg pour expliquer la prévalence dans un troupeau. Pour chaque vache en stade avancé il y a probablement 15 à 25 vaches infectées au stade 1 de la maladie (Fecteau and Whitlock, 2010).

Figure 1.1 Évolution de l'infection par *Mycobacterium avium* ssp. *paratuberculosis* et stades de la maladie



Source : Modifiée de la thèse de doctorat de Dr Juan Carlos Arango Sabogal. « Détection des troupeaux laitiers infectés par *Mycobacterium avium* ssp. *paratuberculosis* via la culture fécale et impact des mesures de contrôle des maladies entériques contagieuses sur l'incidence d'excrétion fécale individuelle » avec permission.

1.6 Diagnostic

1.6.1 Tests diagnostiques disponibles

Il y a deux types de tests diagnostiques pour MAP : ceux qui identifient l'organisme directement, et ceux qui identifient la réponse immunitaire de l'hôte. Aussi, l'identification des lésions histopathologiques caractéristiques de la maladie pourrait être considéré comme le troisième moyen pour diagnostiquer la maladie.

Le plus grand défi diagnostique est la détection des animaux aux stades initiaux de la maladie. En effet, l'excrétion fécale et la réponse humorale augmentent avec la progression de la maladie et sont peu développés dans les stades précoces (Dargatz et al., 2001; Harris and Barletta, 2001; Nielsen and Toft, 2008).

1.6.1.1 Culture bactérienne des échantillons fécaux, des tissus ou du lait

La culture de MAP est un processus long et sa croissance est fastidieuse. Toutefois, un avantage de la culture fécale c'est qu'un résultat positif confirme la présence de MAP

viable (Sweeney et al., 2012). Elle est considérée comme la méthode de référence *ante-mortem* (Whittington, 2010) et peut être réalisée sur des échantillons fécaux ou des tissus.

La première étape dans les processus de culture est la décontamination de l'échantillon pour réduire le nombre d'organismes à croissance rapide. Les échantillons fécaux représentent un défi en raison de la grande quantité de bactéries entériques présentes. La deuxième étape est l'incubation pour que la bactérie puisse croître, soit dans un milieu solide ou un milieu liquide. Ces milieux sont souvent enrichis avec des antibiotiques qui réduisent la croissance d'organismes qui auraient survécu à la décontamination. Les milieux solides permettent une identification visuelle du MAP et sont, en général, moins dispendieux. Cependant, le processus est plus long. Les tubes, enrichis avec la mycobactine, doivent être incubés à 37°C pendant 12 à 20 semaines avant de pouvoir déclarer l'échantillon négatif (Whittington, 2010). La culture en milieu liquide est plus rapide. En seulement 8 à 12 semaines, les échantillons sont déclarés négatifs. Le milieu liquide a une meilleure sensibilité que le milieu solide, mais il nécessite une confirmation par coloration acido-alcoolo-résistante ou par PCR (Whittington, 2010). La troisième étape consiste en la reconnaissance des colonies de MAP dans les milieux solides, ou, dans le cas des milieux liquides un signal est émis lors de la croissance et la confirmation génotypique (Whittington, 2010).

La sensibilité diagnostique de la culture fécale est estimée à 60% comparée à la nécropsie, avec une spécificité >99% (Collins et al., 2006). En général, pour la culture en milieu solide, la sensibilité rapportée varie de 39% à 82% (Whittington, 2010). Pour la culture en milieu liquide, la sensibilité est estimée entre 26% et 92% (Collins et al., 1990; Eamens et al., 2000; Motiwala et al., 2005).

Concernant la sensibilité analytique, différentes méthodes de culture sont utilisées dans différents laboratoires, il y a un manque de standardisation ou de mesure de la sensibilité analytique, et le niveau de compétence entre laboratoires est variable (Collins, 1996). La sensibilité analytique a été estimée à 10^1 UFC pour le BACTEC MGIT 960 (le système utilisé au laboratoire d'épidémiosurveillance animale du Québec) (Shin et al., 2007).

1.6.1.2 Détection de l'ADN du MAP par PCR

La PCR détecte une séquence du génome du MAP dans les fèces, les tissus ou le lait (Bosshard et al., 2006; Alinovi et al., 2009; Slana et al., 2009; Bolskë and Herthnek, 2010). Aussi, la PCR peut être utilisée comme confirmation de la présence de l'ADN du MAP après la culture en milieu solide ou liquide (Herthnek and Bolske, 2006) La séquence d'insertion IS900 est la plus utilisée (Bolskë and Herthnek, 2010). L'avantage de la PCR est la rapidité pour obtenir un résultat en comparaison à la culture. La sensibilité et spécificité de la PCR sont similaires à la culture fécale en milieu solide et liquide (Alinovi et al., 2009; Sweeney et al., 2012). Le résultat à la PCR peut être utilisé comme un indicateur de la quantité de MAP excrétée qui est inversement proportionnelle au nombre de cycles nécessaires par la PCR pour amplifier la quantité initiale de ADN (Aly et al., 2010; Sweeney et al., 2012).

1.6.1.3 Détection des anticorps dans le sérum ou dans le lait

L'ELISA est le test indirect le plus utilisé parce qu'il est simple, rapide et peu coûteux (Tiwari et al., 2006; Nielsen, 2010). La sensibilité est moindre que pour la culture bactérienne (Nielsen and Toft, 2008), et varie beaucoup selon le stade de la maladie dans lequel l'animal se trouve (Nielsen and Toft, 2006; Nielsen, 2010). La sensibilité a été rapportée entre 25% et 61% et la spécificité entre 83% et 100% (Collins et al., 2006; Nielsen and Toft, 2008; Nielsen, 2010; Sweeney et al., 2012). Plusieurs kits ELISA pour la détection des anticorps sériques sont disponibles (par exemple : HerdCheck, IDEXX Laboratories, Inc. Westbrook, ME; ParaCheck, Prionics AG, Zurich, Switzerland et ID Screen, ID-Vet, Montpellier, France), et certaines entreprises ont adopté cette technologie pour les échantillons de lait (Shin et al., 2008). Le seuil recommandé par le fabricant varie d'un kit à l'autre (Collins et al., 2005). Selon le seuil, la sensibilité et la spécificité varient pour chaque kit (Collins et al., 2005).

1.6.1.4 Détection des lésions par histopathologie

Les lésions tissulaires produites par MAP peuvent être évidentes à la nécropsie et sont habituellement localisés dans la portion terminale du petit intestin et les nœuds lymphatiques associés (Whitlock and Buergelt, 1996). La coloration de Ziehl-Neelsen est

la technique utilisée pour mettre en évidence la bactérie sur les tissus formolés. C'est une technique qui a une spécificité de 100 % mais une sensibilité inconnue. Par contre, elle est peu pratique à l'échelle du troupeau, car elle nécessite une intervention chirurgicale (biopsie) (Collins et al., 2006). La détection des lésions chez les animaux aux abattoirs peut être utile pour la classification des lésions de la maladie (Buergelt et al., 1978; Gonzalez et al., 2005) et comme outil de surveillance (Okura et al., 2010; Okuni et al., 2013).

1.6.2 Utilisation des tests diagnostiques

1.6.2.1 Diagnostic individuel

Comme présenté dans la section précédente, il y a plusieurs tests disponibles pour le diagnostic individuel. Cependant, ces tests vont avoir des limitations à cause de l'épidémiologie et la pathogénie de la maladie. À cause de la longue période latente de la maladie, les animaux doivent avoir au moins 36 mois (Nielsen and Toft, 2006; Collins, 2011; Mortier et al., 2015). Comme mentionné avant, la production d'anticorps et l'excrétion du pathogène ne se produisent pas nécessairement en même temps (Nielsen and Toft, 2006, 2008; Mortier et al., 2015).

La culture fécale individuelle détermine si l'animal est infectieux, étant donné qu'il excrète le MAP dans les fèces. Certains animaux peuvent excréter le MAP sans être infectés (Sweeney et al., 1992a), alors pas tout le temps les animaux infectieux vont être infectés.

Par rapport à la PCR, elle ne peut pas déterminer s'il s'agit du MAP viable ou seulement de l'ADN de MAP. Alors, un résultat positif chez un individu ne démontre pas que l'animal est assurément infecté.

Finalement, le test ELISA est le plus utilisé, le plus vite et le moins cher. Par contre, la sensibilité et la spécificité sont les plus faibles.

1.6.2.2 Stratégies de dépistage à l'échelle du troupeau

Les tests décrits peuvent être utilisés pour identifier les troupeaux positifs de différentes façons. Lorsqu'un test est utilisé chez un individu ou une population apparemment saine on l'appelle un test de dépistage (Dohoo et al., 2014).

1.6.2.2.1 Cultures de prélèvements de l'environnement

Selon les experts, la culture bactérienne de prélèvements de l'environnement (CPE) est la procédure la plus économique pour déterminer si un troupeau est possiblement infecté par MAP (Collins et al., 2006; Sweeney et al., 2012). La technique est simple, moins coûteuse que les test individuels et ne nécessite pas de manipulation des animaux (Collins et al., 2006). Trois aires sont prélevées en duplicata pour un total de six échantillons par ferme selon les recommandations des programmes du USDA (USDA-APHIS-VS, 2010) : premièrement, une aire où les fèces des vaches adultes s'accumulent (dans l'étable principale); deuxièmement, l'aire de récolte du fumier (fosse ou tas à fumier); finalement, aires où le fumier s'accumule, mais différentes de la première (aire de vêlage ou logette des vaches malades). Cette technique a été évaluée dans plusieurs études (Raizman et al., 2004; Berghaus et al., 2006; Lombard et al., 2006; Lavers et al., 2013; Wolf et al., 2014; Arango-Sabogal et al., 2016). La sensibilité rapportée varie de 31 à 90% (Raizman et al., 2004; Berghaus et al., 2006; Lombard et al., 2006; Pillars et al., 2009b; Smith et al., 2011; Lavers et al., 2013; Wolf et al., 2014; Arango-Sabogal et al., 2016). La spécificité est toujours estimée à près de 100%. Les troupeaux qui n'ont aucun échantillon positif de l'environnement sont identifiés comme négatifs ou à faible prévalence. Une étude a déterminé que les troupeaux négatifs à la CPE avaient une prévalence d'infection à MAP inférieure à 2% (Pillars et al., 2009a).

1.6.2.2.2 PCR utilisée à l'échelle du troupeau

Selon les experts (Collins et al., 2006), la PCR peut être utilisée sur des échantillons fécaux poolés ou sur des prélèvements de l'environnement pour déclarer un troupeau positif. La PCR a aussi été utilisé dans le lait du réservoir ou le filtre du réservoir (Slana et al., 2012). Un résultat de PCR positif dans un prélèvement au sein d'un troupeau indique que le MAP circule dans le troupeau et que l'on peut le considérer infecté et instaurer des mesures de contrôle.

1.6.2.2.3 Test du troupeau entier ou un groupe d'animaux

La culture fécale individuelle peut être utilisée pour tester tous les animaux du troupeau (Collins et al., 2006). Aussi on peut faire des ELISA de tous les animaux du

troupeau, puis confirmer par culture fécale les animaux positifs (Collins et al., 2006; Sweeney et al., 2012). Le testage ciblé est une autre option, en réalisant des cultures fécales individuelles des animaux avec un bas état de chair (Collins et al., 2006; Sweeney et al., 2012). Pour le testage d'un sous-groupe aléatoire d'animaux, le USDA recommande tester tous les animaux de moins de 36 mois dans des troupeaux de moins de 300 vaches (le plus commun au Québec) (USDA-APHIS-VS, 2010). Si le nombre est plus petit que 30 animaux, les animaux de plus de 24 mois devraient être aussi inclus (USDA-APHIS-VS, 2010).

Tableau 1.2. Tests disponibles pour le diagnostic de la paratuberculose et ses caractéristiques

Test	**Substrat**	**Avantages**	**Désavantages**
Culture bactériologique	Fèces, lait, tissus, prélèvements de l'environnement	Bonne valeur prédictive négative (haute ou base prévalence)	Long processus Sensibilité varie avec le stade d'infection Valeur prédictive négative base dans des populations à base prévalence
PCR	Fèces, lait, tissus, prélèvements de l'environnement	Rapide Haute spécificité	Base sensibilité N'indique pas la présence de MAP viable
ELISA	Sérum, lait	Économique et rapide	Sensibilité varie avec le stade d'infection
Histopathologie	Tissus	Spécificité 100%	Peu pratique ante-mortem (nécessite intervention chirurgicale)

Adapté de (Nielsen et al., 2001)

1.7 Traitements

La Paratuberculose est une maladie incurable. Les traitements visent à réduire les signes cliniques, mais n'éliminent pas la bactérie des tissues et ne limitent pas l'excrétion (Fecteau and Whitlock, 2011). Les vaches excrétrices qui sont traitées seront gardées pendant une période plus longue au sein du troupeau augmentant le risque de contamination environnementale (Bakker, 2010).

L'isoniazid, le rifampin, la clofazimine et les aminoglycosides ont été utilisés dans le traitement qui peut se réaliser en monothérapie ou en combinaison (Baldwin, 1976; Zanetti et al., 2006; Fecteau and Whitlock, 2011), mais aucun n'élimine la bactérie.

Le monensin a été utilisé comme chimioprophylaxie, ajouté à la ration des animaux, mais dans la littérature, il est toujours classé comme traitement. Il pourrait jouer un rôle dans la prévention des infections chez les jeunes et diminuer l'excrétion fécale chez des adultes infectés (Hendrick et al., 2006; Fecteau and Whitlock, 2011). De façon similaire, le nitrate de gallium a été testé et pourrait être aussi utilisé pour la prévention de la maladie chez les jeunes (Fecteau and Whitlock, 2011; Fecteau et al., 2011).

1.8 Contrôle

1.8.1 Bases pour le contrôle de la paratuberculose

Étant donné que la paratuberculose est une maladie incurable, le contrôle de la maladie s'appuie sur la prévention. Le contact des jeunes avec les fèces des animaux adultes est le facteur de risque le plus important pour la transmission de MAP (Doré et al., 2012). La plupart des programmes de contrôle visent à diminuer le contact entre les animaux susceptibles et les animaux excréteurs. Cette approche est plus efficace pour réduire la prévalence de la maladie que de tester et de réformer les vaches infectées par MAP (Garry, 2011)

La vaccination a été décrite et est utilisée comme un moyen pour améliorer la résistance des animaux contre le pathogène (Sweeney et al., 2009; Sweeney et al., 2012), mais ne protège pas complètement. Le vaccin n'évite pas l'excrétion de MAP dans les fèces et ne

prévient donc pas la transmission de MAP (Kalis et al., 2001; Fecteau and Whitlock, 2011). La vaccination est une option pour améliorer la résistance, mais ne remplace pas un plan de bonne gestion. De plus, la vaccination n'est pas disponible au Canada.

Récemment, la sélection génétique a été évaluée comme une stratégie de contrôle (Koets et al., 2000; Gonda et al., 2006). Le statut d'infection de chaque animal est une combinaison de facteurs génétiquement déterminés (gènes de susceptibilité et de résistance) et de facteurs environnementaux (exposition à MAP) (Sweeney et al., 2012).

1.8.2 Approches pour le contrôle de la paratuberculose

1.8.2.1 Troupeaux négatifs

Si le troupeau n'est pas infecté, le but principal est de maintenir le statut négatif (Sweeney et al., 2012). Pour cela, il est recommandé d'éviter l'achat d'animaux et d'éviter le contact avec les animaux d'autres troupeaux. Il faut mieux élever les génisses de remplacement plutôt que de les acheter. Si l'achat d'animaux est nécessaire, il faut acheter d'un troupeau avec des bonnes pratiques de gestion et des règles de biosécurité strictes. Dans le troupeau, éviter le contact direct ou indirect des veaux avec les adultes ou les fèces des adultes est toujours primordial (Sweeney et al., 2012).

1.8.2.2 Troupeaux infectés

Si le troupeau est déjà infecté, trois stratégies sont recommandées : 1) prévenir les nouvelles infections, 2) gérer les animaux infectés et 3) améliorer la résistance. La prévention des nouvelles infections se fait principalement en brisant le cycle de transmission. Une attention particulière est portée aux animaux plus susceptibles (alors les plus jeunes). Une amélioration des pratiques de gestion afin d'éviter que les veaux entrent en contact avec les fèces des adultes, et de s'assurer que du lait ou colostrum provenant des animaux qui pourraient être infectés, est très importante. Des mesures doivent être prises par rapport aux animaux infectés. Les animaux avec la maladie clinique ou les fortes excrétrices devraient être reformés. Les animaux excréteurs sans signes cliniques devraient être reformés à la fin de la lactation.

1.8.2.3 Identification des facteurs de risque dans un élevage : questionnaires d'analyse de risque

Dans les régions où la paratuberculose est endémique, les programmes de contrôle se basent sur l'évaluation des pratiques à risque pour le troupeau et gèrent la paratuberculose comme une infection subclinique (Kennedy, 2011). Le programmes de contrôle encouragent la mise en place des meilleures pratiques de gestion pour prévenir l'introduction et la transmission du MAP (McKenna et al., 2006).

La première étape vers le succès du contrôle de la paratuberculose consiste à identifier les faiblesses dans les pratiques et à proposer des changements (Garry, 2011). Les questionnaires d'analyse de risque (QAR) sont utilisés dans plusieurs programmes de contrôle. Les QAR sont utiles pour le médecin vétérinaire et le producteur pour cibler des recommandations visant l'amélioration de la gestion et la prévention à la ferme (Pieper et al., 2015). Si le QAR est chiffré et donne un score au troupeau, on peut les classer comme des troupeaux à faible, moyen ou haut risque (Whitlock, 2010). Le seuil pour faire cette classification n'a pas été uniformisé et certains auteurs, déclarent avoir choisi un seuil par consultation d'experts (Raizman et al., 2006). Une autre étude a établi un seuil pour dichotomiser le risque de transmission en deux catégories (faible et haut risque) en utilisant les ratios de vraisemblance positifs (Arango-Sabogal et al., 2017).

Après avoir répondu au questionnaire on peut voir quelles sont les pratiques d'un producteur qui sont des facteurs de risque connus pour la paratuberculose. Plusieurs études ont utilisé l'information des questionnaires pour les associer à un statut de la paratuberculose au sein du troupeau (statut positif à MAP, prévalence de MAP dans le troupeau, incidence de MAP dans le troupeau, entre autres). Cependant l'information disponible est très variable. D'une étude à l'autre les populations source vont différer, aussi que le contenu du questionnaire, l'issue d'intérêt et l'approche statistique.

1.9 Situation au Québec

L'industrie laitière au Québec a des caractéristiques particulières. En premier lieu, le climat continental humide et les hivers longs et froids, exposent les productions agricoles

à de cycles gel-dégel qui pourraient affecter la survie de certains agents pathogènes, incluant le MAP.

Pour contextualiser, avec 5500 troupeaux répartis sur tout le territoire, le Québec produit près de trois milliards de litres de lait annuellement qui représentent environ 50% du lait canadien (Groupe AGÉCO, 2016a). La production laitière contribue à 24.000 emplois directes, ce qui place le secteur comme le 4ième des 500 plus gros employeurs au Québec (Les producteurs de lait du Québec, 2017a). La province compte aussi avec un système strict de traçabilité. Chaque bovin a un numéro unique d'identification qui permet le suivi de chaque animal tout au long de sa vie, outil important pour la sécurité alimentaire au bénéfice des consommateurs (Agri-Traçabilité Québec, 2015).

À son tour, les fermes laitières québécoises ont aussi des caractéristiques uniques. Il s'agit d'entreprises agricoles formées d'associés provenant de la même famille (Les producteurs de lait du Québec, 2017b). La taille moyenne des troupeaux est de 64 vaches laitières par ferme (Groupe AGÉCO, 2016b). Quatre-vingt-treize pourcent des troupeaux sont en stabulation entravée (Canadian Dairy Information Center, 2016). Ces caractéristiques sont similaires aux fermes du nord de l'Europe mais différente des grands troupeaux du sud-ouest des États-Unis.

1.9.1 Prévalence de MAP

Une enquête de séroprévalence a été réalisée en 2002 par le Ministère de l'Agriculture, Pêcherie et Alimentation du Québec (MAPAQ). Au niveau individuel, 2,4% des vaches avaient des anticorps contre MAP au test ELISA (Côté, 2003). À l'échelle du troupeau, 12,1% des troupeaux avaient au moins 2 vaches séropositives (Côté, 2003). En 2010, 7% des troupeaux inscrits au programme volontaire de prévention et de contrôle de la paratuberculose au Québec (PVPCPQ) étaient positifs à la CPE (MAPAQ, 2012b).

Le reste du Canada semble avoir une prévalence plus élevée. La prévalence de troupeaux positifs a été estimée à 68% à l'Alberta et à 76% au Saskatchewan (Wolf et al., 2014). Au Canada de l'est 12% des troupeaux sont positifs à la CPE ou la PCR dur le lait du réservoir (Kelton et al., 2016).

1.9.2 Programme de contrôle

Le PVPCPQ a été lancé en 2007. Son objectif primaire est de diminuer la prévalence de la paratuberculose dans les troupeaux québécois et par conséquent, améliorer la santé animale et réduire l'impact économique négatif de la paratuberculose sur l'industrie bovine. Ce programme vise à éduquer les clients, prévenir la maladie par le contrôle des facteurs de risque du MAP, identifier les troupeaux infectés par MAP et faire un suivi dans le temps. À l'enrôlement, chaque producteur complète un questionnaire d'analyse de risque avec son médecin vétérinaire et des recommandations sont faites. Lors de la deuxième année, et annuellement, des prélèvements de l'environnement sont prélevés pour déterminer la présence de MAP à l'aide de culture bactérienne. Le questionnaire et les recommandations sont répétés aussi annuellement.

2. Problématique, hypothèses et objectifs

La paratuberculose est une maladie avec une longue période d'incubation et de latence. De plus, pour chaque cas clinique plusieurs animaux subcliniques sont présents dans un troupeau. Ainsi, le contrôle de la maladie se base principalement sur la prévention. Identifier les facteurs de risque d'introduction et de transmission du MAP dans un troupeau est essentiel pour le succès d'un programme de contrôle. L'étude de ces facteurs de risque est alors un point principal à considérer. Il est important de savoir qu'est qui est rapporté dans la littérature pour pouvoir le comparer avec les facteurs de risque plus importants au Québec.

Les hypothèses suivantes sont considérées :

1) Il existe des facteurs de risque qui seront rapportés de façon constante dans la littérature peu importe la zone géographique ou les caractéristiques des fermes laitières.
2) Au Québec, certains facteurs de risque pourraient être différents étant donné les caractéristiques particulières des fermes laitières.

Pour évaluer ces hypothèses, ce mémoire de maîtrise a les objectifs suivants :

1) Examiner et résumer de façon critique la littérature scientifique disponible qui étudie les pratiques de gestion (mesurées à l'aide d'un questionnaire) associées au statut du troupeau à MAP dans les troupeaux laitiers.
2) Identifier l'association entre les pratiques de gestion associées à un statut de troupeau positif à MAP (déterminé à l'aide de culture bactériologique d'échantillons environnementaux) dans les troupeaux laitiers du Québec.

3. Facteurs de risque associés à un statut positif à *Mycobacterium avium* ssp. *paratuberculosis* dans les troupeaux laitiers : une revue systématique

Risk factors associated with *Mycobacterium avium* subsp. *paratuberculosis* herd status in dairy farms: A systematic review.

Maria Puerto-Parada[1], Juan Carlos Arango-Sabogal[1], Sébastien Buczinski[1], Jean-Philippe Roy[1], Geneviève Côté[2], Olivia Labrecque[3], Vincent Wellemans[1], Gilles Fecteau[1]

(1) Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.
(2) Direction générale des laboratoires et de la santé animale, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Québec, Canada.
(3) Laboratoire d'épidémiosurveillance animale du Québec, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Saint-Hyacinthe, Québec, Canada.

Abstract

Prevention and control of paratuberculosis (PTB) is based on application of management practices to reduce exposure of uninfected animals to *Mycobacterium avium* subsp *paratuberculosis* (MAP). Management practices that expose (directly or indirectly) susceptible animals to fecal material from MAP shedders can be considered a risk factor for infection. Our objective was to critically review available scientific literature that evaluates risk factors associated with PTB herd status of dairy farms.

We included studies that assessed PTB risk factors using a risk assessment questionnaire (RAQ) and measured the association between risks factors and PTB. Studies written in English, French and Spanish were included. No exclusion was implemented based on type of study, sample size, diagnostic test used, or geographical location.

Online research databases CAB Abstracts, Medline, Embase, Biological Abstracts, and Scielo were screened in April 2016 without limit of publication date. The search strategy targeted 5 concepts: paratuberculosis, risk factors, MAP, bovine and herd. After removing duplicates, 2 reviewers screened the studies by title, abstract and by reading the full text. Risk of bias (RoB) was assessed by 3 reviewers using an instrument including Grading of Recommendations, Assessment, and Evaluation (GRADE) criteria.

Out of 1,675 initially eligible studies, 21 cross-sectional, 5 case control and 3 longitudinal studies met the inclusion criteria. To diagnose MAP, 14 studies used MAP antibodies detection tests, 7 used organism detection tests, 3 used detection of clinical disease, and 5 used a combination of tests. Risk factors were studied but they were not consistently evaluated in all 29 studies. Only 8 studies considered and adjusted their estimates of association for confounding factors. Herd size was significantly associated with MAP herd status in 12 (4 with low RoB) out of 16 studies, introduction of new animals was significantly associated with MAP herd status in 10 (4 with low RoB) out of 24, history of PTB was significantly associated with MAP herd status in 6 (4 with low RoB) out of 13, and management of colostrum and milk were significantly associated with MAP herd status in 5 (none with low RoB) out of 18.

This review provides a large compilation of available information about risk factors associated with MAP herd status evaluated using a RAQ. The large heterogeneity between studies made comparisons difficult. Some risk factors were apparently more consistent across studies. However, results should be interpreted in the light of the quality and risk of bias of each study.

3.1 Introduction

Paratuberculosis (PTB) is a contagious, and chronic granulomatous enteritis caused by Mycobacterium avium subspecies paratuberculosis (MAP). The disease is characterized by a long subclinical period followed by diarrhea, weight loss and eventually death [1]. Milk production [2-4], reproductive performance [5], longevity [2-4] and carcass weight at slaughter [6] are negatively affected by the disease, leading to economic losses [7, 8]. The most important route of transmission is feco-oral [9]. The MAP can also be excreted in colostrum and milk [10-13] from subclinically or clinically affected cows.

Any management practices that expose (directly or indirectly) susceptible animals to fecal material from MAP shedders can be considered a risk factor for infection [8]. Reducing exposure to risk factors is one of the key component to decrease MAP prevalence in a herd [14]. Identifying these risk factors is a key element in PTB control. A vast body of literature reports associations between various management practices applied on dairy farms and MAP herd status. These management practices have not been systematically reviewed. Herd status is one of the most frequent parameter studied and using a questionnaire a frequent way to collect data. Since 1990, risk assessment questionnaires (RAQ) are more frequently used in control programs around the world (reviewed in: [15]). The RAQ are an efficient way to know and evaluate the management practices in place in a herd. Based on a RAQ, several studies have been conducted to determine which management practices are associated with MAP herd status.

The main objective of this comprehensive review was to critically review the available scientific literature that evaluates the association between management practices (measured by a RAQ) and MAP herd status. A secondary objective was to identify, appraise and summarize consistently reported risk factors associated with MAP herd status.

3.2 Material and methods

This review was designed following a systematic approach to identify and select studies. Main question was: Which are the management practices (measured using a RAQ) used in dairy farms most consistently reported to be associated with MAP herd status? Online research databases and reference literature were screened in order to find studies that evaluated management practices that represent potential risk factors of PTB. We focused on the studies using a questionnaire as a primary research method of data collection.

3.2.1 Search strategy

The internet search was performed in April 21, 2016. The search was conducted in the following databases: CAB Abstracts (1910-2016), Medline (1946-2016), and Embase (1974-2016) using the Ovid platform; Biological Abstracts (1969-2016) and Scielo (1997-2016) using the Web of Science platform. The search strategy was developed with the help of a librarian:

(Mycobacterium avium paratuberculosis or Mycobacterium paratuberculosis or paratuberculosis or Johne* disease* or mycobacterium avium subsp paratuberculosis or mycobacterium avium subspecies paratuberculosis or mycobacterium avium ssp paratuberculosis) AND (risk* or risk factor* or management practice* or measure* or management procedure* or management* or calf-rearing practice* or biosecurity or hygiene) AND (herd status or prevalence* or status or infect* or seroprevalence or transmission or shed* or excret* or clinical or positive result) AND (Cattle or Bovi* or Cow* or Dairy or Calf or Calves or Heifer* not sheep* not goat*) AND (Herd* or Farm*). Citations were imported into EndNote 7.5, duplicates were removed and when two versions of the same study were available, the most complete was selected.

3.2.2 Identification of relevant studies

The inclusion criteria were studies that: 1) studied herd characteristics and management practices applied or observed at the herd level that can be risk factors for MAP; 2) those risk factors were evaluated using a risk assessment questionnaire (studies based on selected data from farmers registers or studies evaluating a unique specific herd characteristic were

excluded); 3) evaluated or described the association between these risk factors and MAP herd status; 4) were written in English, French or Spanish. The studies were excluded if they were related evident and strictly to evaluation of diagnostic tests, vaccines, economic parameters, productivity parameters, genomics, Crohn's disease, pharmacology, pathophysiology, immunology, season effect, nutritional parameters, exclusively vertical transmission, exclusively transmission by embryo transfer and semen, species other than bovine or production other than dairy. Only observational original studies were considered, case reports and review articles were excluded. No exclusion was implemented based on geographical location or sample size.

Initially, screening by title was performed by two authors (M.P. and V.W.). Then, selected manuscripts were screened by abstract (M.P. and V.W.). At this point, all studies that seemed (by reading the abstract) to provide insight to our objective were kept. A full manuscript lecture was performed to confirm the presence of the inclusion criteria. Agreement between the reviewers was assessed after each step using the Cohen's kappa (*kappa*) statistic. Disagreement between reviewers were discussed and solved by consensus or by consulting a third reviewer (J.C.A.) and then by majority. Reference lists of eligible studies were checked for additional studies not retrieved by our literature search (snowballing) in a similar manner by one author (M.P).

3.2.3 Quality appraisal

Risk of bias (RoB) for all studies was evaluated independently by 3 researchers (M.P., V.W. and J.C.A.), using a pre-designed RoB assessment form suggested by Waddell *et al*. in a previous study [16], (included as supplementary material, Annexes 1 and 2). Briefly, this form is adapted from The Cochrane Collaboration's tool for assessing RoB and updated to include Grading of Recommendations, Assessment, and Evaluation (GRADE) criteria for GRADING the evidence. The form uses several questions to evaluate generic and individual components of study methodology that have a potential relation to bias (selection, confounding, and losses to follow-up) and validity of the studies. There are 21 questions to appraise cross-sectional and case-control studies, and 23 questions to appraise cohort studies (18 are the same for both study designs). For each study, the reviewer responds all the questions. Finally, based on the questions, each reviewer indicates that the

article has low, high, or unclear RoB. Results of the 3 reviewers' evaluations were then compared. For each question, a coefficient of agreement was estimated. When agreement coefficient between the three reviewers was >0.4, final answer for the question was obtained using the majority rule (≥2 out of 3). When agreement was <0.4, or when all 3 reviewers responded differently, a fourth reviewer was solicited to reread the article and decide (G.F.). Finally, each article had a global answer to each question and three (each one of the reviewers) RoB classification. Based on the risk classification that each reviewer provided and the response to each question, each study received an overall assessment of RoB. Studies minimizing bias (majority of responses indicating low RoB) in the results were assigned a low risk of bias. For studies with a plausible bias that raises doubt about results (majority of questions indicating unclear RoB), an unclear risk of bias was appointed. Studies received a high risk of bias if several domains indicated serious plausible bias (majority of responses indicating high RoB).

3.2.4 Data extraction

Relevant information was collected from eligible articles using an individual collection form (supplementary material, Annexe 3). Relevant information included first author, journal and year of publication, country where the study was performed, study design, sample size, sample unit, type of sample, diagnostic test(s) used, case (positive herd) definition, statistical approach, availability of the questionnaire, and factors associated with MAP herd status (risk or protective factors). Analysis of the data was descriptive.

3.3 Results

3.3.1 Study selection

A flowchart of the manuscript selection process (including reasons for exclusion) is shown in Figure 1. A total of 1,625 potential manuscripts were identified with the search strategy performed on the electronic databases. After duplicate removal, 848 articles were screened by title and 357 were considered for abstract screening (*kappa* agreement coefficient between both reviewers=0.65; 95% CI:0.59-0.70). Among the 357 articles screened by abstract, 97 were considered for full text reading (*kappa*=0.53; 95% CI: 0.44-0.71). Of the 97 articles evaluated, 29 were of interest and suitable for data extraction (*kappa*=0.61; 95% CI:0.47-0.77). No articles were included after bibliography screening.

3.3.2 General characteristics

General characteristics of the 29 selected studies are summarized in Table 1. Articles were published between 1992 and 2016, in 19 different journals. Preventive Veterinary Medicine was the most common journal (*n*=7; 24%). The studies were carried out in 17 different countries. 8 studies (28%) were performed in USA. Cross-sectional was the most common study design (*n*=21; 73%) followed by case-control studies (*n*=5, 17%) and longitudinal studies (*n*=3, 10%). All studies reported the number of herds followed. The mean number of herds included in the studies was 332 and the median number was 122, ranging from 20 to 2,953. Mean number of sampled animals was 8,260 and median number 4,484 (range: 820 to 31,745). The entire questionnaire was available either as supplementary material or included in the text for 13 studies (45%). The remaining 16 studies mentioned the use of a questionnaire, but the entire version was not readily available for the readers.

3.3.3 Risk of bias

Overall RoB was considered low in 8 studies (28%), unclear in 14 studies (48%) and high in 7 studies (24%). The most common reason for an unclear RoB score was a lack of clarity on whether tools to measure exposure (e.g., questionnaires) were available. Risk of bias attributed to each study is presented in Table 3.1.

3.3.4 Paratuberculosis diagnostic tests

Five studies (17%) performed herd level sampling (no individual sampling was performed). Of the other 24 studies, only 6 studies reported the number of sampled animals. Among the 29 studies, 24 studies used only one type of diagnostic test for PTB detection (14 used MAP antibodies detection tests, 7 used organism detection tests, and 3 used detection of clinical disease). Five studies used a combination of tests for PTB diagnostic (4 used antibodies and organism detection tests, and 1 used antibodies detection tests and detection of clinical disease). Type of diagnostic tests and the specific tests used in each study are shown in supplementary files (Annexe 4). ELISA was the most common test used among the studies. The different ELISA kits used in these studies are shown in supplementary files (Annexe 5).

3.3.5 Herd status case definitions

Definition of herd status (positive *vs.* negative) for all studies is presented in Table 3.2.

3.3.6 Statistical approaches

In 26 of the 29 studies (90%) the univariable analysis was explicitly described [17-42]. Among them, the two most common univariable approaches were logistic regression (n=10, 38%) and chi square test (n=7, 27%). The *p* value of significance for univariable analysis (cut-off) was reported in 14 studies [17, 20, 21, 23-26, 28, 30, 31, 39-42] and varied from 0.05 to 0.25, with 86% (n=12) using a *p* value between 0.15 and 0.25.

Of the 29 eligible studies, 25 studies (90%) reported using a multivariable logistic regression [17-21, 23-28, 30-43] and 1 study [44] used a linear regression.

Assessment of confounding factors was reported in 8 studies [17, 18, 20, 21, 24, 31, 34, 40], while the interaction between predictors was evaluated in 6 studies [24-27, 31, 40]. Only 2 studies presented a directed acyclic graph [20, 40].

3.3.7 Risk factors associated with PTB

For the purpose of this study, the risk factors reported in the eligible studies were grouped into epidemiological risk categories as shown in Table 3.3. Also, risk factors were

classified if the study was of low, unclear or high RoB. However, the measure and the definition of the risk factors may vary within the same category.

For example, herd size was reported to be a risk factor in 12 of 18 studies. However, the cut-offs for the categorization of the variable herd size varied between articles. More than 12 [17], 70 [44], 100 [33, 42], 500 [24] cows were used to classify a herd as large. One study [20] evaluated specifically the increase of herd size by every 100-cows. In other studies, herd size was measured as a continuous variable [23, 27, 30, 35, 36, 43].

The introduction of animals was found to be a risk factor in 10 of 24 studies. Some of these studies measured the number of animals purchased in the last 4 [25], 5 [27] or 20 years [21]. One study [20], evaluates not only the number of animals purchased in the last 5 years, but also took into account preventive measures undertaken when purchasing like purchasing from a single herd and information about origin herd MAP status. Other study estimated the number of animals purchased in the last year [19]. Others evaluated whether the herd had imported cattle [23] or introduced animals from other herds (without mentioning if these cattle were purchased) [44]. Other study [42] assessed the percentage of cows born in other dairies. Two other studies [23, 30] evaluated if the herd was partially or fully depopulated because of Bovine Spongiform Encephalopathy or Bovine Tuberculosis which resulted in partial or full restocking.

History of PTB (clinical signs or positive tests) was reported to be a risk factor in 6 of 13 studies using different definitions. One study evaluated if there have been positive animals in the farm in the last 3 years [37]. Three studies only mentioned “history of paratuberculosis” [26, 38]. Two evaluated specifically if clinical signs were present as classical cases [31, 39] and one considered if prior testing for MAP was performed [27].

Management and administration of colostrum and milk were reported as risk factors in 5 of 18. Risk factors related to colostrum and milk management and administration included: colostrum from cows with previous MAP diagnosis [28], feeding waste milk or milk with antibiotics [30, 32], milk replacer only [43], and no raw milk [41].

Risk factors related to the direct or indirect contact of calves with adult cows or their feces were reported in 2 of 20 studies and included: 0-6 weeks old calves exposure to adult cows feces [39] and housing <6 months-old calves with adult cows [28].

Presence of other conditions and practices related to other diseases were reported as risk factors for PTB in 2 of 4 studies. Presence of calves with diarrhea [21] and incidence of clinical mastitis [28] were the two conditions other than paratuberculosis associated with a positive MAP herd status.

Breed was reported as a risk factor in 2 of 5 studies. Having more than 40% of Holstein animals [44], and *Channel Islands* breeds [40], were associated with a positive MAP herd status.

Contact of cows with other cattle was reported as a risk factor in 3 of 8 studies [25, 34, 41]. Specifically, cows sharing pastures with other herds [34], and contact of dairy cows with beef cattle during the winter [41] were associated with a positive MAP herd status.

Two of 4 studies reported factors related to neonatal environmental hygiene. Specifically, contamination of udders of periparturient cows with manure [26] and extent of manure build up in calving area [33], were found to be risk factors associated with a positive MAP herd status.

Other risk factors associated with positive MAP herd status were reported only once and were not included in Table 3.3: not using a maternity pen [17], washing udders prior to parturition [37], high calving area stocking density [24], presence of Johne's suspects in the calving area [24], giving water to calves from birth [32], individually tied heifers rather than indoor on outdoor pens or lots [41], group housing pre-weaned calves [42], manure contamination of animal pens and feeders [20], manure contamination of adult cattle's feed [24], and increased soil iron content [38] were associated to a positive MAP herd status. In one study [45], herds keeping or culling MAP positive cows had higher bulk tank milk ELISA optical densities than herds that never observed clinical signs [45]. Commercial hers were more likely to be MAP positive than registered herds in one study [39]. One study reported that, surprisingly, purchasing replacements from private sources was associated with positive MAP herd status [40].

3.3.8 Management practices reported as protective factors

Some studies reported management practices as protective factors by being associated with a negative MAP herd status. The protective factors that are biologically plausible according to the characteristics of the disease and existing knowledge [1], are presented in Table 3.4.

However, some management practices thought to increase MAP introduction or transmission were reported as negatively associated to paratuberculosis. For example, feeding colostrum from other herds [23], feeding pooled non-pasteurized milk [33], contact of bred heifers with adult manure [24], contamination of heifer's food with feces [30], pre-weaned calves housed near adult cows [33], and the use the same equipment to feed and manure [38]. Other inadequate management practices reported as protective factors were: manure build up and humidity of calving area [24, 41], cows calving in a paddock compared to shed area and calving pad [32].

Also, management practices not directly related to MAP transmission were reported as protective factors for PTB: spreading fertilizer on pastures [38], lime application to pasture [37, 38], type and quantity of concentrates fed to lactating cows [40, 41], teat dipping after milking [41], using a "gutter cleaning" [38] and high soil pH [38].

3.4 Discussion

This comprehensive review identified and summarized 29 studies evaluating potential risk factors associated with MAP herd status using a RAQ in several parts of the world. The analysis of PTB risk factors around the world is important to better understand the epidemiology of the disease and to better aim the actions to control the disease. This comprehensive review summarizes evidence and identifies direction for further research efforts. Despite some differences in geography, study design and statistical approach, some risk factors are more commonly reported to be associated with MAP herd status.

3.4.1 Risk of bias

Statistically significant risk factors should be analyzed in the light of the RoB of the study. A high RoB might limit the validity of the obtained results depending of the sources of bias identified in the study. Our results suggest that herd size, introduction of animals to the herds, history of PTB, are important global risk factor for PTB as they were found significantly associated to PTB in 4 low RoB studies conducted in different countries (Canada, Brazil, Japan and United States). If we concentrate only in studies with low RoB, herd size was significantly associated with PTB in 4 of 5 studies which evaluated this variable; introduction of animals to the herd was significantly associated with PTB in 4 of 5 studies which evaluated this variable; and having history of PTB was significantly associated with current herd status in 4 of 6 studies which evaluated this variable. All these factors, but introduction of animals to the herd, correspond to herd characteristics rather than management practices that may be modified by producers. So, control programs cannot enforce changes related to those characteristics. However, herds with these characteristics could be targeted as high-risk herds.

The MAP within herd transmission and introduction risk factors presented here should be considered as important management practices or herd characteristics associated to PTB as they were reported as being statistically associated to a positive MAP herd status in many studies and, most of them, in at least 1 low RoB study.

One of the most common reason for an unclear RoB score was a lack of clarity on whether tools to measure exposure (e.g., questionnaires) were available. Also, not all the studies

validate the questionnaire used. Validity for a study with an unclear RoB is more difficult to assess if the source of incertitude comes from a material that is not available at the moment of publication. One might think that with the increasing online publication the inclusion of supplementary material has been more accessible to authors leading to manuscripts with more complete information.

3.4.2 Protective factors

Some factors were associated with a negative MAP herd status (protective factors). Having individual calving pens was reported as a protective factor in 2 unclear RoB studies [30, 40] and in one high Rob study [29]. Herds cleaning calf hutches or pens were less likely to be MAP positive in one Low RoB study [37]. The chances to be MAP positive were reduced in herds were most of calvings were attended [29]. Having been previously tested negative for PTB was a protective factor in on unclear RoB study [43].

However, some protective factors were not intuitively suspected. This can occur in cross-sectional and case-control studies given that some management practices may have been implemented in herds diagnosed with the disease recently. In those herds, awareness of the presence of the disease may have motivate the producers to change a management practice to reduce MAP prevalence.

3.4.3 Type of study

The most frequent study design was cross-sectional. Cross-sectional studies are considered to bring low level of evidence and cannot provide evidence of causal relationship [46]. They are excellent for hypothesis generation and establishing directions for future research [46]. Furthermore, one may think that the long incubation and latency periods of PTB makes it even more complicated to completely understand the epidemiology of this disease in cross-sectional studies. Contrary to other diseases (e.g. mastitis) which can be evaluated in a more immediate way, PTB requires long periods of evaluation. Given the slow development and the long incubation period of MAP infection, a minimum of 4–5 years of follow up is required to study the evolution of the disease [47]. So, it has been suggested that longitudinal studies are more suitable to evaluate the changes in MAP incidence after the implementation of better management practices [48]. However, those were not common

(n=3) in the present study. Longitudinal studies are laborious and time consuming. Besides, keeping the participation of producers in such long studies is challenging. Sometimes, important aspects that may affect the RoB, are sacrificed to maintain the participation of producers. This trade off may affect the quality of the study and the validity of the results. For example, random sampling is easier in cross-sectional studies. In longitudinal studies, random sampling becomes more difficult. Non-probabilistic sampling (e.g: convenience sampling) can be misleading and have to be considered when appraising each study [46].

Long randomized controlled trials could be useful to evaluate the causality of management practices in MAP herd status, but ethically they are not feasible.

3.4.4 Associations found in studies

Failure to find a significant association for a particular risk factor may be due to a high RoB (e.g. confounding bias, selection bias), and/or a lack of power of study, or a weak association with the outcome. A low power reduces the chance of identifying a potential risk factor associated with the outcome [46]. The only way to increase the power of a study is by increasing the sample size (to include more herds) [46]. However, the budget available for research studies often limits the number of herds enrolled. To better appraise non-significant results, we estimated the post-hoc power of studies that could not identify as statistically significant the most frequent studied risk factors (e.g. introduction of animal and herd size). One may hypothesize that those factors would be detected as significant with an adequate sample size given that power was relatively low for all those studies (the average post hoc power was 25.5% to detect a difference varying between 0.5 and 3.9).

The opposite situation may occur if a high RoB is derived from a study design or analysis (e.g: selection, measure of exposure, measure of outcome, confounding). This may hamper confidence in the findings. In those cases, an association would then be identified when in fact it may not exist. The quality appraisal is one way to make an effort to emit a concept of each paper (Table 6).

3.4.5 Using a questionnaire

In this comprehensive review were targeted studies that used a RAQ to measure the exposure. Around the world, questionnaires are used to better understand and control all kind of diseases [49]. More specifically, they are considered an important key element in PTB control programs [50, 51]. The use of a questionnaire as data-collection tool allows to investigate specific subjects related to a disease (by previous knowledge). A well designed questionnaire should be objective, reliable, valid and precise [46]. Some studies [52] have found associations using only information available on records that producers routinely fill (e.g: milk production, entry/exit of animals). This kind of study was not included in our review. Using a RAQ for identifying management practices and herd characteristics allows to build a more complete multivariable analysis. Complex analysis such as confounding factors and interaction could be assessed since several variables are described. A RAQ can be designed to evaluate specific practices related to the subject studied [53]. However, the quality of the data collected with questionnaires can vary. Method of administration (e.g. self-administered versus interview modes) has been identified to have important effect on quality of collected data [54]. We can also hypothesize, that quality of the data will be better when producers are part of a voluntary program. Response rate could be higher and more carefully provided when producers are motivated. One might think that producers participating in control programs can be more familiar with the disease, and so answers can be biased by previous knowledge.

Target the observational studies using a questionnaire can lead to miss studies using direct observation as measure of exposure. Farm audit was included only if a questionnaire or a check list was used. Eighteen studies were excluded for not using of a questionnaire, but most of them have another reason (e.g. not association with MAP herd status reported). Only 5 studies had an association reported, but the variables were specific (e.g. herd size only) and not evaluated at the light of the rest of practices used in the herd.

3.4.6 Heterogeneity among the studies

Heterogeneity among the studies included in the present review did not allow us to perform a meta-analysis. However, we consider that the descriptive results provide an interesting

summary of research conducted by different groups around the world. The most common risk factors are listed, and the quality appraisal allows the reader to establish a relative degree of quality or certainty in the conclusions reported.

The variation in the herd status definition observed in the present study was expected. We can hypothesize that the magnitude of the PTB problem in the herd will be estimated with more or less precision depending on the herd status definition used in a study. It has been suggested that standardization of case definitions and outcomes would improve the comparability of results across studies [55], making them easier to synthesize and to draw more generalized conclusions about the consistency, direction, and magnitude of results. The ideal case definition for a positive herd still not defined. However, bacteriological culture of environmental samples seems a good option. The advantage of detecting live MAP by culture is that a positive result confirms the presence of viable MAP on the farm [56]. Given the high specificity of fecal culture [57], a minimum of just 1 positive sample can be used as the cut-off to declare a herd infected. Including 2 consecutive negative tests as the criteria to select control herds increase the likelihood that MAP was absent in those herds.

Herd status definition depends on the unit of study and the on the diagnostic test used. All three components substantially varied among the studies. ELISA was the test most commonly used. This is not surprising since ELISA is simple, fast, and inexpensive [58, 59]. However, variation can be expected among studies, as several different ELISA kits were used. Threshold recommended by the manufacturer varied from kit to kit making the agreement between the test results inconsistent [60]. It has been reported that using different cut-offs make a better agreement between different ELISA kits [60]. If the objective is to determine MAP herd status, bacteriological culture of environmental samples is considered the most cost-effective strategy by some experts [56]. In this systematic review, only 2 studies used bacteriological culture of environmental samples to define herd status.

Depending of the study design, the statistical approach also varied among the studies. Since the studies used a questionnaire, they all have multiple predictors (independent variables) to be associated with the MAP herd status (dependent variable). Therefore, it was not

surprising that logistic regression was the most commonly used statistical approach. The evaluation of confounding and interactions was not always considered or adequately described. Confounding, interactions and directed acyclic graphs should be more often used and reported in epidemiologically studies to improve modelling strategy and avoid misinterpretation [46]. Confounders identification allow to adjust the final model in order to isolate the causal effect of interest [61].

3.4.7 Limitations

The search strategy used was designed trying to miss the least amount of studies. However, it is possible that some studies have been missed. Using the exclusion operator "not" can be too exclusive, decreasing the number of manuscripts found. In the present study the difference between using the operator "not" and not using it was not meaningful.

It is also possible to miss studies in the selection process, even if a systematic screening process is used. Efforts to reduce this selection bias were done by repeating the selection process by two researchers as recommended [62]. Even if studies written in three languages (English, French and Spanish) were included in the present review, selection bias remains possible due to lack of resources to translate studies published in other foreign languages. *Kappa* coefficient of agreement was moderate between the two reviewers in the selection process. Careful discussion of each disagreement corrected this.

One reviewer performed data extraction. Most of the data extracted were factual and explicit data. Considering the type of information extracted, we consider that the potential bias introduced when extracting data by a single reviewer was minimal.

Finally, the quality of underlying studies limits the results of our systematic review. Of the 46 studies, 13 studies (28%) were classified as low RoB. However, we achieved our objective of describing all the scientific evidence published on the subject.

3.5 Conclusions

The global evidence reported in this systematic review shows that although there are some regional differences, some risk factors associated to PTB appears more relevant and should be prioritized in the prevention and control of the disease. There is a need for further

evidence-based longitudinal studies, in order to obtain more accurate information about the most important risk factors associated with PTB.

Figure 3.1 Flow chart describing the selection steps of scientific studies evaluating the association between risk factors (identified using a risk assessment questionnaire) and paratuberculosis.

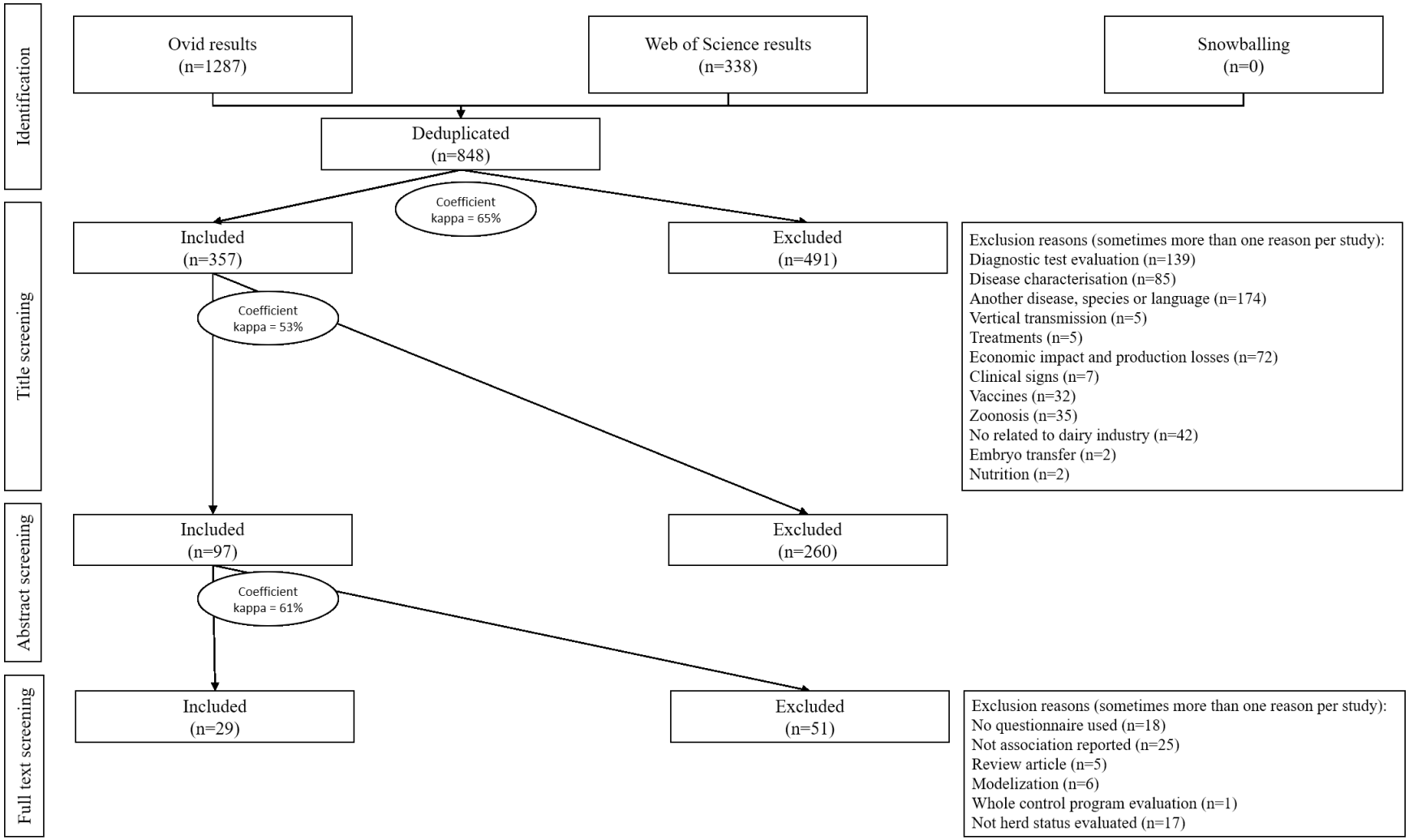


Table 3.1 General characteristics of 29 studies using a risk assessment questionnaire to evaluate the risk factors associated with *Mycobacterium avium* subsp. *paratuberculosis* herd status.

Ref	First author	Year	Country	Study design	Number of herds	Sampling unit	Number of animals	Risk of bias
[20]	Wolf, R.	2016	Canada	Cross-sectional	354	Herd	-	Low
[17]	Vilar, A. L. T.	2015	Brazil	Cross-sectional	480	Animal	2,504	Low
[18]	Sun, W.	2015	China	Cross-sectional	113	Animal	3,674	Unclear
[19]	Kunzler, R.	2014	Switzerland	Case-control	85	Animal	NR	Unclear
[45]	Cazer, C. L.	2013	United States	Cross-sectional	233	Herd	-	Unclear
[21]	Sorge, U. S.	2012	Canada	Longitudinal	226	Animal	NR	Low
[22]	Erume, J.	2012	Uganda	Cross-sectional	69	Animal	820	Unclear
[23]	Barrett, D. J.	2011	Ireland	Case-control	152	Animal	NR	Unclear
[24]	Kinsel, M.	2010	United States	Cross-sectional	312	Animal	NR	Unclear
[25]	Correia-Gomes, C.	2010	Portugal	Cross-sectional	122	Animal	5,294	Unclear
[26]	Ansari-Lari, M.	2009	Iran	Cross-sectional	110	Herd	-	Unclear
[27]	Pillars, R. B.	2009	United States	Cross-sectional	94	Herd	-	Low
[28]	Dieguez, F, J.	2008	Spain	Cross-sectional	101	Animal	5,528	Unclear
[29]	Cashman, W.	2008	Ireland	Cross-sectional	20	Herd	-	High
[30]	Barrett, D.	2008	Ireland	Case-control	152	Animal	NR	Unclear
[44]	Pozzato, N.	2007	Italy	Cross-sectional	23	Animal	NR	High

[31]	Kobayashi, S.	2007	Japan	Longitudinal	594	Animal	NR	Low
[43]	Weering, H. J.	2005	Netherlands	Longitudinal	1083	Animal	NR	Unclear
[32]	Ridge, S. E.	2005	Australia	Cross-sectional	54	Animal	NR	High
[33]	Berghaus, R. D.	2005	United States	Cross-sectional	482	Animal	NR	High
[34]	Fredriksen, B.	2004	Norway	Case-control	128	Animal	NR	High
[35]	Muskens, J.	2003	Netherlands	Cross-sectional	309	Animal	NR	High
[36]	Daniels, M, J.	2002	Scotland	Cross-sectional	86	Animal	NR	High
[42]	Wells, S. J.	2000	United States	Cross-sectional	967	Animal	31,745	Low
[38]	Johnson-Ifearulundu, Y.	1999	United States	Cross-sectional	83	Animal	NR	Low
[37]	Johnson-Ifearulundu, Y.	1998	United States	Cross-sectional	83	Animal	NR	Low
[39]	Obasanjo, I. O.	1997	United States	Cross-sectional	33	Animal	NR	Unclear
[40]	Cetinkaya, B.	1997	England	Cross-sectional	2953	Animal	NR	Unclear
[41]	McNab, W. B.	1992	Canada	Case-control	120	Animal	NR	Unclear

Ref: reference. NR: Not reported.

Table 3.2 Positive herd case definition of 29 studies evaluating the association between risk factors (identified using a risk assessment questionnaire) and *Mycobacterium avium* subsp *paratuberculosis* positive herd status.

Positive herd case definition	Diagnostic test	References
Test performed at the herd level		
Positive sample	Bulk tank milk ELISA	[45]
	Bulk tank milk PCR	[26]
	Milk sock filter residue culture	[29]
At least 1 positive sample	Environmental culture	[27], [20]
Test performed at the individual level		
At least 1 positive animal	Serum ELISA	[18], [22], [24], [43], [32], [35]
	Clinical signs	[32], [33], [36], [40]
	Milk ELISA	[25], [21]
	Individual fecal culture	[23], [24], [30], [39]
	Fecal PCR	[24]

	ELISA (no sample specified)	[31]
	Culture (no sample specified)	[31]
	Serum ELISA, fecal culture and fecal PCR	[19]
	Bacterioscopy	[31]
	Complement fixation	[31]
	Johnin intradermal hypersensitivity	[31]
At least 2 positive animals	Serum ELISA	[38], [37]
≥1 positive animal in herds up to 24 cows and ≥2 positive animals in herds with more than 24 cows	Serum ELISA	[17]
2-4 ELISA positive cows, or 1 ELISA positive cow and ≥1 cow culled with clinical signs in the last year	Serum ELISA	[28]
≥5 animals with S/P ≥0.15 or ≥1 animal with S/P=0.5	Serum ELISA	[34]
≥2 positive cows or 1 positive cow and JD in >5% of culled cows the previous year	Serum ELISA or clinical signs	[42]

60 herds with highest risk and level of infection based on their mean LAM-ELISA	Serum ELISA	[41]
Not mentioned	Serum ELISA and fecal culture	[44]

S/P: sample to positive ratio. JD: Johne's Disease.

Table 3.3 Risk factors* (identified using a risk assessment questionnaire) reported to be significantly associated with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) positive herd status in more than 1 study.

Risk factor category	**Number of studies that found the factor significantly associated with MAP herd status / Number of studies evaluating the factor** (%)**	**Low RoB**		**Unclear RoB**		**High RoB**	
		Number of studies that found the factor significantly associated with MAP herd status	**Number of studies that did not find the factor significantly associated with MAP herd status**	**Number of studies that found the factor significantly associated with MAP herd status**	**Number of studies that did not find the factor significantly associated with MAP herd status**	**Number of studies that found the factor significantly associated with MAP herd status**	**Number of studies that did not find the factor significantly associated with MAP herd status**
Herd size	12/18 (71)	4 [17, 20, 27, 42]	1 [37]	4 [23, 24, 30, 43]	5 [18, 25, 26, 40, 45]	4 [33, 35, 36, 44]	0
Introduction of animals to the herd	10/24 (42)	4 [20, 21, 27, 42]	1 [17]	5 [19, 23, 25, 30, 43]	8 [18, 22, 24, 26, 28, 39, 40, 45]	1 [44]	5 [29, 32, 34, 35, 36]
History of paratuberculosis	6/13 (46)	4 [27, 31, 37, 38]	2 [20, 21]	2 [26, 39]	3 [24, 25, 43]	0	2 [35, 36]

Colostrum and milk management***	5/18 (28)	0	3 [20, 21, 31]	4 [28, 30, 41, 43]	6 [22, 23, 24, 25, 26, 40]	1 [32]	4 [29, 33, 34, 35]
Specific geographic region of the country	3/9 (33)	2 [17, 42]	2 [21, 37]	0	4 [18, 26, 40, 45]	1 [34]	0
Contact with cattle from other herds	3/8 (38)	0	1 [17]	2 [25, 41]	2 [19, 28]	1 [34]	2 [29, 35]
Contact with wild animals (Deer and/or rabbits)	3/6 (50)	0	1 [17]	1 [40]	1 [25]	2 [34, 36]	1 [29]
Contact of calves with adult cows or adult feces between birth and 6 months	2/20 (10)	0	4 [20, 21, 38, 42]	2 [28, 39]	9 [19, 22, 24, 25, 26, 30, 40, 41, 43]	0	5 [29, 32, 33, 34, 35]
Calving area hygiene	2/9 (22)	0	2 [20, 42]	1 [26]	4 [19, 24, 28, 43]	1 [33]	1 [35]
Manure spread to fields used to feed animals	2/7 (29)	1 [38]	[20, 37]	0	[39, 45]	1 [36]	[29]
Breed	2/6	0	1	1	3	1	0

	(33)		[17]	[40]	[22, 26, 45]	[44]	
Other diseases (management practices	2/4	1	0	1	1	0	1
and presence of)	(50)	[21]		[28]	[18]		[32]
Exercise lot for lactating cows	2/2	2	0	0	0	0	0
	(100)	[37, 38]					

RoB: risk of bias

* Risk factors reported in the eligible studies were grouped into categories following an epidemiological sense.

** Studies where it was evident that the factor was studied

*** Studies that evaluated any management practices related to colostrum and milk management and administration (colostrum from cows with previous MAP diagnosis, feeding waste milk or milk with antibiotics, milk replacer only, and no raw milk.

References

1. Fecteau ME, Whitlock RH. Paratuberculosis in Cattle. In: Behr MA, Collins D, editors. Paratuberculosis: Organism, Disease, Control. Wallingford, Oxfordshire: CAB International; 2010. p. 144-56.
2. Hendrick SH, Kelton DF, Leslie KE, Lissemore KD, Archambault M, Duffield TF. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. Journal of the American Veterinary Medical Association. 2005;227(8):1302-8.
3. Goodell G, Hirst H, Garry F, Dinsmore P. Comparison of cull rates and milk production of clinically normal dairy cows grouped by ELISA *Mycobacterium avium paratuberculosis* serum antibody results. Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics. 2000.
4. Lombard JE, Garry FB, McCluskey BJ, Wagner BA. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. J Am Vet Med Assoc. 2005;227(12):1975-81.
5. Johnson-Ifearulundu YJ, Kaneene JB, Sprecher DJ, Gardiner JC, Lloyd JW. The effect of subclinical *Mycobacterium paratuberculosis* infection on days open in Michigan, USA, dairy cows. Preventive veterinary medicine. 2000;46(3):171-81.
6. Kudahl AB, Nielsen SS. Effect of paratuberculosis on slaughter weight and slaughter value of dairy cows. J Dairy Sci. 2009;92(9):4340-6.
7. Tiwari A, VanLeeuwen JA, Dohoo IR, Stryhn H, Keefe GP, Haddad JP. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, *Mycobacterium avium* subspecies *paratuberculosis,* and Neospora caninum on culling in dairy cattle in four Canadian provinces. Veterinary Microbiology. 2005;109(3/4):147-58.
8. McKenna SL, Keefe GP, Tiwari A, VanLeeuwen J, Barkema HW. Johne's disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. The Canadian veterinary journal = La revue veterinaire canadienne. 2006;47(11):1089-99.
9. Manning EJB, Collins MT. Epidemiology of paratuberculosis. In: Behr MA, Collins D, editors. Paratuberculosis: Organism, Disease, Control. Wallingford, Oxfordshire: CAB International; 2010. p. 22-8.
10. Giese SB, Ahrens P. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in milk from clinically affected cows by PCR and culture. Vet Microbiol. 2000;77(3-4):291-7.
11. Streeter RN, Hoffsis GF, Bech-Nielsen S, Shulaw WP, Rings DM. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. American journal of veterinary research. 1995;56(10):1322-4.
12. Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. Journal of clinical microbiology. 1992;30(1):166-71.
13. Taylor TK, Wilks CR, McQueen DS. Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. The Veterinary record. 1981;109(24):532-3.
14. Garry F. Control of paratuberculosis in dairy herds. The Veterinary clinics of North America Food animal practice. 2011;27(3):599-607, vii.
15. Kennedy D. International efforts at paratuberculosis control. The Veterinary clinics of North America Food animal practice. 2011;27(3):647-54, viii.
16. Waddell LA, Rajic A, Stark KD, Mc ES. The zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: a systematic review and meta-analyses of the evidence. Epidemiology and infection. 2015;143(15):3135-57.

17. Vilar ALT, Santos CSAB, Pimenta CLRM, Freitas TD, Brasil AWL, Clementino IJ, et al. Herd-level prevalence and associated risk factors for *Mycobacterium avium* subsp. *paratuberculosis* in cattle in the State of Paraiba, Northeastern Brazil. Preventive veterinary medicine. 2015;121(1/2):49-55.
18. Sun WW, Lv WF, Cong W, Meng QF, Wang CF, Shan XF, et al. *Mycobacterium avium* Subspecies *paratuberculosis* and Bovine Leukemia Virus Seroprevalence and Associated Risk Factors in Commercial Dairy and Beef Cattle in Northern and Northeastern China. BioMed Research International. 2015;2015 (no pagination)(315173).
19. Kunzler R, Torgerson P, Keller S, Wittenbrink M, Stephan R, Knubben-Schweizer G, et al. Observed management practices in relation to the risk of infection with paratuberculosis and to the spread of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss dairy and beef herds. BMC Veterinary Research. 2014;10(132).
20. Wolf R, Barkema HW, De Buck J, Orsel K. Dairy farms testing positive for *Mycobacterium avium* ssp. *paratuberculosis* have poorer hygiene practices and are less cautious when purchasing cattle than test-negative herds. Journal of dairy science. 2016;99(6):4526-36.
21. Sorge US, Lissemore K, Godkin A, Jansen J, Hendrick S, Wells S, et al. Risk factors for herds to test positive for *Mycobacterium avium* ssp. *paratuberculosis*-antibodies with a commercial milk enzyme-linked immunosorbent assay (ELISA) in Ontario and western Canada. Canadian Veterinary Journal. 2012;53(9):963-70.
22. Erume J, Mutebi F. The prevalence and risk factors of para tuberculosis in indigenous and exotic cattle in Wakiso and Masaka Districts, Uganda. International Journal of Animal and Veterinary Advances. 2012;4(4):244-51.
23. Barrett DJ, Mee JF, Mullowney P, Good M, McGrath G, Clegg T, et al. Risk factors associated with Johne's disease test status in dairy herds in Ireland. Veterinary Record. 2011;168(15):410.
24. Kinsel M, Mueller B, Honstead J. A retrospective analysis of risk factors associated with Johne's disease in Pacific Northwest dairy herds. Bovine Practitioner. 2010;44(2):114-22.
25. Correia-Gomes C, Mendonca D, Niza-Ribeiro J. Risk associations to milk ELISA result for paratuberculosis in dairy cows in northern Portugal using a multilevel regression model. Revue de Medecine Veterinaire. 2010;161(6):295-301.
26. Ansari-Lari M, Haghkhah M, Bahramy A, Novin Baheran AM. Risk factors for *Mycobacterium avium* subspecies *paratuberculosis* in Fars province (Southern Iran) dairy herds. Trop Anim Health Prod. 2009;41(4):553-7.
27. Pillars RB, Grooms DL, Woltanski JA, Blair E. Prevalence of Michigan dairy herds infected with *Mycobacterium avium* subspecies *paratuberculosis* as determined by environmental sampling. Prev Vet Med. 2009;89(3/4):191-6.
28. Dieguez FJ, Arnaiz I, Sanjuan ML, Vilar MJ, Yus E. Management practices associated with *Mycobacterium avium* subspecies *paratuberculosis* infection and the effects of the infection on dairy herds. Veterinary Record. 2008;162(19):614-7.
29. Cashman W, Buckley J, Quigley T, Fanning S, More S, Egan J, et al. Risk factors for the introduction and within-herd transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection on 59 Irish dairy herds. Irish Veterinary Journal. 2008;61(7):464-7.
30. Barrett D, Mee J, Good M, Mullowney P, Clegg T, More S. Risk factors for Johne's disease in Irish dairy herds: a case-control study. Cattle Practice. 2008;16(3):209-15.

31. Kobayashi S, Tsutsui T, Yamamoto T, Nishiguchi A. Epidemiologic indicators associated with within-farm spread of Johne's disease in dairy farms in Japan. J Vet Med Sci. 2007;69(12):1255-8.
32. Ridge SE, Baker IM, Hannah M. Effect of compliance with recommended calf-rearing practices on control of bovine Johne's disease. Aust Vet J. 2005;83(1-2):85-90.
33. Berghaus RD, Lombard JE, Gardner IA, Farver TB. Factor analysis of a Johne's disease risk assessment questionnaire with evaluation of factor scores and a subset of original questions as predictors of observed clinical paratuberculosis. Preventive veterinary medicine. 2005;72(3/4):291-309.
34. Fredriksen B, Djonne B, Sigurdardottir O, Tharaldsen J, Nyberg O, Jarp J. Factors affecting the herd level of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. Vet Rec. 2004;154(17):522-6.
35. Muskens J, Elbers ARW, van Weering HJ, Noordhuizen JPTM. Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. J Vet Med B Infect Dis Vet Public Health. 2003;50(8):372-7.
36. Daniels MJ, Hutchings MR, Allcroft DJ, McKendrick J, Greig A. Risk factors for Johne's disease in Scotland--the results of a survey of farmers. Vet Rec. 2002;150(5):135-9.
37. Johnson-Ifearulundu YJ, Kaneene JB. Management-related risk factors for M. paratuberculosis infection in Michigan, USA, dairy herds. Preventive veterinary medicine. 1998;37(1-4):41-54.
38. Johnson-Ifearulundu Y, Kaneene JB. Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. American journal of veterinary research. 1999;60(5):589-96.
39. Obasanjo IO, Grohn YT, Mohammed HO. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. Prev Vet Med. 1997;32(3-4):243-51.
40. Cetinkaya B, Erdogan HM, Morgan KL. Relationships between the presence of Johne's disease and farm and management factors in dairy cattle in England. Prev Vet Med. 1997;32(3-4):253-66.
41. McNab WB, Meek AH, Martin SW, Duncan JR. Associations between lipoarabinomannan enzyme-immuno-assay test results for paratuberculosis and farm-management factors. Preventive veterinary medicine. 1992;13(1):39-51.
42. Wells SJ, Wagner BA. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. Journal of the American Veterinary Medical Association. 2000;216(9):1450-7.
43. Weering HJv, Mars MH, Muskens J, Middelesch H, Schaik Gv. The effect of biosecurity measures for paratuberculosis on the seroprevalence in Dutch dairy herds. Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark. 2005.
44. Pozzato N, Paoli M, Stefani E, Busani L, Farina G, Valorz C, et al. Study design and application of a paratuberculosis assurance program in Brown breeders' herds in the Italian central Alps. Proceedings of the 9th International Colloquium on Paratuberculosis, Tsukuba, Japan. 2007;29(2):317-8.
45. Cazer CL, Mitchell RM, Cicconi-Hogan KM, Gamroth M, Richert RM, Ruegg PL, et al. Associations between *Mycobacterium avium* subsp. *paratuberculosis* antibodies in bulk

tank milk, season of sampling and protocols for managing infected cows. BMC Veterinary Research. 2013;9(234).
46. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Second ed. Charlottetown, PEI, Canada: VER Inc; 2014.
47. Pillars RB, Grooms DL, Gardiner JC, Kaneene JB. Association between risk-assessment scores and individual-cow Johne's disease-test status over time on seven Michigan, USA dairy herds. Prev Vet Med. 2011;98(1):10-8.
48. Schukken YH, Whitlock RH, Wolfgang D, Grohn Y, Beaver A, VanKessel J, et al. Longitudinal data collection of *Mycobacterium avium* subspecies *Paratuberculosis* infections in dairy herds: the value of precise field data. Veterinary research. 2015;46:65.
49. Nielsen TD, Vesterbaek IL, Kudahl AB, Borup KJ, Nielsen LR. Effect of management on prevention of Salmonella Dublin exposure of calves during a one-year control programme in 84 Danish dairy herds. Prev Vet Med. 2012;105(1-2):101-9.
50. Kelton D, Barkema HW, Keefe GP, Fecteau G, A. BR, editors. Johne's Disease Control in Canada - 2010-2013 - Successes and Challenges. 4th Paratuberculosis Forum; 2014; Parma, Italy.
51. Geraghty T, Graham DA, Mullowney P, More SJ. A review of bovine Johne's disease control activities in 6 endemically infected countries. Preventive veterinary medicine. 2014;116(1-2):1-11.
52. Hirst HL, Garry FB, Morley PS, Salman MD, Dinsmore RP, Wagner BA, et al. Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity. Journal of the American Veterinary Medical Association. 2004;225(1):97-101.
53. Boynton PM, Greenhalgh T. Selecting, designing, and developing your questionnaire. BMJ. 2004;328(7451):1312-5.
54. Bowling A. Mode of questionnaire administration can have serious effects on data quality. Journal of public health (Oxford, England). 2005;27(3):281-91.
55. Whittington RJ, Begg DJ, de Silva K, Purdie AC, Dhand NK, Plain KM. Case definition terminology for paratuberculosis (Johne's disease). BMC Vet Res. 2017;13(1):328.
56. Sweeney RW, Collins MT, Koets AP, McGuirk SM, Roussel AJ. Paratuberculosis (Johne's disease) in cattle and other susceptible species. Journal of veterinary internal medicine. 2012;26(6):1239-50.
57. Collins MT, Gardner IA, Garry FB, Roussel AJ, Wells SJ. Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. J Am Vet Med Assoc. 2006;229(12):1912-9.
58. Nielsen SS. Immune-based diagnosis of Paratuberculosis. In: Behr MA, Collins D, editors. Paratuberculosis: Organism, Disease, Control. Wallingford, Oxfordshire: CAB International; 2010. p. 284-93.
59. Tiwari A, VanLeeuwen JA, McKenna SL, Keefe GP, Barkema HW. Johne's disease in Canada Part I: clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. The Canadian veterinary journal. 2006;47(9):874-82.
60. Collins MT, Wells SJ, Petrini KR, Collins JE, Schultz RD, Whitlock RH. Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clinical and diagnostic laboratory immunology. 2005;12(6):685-92.

61. Williamson EJ, Aitken Z, Lawrie J, Dharmage SC, Burgess JA, Forbes AB. Introduction to causal diagrams for confounder selection. Respirology (Carlton, Vic). 2014;19(3):303-11.
62. Pham MT, Waddell L, Rajic A, Sargeant JM, Papadopoulos A, McEwen SA. Implications of applying methodological shortcuts to expedite systematic reviews: three case studies using systematic reviews from agri-food public health. Research synthesis methods. 2016;7(4):433-46.

4. Facteurs de risque associés à un statut positif à *Mycobacterium avium* ssp. *paratuberculosis* dans les troupeaux laitiers du Québec.

Risk factors associated with *Mycobacterium avium* subsp. *paratuberculosis* herd status in Québec dairy herds

Maria Puerto-Parada[1], Juan Carlos Arango-Sabogal[1], Julie Paré[2], Elizabeth Doré[1], Geneviève Côté[3], Vincent Wellemans[1], Sébastien Buczinski[1], Jean-Philippe Roy[1], Olivia Labrecque[4], Gilles Fecteau[1]

(1) Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada, J2S 8H5

(2) Agence canadienne d'inspection des aliments, Saint-Hyacinthe, Québec, Canada, J2S 7C6

(3) Direction générale des laboratoires et de la santé animale, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Québec, Canada, G1P 4S8

(4) Laboratoire d'épidémiosurveillance animale du Québec, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Saint-Hyacinthe, Québec, Canada, J2S 7X9

Accepté dans Preventive Veterinary Medicine

Abstract

Paratuberculosis is a chronic and contagious enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Control of paratuberculosis is justified given the associated economic losses and the potential role of MAP in Crohn's disease in humans. Management practices that limit exposure of susceptible animals to MAP are more effective at reducing disease prevalence than testing and culling infected cows. The objective of this retrospective case-control study was to study the association between management practices and MAP status in dairy herds in Québec, Canada. A total of 26 case herds (MAP had been isolated from at least 1 environmental sample in each herd) and 91 control herds (no clinical cases of paratuberculosis and negative on 2 consecutive yearly environmental samplings) were selected among herds enrolled in the Québec Voluntary Paratuberculosis Control Program. A risk assessment questionnaire, completed at enrolment, was available for the selected herds. Culture of MAP was achieved using liquid media and the BACTEC 960 detection system. Multivariable logistic regression was used to evaluate the association between selected risk factors and MAP herd status. Herd size (OR=1.17; 95% CI: 1.02-1.33) and proportion of cows purchased per year in the last 5 years (OR=5.44; 95% CI: 1.23-23.98) were significantly associated with a positive MAP herd status.

The management risk factors identified in the present study are in accord with previous studies. Management practices aiming to prevent the introduction of new animals into the herd and to reduce the contact of newborn calves with adult animals or their feces are key elements to minimize MAP introduction and transmission into a herd. These elements should be prioritized in control programs**.**

Key words: *Mycobacterium avium* subsp. *paratuberculosis*, risk factors, management practices, case-control, epidemiology, MAP control.

Highlights

- Risk factors associated with a positive MAP status in dairy herds in Québec, Canada are reported.
- A herd's risk of being MAP positive increases as herd size increases.

- Farms buying more than 4% of the cows in their herds per year in the last 5 years have significantly greater odds of being MAP positive compared to closed herds.

4.1 Introduction

Paratuberculosis is a chronic and contagious enteric disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Economic losses associated with paratuberculosis are related to lower milk production (Hendrick et al., 2005; Lombard et al., 2005), increased culling rate (Tiwari et al., 2005), and decreased carcass weight at slaughter (Kudahl and Nielsen, 2009). The pathogen MAP is often found in patients with Crohn's disease, a human chronic inflammatory bowel disease, but a causal relationship has not been confirmed (Waddell et al., 2015).

Cattle usually become infected at a young age, primarily by the fecal-oral route (Manning and Collins, 2010). After a long incubation period (up to several years), infected cows may start shedding MAP without showing clinical signs, thus perpetuating MAP infections on the farm (Benedictus et al., 2008). Management procedures that limit exposure of susceptible animals to MAP are more effective at reducing disease prevalence than simply testing and culling MAP infected cows (Garry, 2011). Thus, reducing the risk of transmission to susceptible young stock is of primary importance (Doré et al., 2012).

The possibility of direct or indirect contact between calves and adult cows or adult manure has been associated with paratuberculosis in previous studies. However, the definition of calf exposure to adult manure varies among these studies. For example, exposure to adults other than the dam at birth was associated with cows testing positive to MAP (Pillars et al., 2011), housing <6 month old calves with adults (Dieguez et al., 2008), and exposure of 0-6-week-old calves to the feces of adults (Obasanjo et al., 1997) were associated with positive MAP herd status. Hygiene of the neonatal environment has been found to be an important risk factor. For example, contamination of the udders of periparturient cows with manure (Ansari-Lari et al., 2009) and the extent of manure buildup in calving area (Berghaus et al., 2005) have been reported to be associated with positive MAP herd status. Also, herd size (Wells and Wagner, 2000; Ridge et al., 2010; Bolton et al., 2011; Vilar et al., 2015) and introduction of new animals to the herd (Pillars et al., 2009; Correia-Gomes et al., 2010; Kunzler et al., 2014; Pieper et al., 2015) are frequently found to be risk factors.

Among the studies evaluating the association between risk factors and MAP herd status, serum ELISA and milk ELISA are the most common diagnostic tests used (Nielsen and Toft, 2011; Sorge et al., 2012; Pieper et al., 2015; Vilar et al., 2015). One recent study in Alberta (Wolf et al., 2016) determined MAP herd status using environmental fecal sampling. Bacteriologic culture of environmental samples is considered the most cost-effective strategy to determine herd status (Sweeney et al., 2012). It has been suggested that protocols to monitor MAP prevalence that include environmental sampling may be of great benefit to the global effort in the control and prevention of paratuberculosis (Barkema et al., 2010).

In Québec dairy herds, risk factors associated with MAP herd status might be different from those already reported in the literature. The distinctive characteristics of the dairy operations in this province – housing (92% of the herds are housed in tie-stall barns) (Groupe AGÉCO, 2013), herd size (average of 57 cows per farm) (Groupe AGÉCO, 2013), and the humid continental climate with long, cold winters – could affect the epidemiology of MAP on farms in this area.

The objective of this retrospective case-control study was to identify the association between management practices and MAP herd status (determined using bacteriologic culture of environmental samples) of dairy herds in Québec, Canada.

4.2 Material and methods

4.2.1 Study design

A retrospective case-control study was conducted to identify management practices associated with positive MAP herd status.

4.2.2 Source population, study sample and case definition

The unit of interest was the herd. The source population was composed of dairy herds enrolled in the Québec Voluntary Paratuberculosis Prevention and Control Program (QVPPCP) in 2012. This program was initiated in 2007. Upon enrollment, producers completed a general survey which included a section on herd characteristics and a section on risk factors. Fifty-nine potential risk factors were studied (Risk Assessment Questionnaire – RAQ, included in the supplementary material, Annexe 6). No sampling was performed the first year of enrollment. Starting after the first year, yearly environmental samples were collected and cultured to detect the presence of MAP. The sampling frame included 330 herds that met the following inclusion criteria: enrolled for a minimum of 2 years in the QVPPCP, completion of a risk assessment questionnaire at enrollment, and raises replacement heifers on-site.

To complete the study sample, case herds were initially selected among the 330 herds of the sampling frame. A case herd was defined as a herd from which MAP had been isolated from at least 1 environmental sample. All available case herds were included (n=26). The criteria to define a control herd were: no clinical cases of paratuberculosis and culture negative on 2 consecutive yearly environmental samplings. All herds meeting the criteria were included as controls (n=92). Selection of cases and controls resulted in a ratio of approximately 3 controls per case. In the case of the present study, (26 case herds), if a predictor of interest has a 20% exposure in the control group, a ratio of 3 controls per case would have a 70% power to detect an odds ratio (OR) of 3 or higher with 95% confidence (Lewallen and Courtright, 1998).

4.2.3 Sample collection

Environmental samples were collected during the second year of enrolment of each herd in the QVPPCP. Sample collection and laboratory analysis were completed between 2009 and 2011.

The following 3 sites were sampled in duplicate by the attending veterinarian for a total of 6 composite environmental samples per farm: 1) areas where feces from adult cows accumulate, 2) areas of manure storage, and 3) areas other than site 1 where feces accumulate (e.g.: calving area). Each composite sample consisted of about 20g of manure or feces collected from 4 different surfaces within each site. Samples were stored in a plastic container, refrigerated at 4ºC, and shipped to the laboratory within 48 hours after collection.

4.2.4 Laboratory Testing

Bacteriologic culture was performed at the provincial laboratory (Laboratoire d'épidémiosurveillance animale du Québec, LÉAQ - Saint-Hyacinthe, Québec, Canada), which is an accredited laboratory for MAP culture by the United States Department of Agriculture. After arrival, samples were stored at -70ºC until the analyses were performed. Samples were processed as described elsewhere (Arango-Sabogal et al., 2016). Isolation of MAP was achieved using the MGIT Para TB culture media and the BACTEC 960 system (Becton, Dickinson and Company, Sparks, MD, USA). For BACTEC 12B, sensitivity to detect shedders was estimated between 26% and 89% (Eamens et al., 2000). Analytic sensitivity of the BACTEC 969 MGIT system has been reported to be 101 CFU (Shin et al., 2007). Sensitivity of environmental sampling for dairy herds has been reported to be between 32% and 71% with a specificity of 100% (Lavers et al., 2013; Arango-Sabogal et al., 2016).

4.2.5 Exposure measure

Exposure was measured using 59 questions related to MAP introduction and transmission (i.e. RAQ). Questionnaires were stored in a database (Access® 1997; Microsoft Corporation, Redmond, Washington, USA) and analyzed using Stata® Statistical Software (Release 14. College Station, TX: StataCorp LP).

4.2.6 Statistical analysis

Descriptive analysis

The unit of analysis was the herd. A descriptive analysis was initially performed to explore all variables. Variables were excluded if more than 10% of data was missing (Bennett, 2001).

Categorical variables with multiple answers were dichotomized according to risk and distribution (details in supplementary material). Continuous variable herd size was scaled to evaluate the odds of being MAP positive for every 10 cow increase to facilitate the interpretation of the model estimates. Variables evaluating purchases (number of purchased adult cows in the last year and in the last 5 years, and number of animals (cows, heifers and bulls) purchased in the last 5 years) were transformed to 3 variables expressing the proportion of purchased animals per year. Distribution of data was graphically assessed with histograms and normal probability plots.

Univariable analysis

Univariable analyses were carried out to assess the association between the dependent variable (MAP herd status: case or control) and each independent variable using the Pearson's Chi-square and Fisher exact test for categorical variables, and Wilcoxon rank-sum test for continuous variables.

Multivariable analysis

Independent variables with $P<0.2$ in the univariable analysis were considered for inclusion in the multivariable logistic regression model. The log odds plot was used to verify the assumption of linearity for continuous variables. Briefly, data was divided into quartiles; then a plot of the log odds against the median of each quartile was performed. Assumption of linearity was considered valid if R-squared of the fitted line was at least 0.8. If the linearity assumption was not respected, the variable was then categorized based on the quartiles. Categories were put together if log odds were similar.

Unconditional associations between the independent variables were carried out in order to identify correlated variables using Spearman's rank correlation coefficient (*Rho*). When variables were correlated, a model was constructed with each one. The choice between correlated variables was made based on model comparison using the Bayesian information criteria (BIC).

Potential confounders were initially identified using a causal web diagram (Figure 1). Any causal factor prior to the exposure factor that was on a pathway connecting the exposure and the

outcome was a likely candidate for confounding. Thus, confounder identification was based on the presence of spurious exposure-outcome associations (Shrier and Platt, 2008; Dohoo et al., 2014; Williamson et al., 2014). Evaluation of potential confounders was then performed by assessing the change in the β-coefficient of the variables of the adjusted model compared to the non-adjusted model. Confounders were only retained if a change greater than 20% was observed, regardless of the significance of the coefficient of the confounding variable in the model (Dohoo et al., 2014).

Selection of the independent variables included in the final model was performed based on statistical considerations using a backward elimination procedure with *P*-values of entry and removal of 0.2 and 0.25, respectively. Two-factor interactions between the independent variables of the final model were tested. The goodness-of-fit of the final model was evaluated using Pearson's and the Hosmer-Lemeshow statistics (Dohoo et al., 2014).

4.3 Results

4.3.1 Study population

The sampling frame included 330 herds enrolled in the QVPPCP. Among them, 118 herds were eligible to be included in the study according to the inclusion criteria (cases=26; controls=92). One control herd was excluded from the analysis because of a report of a possible clinical paratuberculosis case, so the final study sample included 26 cases and 91 controls. Distribution of positive samples for each herd is presented in Table 1.

Sixty-three percent (*n*=74) of the herds were kept exclusively in tie-stall housing, 4% (*n*=5) were kept exclusively in free-stall housing, while the remaining 32% (*n*=38) of operations used a combination of both configurations (milking cows in tie-stalls and heifers in free-stalls). Breed information was available for 113 herds. Ninety-three herds were exclusively Holstein, 4 herds were exclusively Ayrshire, 2 herds were exclusively Jersey, 1 herd was exclusively Brown Swiss, and 13 herds had more than 1 breed. The average number of lactating cows per herd (herd size) was 69 (min=19, max=240). Producers had purchased on average 3 adult cows in the last year (min=0; max=40), while in the last 5 years, they had purchased on average 11 adult cows (min=0, max=100) and 12 animals (including adults and young stock) (min=0, max=101). The distribution of other important characteristics by MAP herd status is summarized in Table 2.

4.3.2 Univariable analysis

The distribution and results of univariable analysis of herd characteristics are shown in Table 2. The information concerning risk factors (categorization, distribution and univariable analysis results) is presented in supplementary material (Annexe 7). Univariable analysis of continuous variables is shown in Table 3.

4.3.3 Multivariable analysis

A spurious path including herd size was observed when analyzing the directed acyclic graph (Figure 1), suggesting that this variable could be a potential confounder. Assessment of the change in the β-coefficient (>20% change) of the variable of the adjusted model when compared

to the non-adjusted model confirmed that in fact, herd size was a confounder. Herd size was kept as a continuous variable given that assumption of linearity was respected. The following variables were categorized, given that the assumption of linearity was not respected: proportion of purchased cows in the last year, proportion of purchased cows per year in the last 5 years, and proportion of purchased animals per year in the last 5 years. Also, a correlation was observed between these three variables. Proportion of purchased cows per year in the last 5 years was chosen for inclusion in the final model given that the BIC suggested this model was superior.

The best fit of the model is presented in Table 4. Farms that purchased >4% of the cows in their herds per year in the last 5 years had significantly higher odds of being MAP positive than closed herds (OR=5.44; 95% CI: 1.23-23.98). Herd size was also significantly associated with a positive MAP herd status (OR=1.17; 95% CI: 1.02-1.33). The Pearson's chi-square (X^2=87.4; *P*=0.59) and the Hosmer-Lemeshow (X^2=3.8; 8 degrees of freedom; *P*=0.88) fit statistics suggested a reasonable fit of the model.

4.4 Discussion

In the present study, purchasing cows was associated with a positive MAP status. Specifically, farms buying more than 4% of the cows in their herds per year in the last 5 years have significantly greater odds of being MAP positive compared to closed herds. Purchasing policies and the introduction of new animals have been associated with a positive MAP status in previous studies (Hirst et al., 2004; Pillars et al., 2009; Correia-Gomes et al., 2010; Sorge et al., 2012; Kunzler et al., 2014; Pieper et al., 2015). Another study (Sweeney, 1996) suggested that most of the herds that acquire MAP do so through the introduction of infected animals. Fecal shedding occurs before other clinical signs, so asymptomatic carrier animals may contaminate the premises and infect susceptible animals before they are recognized as infectious. Unfortunately, very few farmers appear to inquire about MAP status (herd or individual) before buying an animal. There were two questions related to this subject in the questionnaire. However, these questions had >10% missing data. If we analyze the herds for which this information was available, the percentage of farmers inquiring the MAP herd or individual status before purchasing an animal was only 10% and 13% respectively. These farms seemed to have lower odds of being positive compared to those farms where the producer does not inquire (herd status: OR=0.8 and individual status: OR=0.5), although not significantly.

Herd size was significantly associated with a positive MAP herd status. This finding is consistent with previous publications (Wells and Wagner, 2000; Hirst et al., 2004; Ridge et al., 2010; Vilar et al., 2015). Qualifying a herd as large depends on the context of the production. In our sample, the largest herd had 240 cows, which for the Québec context can be considered a large herd even though elsewhere, this would be considered a medium herd. It is possible that in larger herds the spread of the disease could be more efficient as calving management and rearing become more challenging (Ridge et al., 2010). Also, in a larger group of animals it could be expected that the opportunity for contact between young animals and adult cows or cow manure increases (Vilar et al., 2015). One might hypothesize that the probability of being MAP positive (as well as other diseases) could be greater in larger herds compared to small herds as it is possible that larger herds need to fulfill the replacement of a higher number of cows. Also, large herds could potentially be the result of the consolidation of several herds with unknown MAP status, increasing the risk of being positive. Given that most of the farmers don't test new

animals, it can be expected that larger herds are more likely to have a higher prevalence rates of paratuberculosis. Simply due to mathematics, the probability of having at least one infected animal increases as herd size increases. Avoiding the introduction of new animals in a herd is a key element for MAP control and, if required, replacement animals should originate from a herd with the same or better MAP risk status (Sweeney et al., 2012).

Frequency of housing more than 1 cow in the maternity pen tended to be significantly associated with being a MAP positive herd. This risk factor has been previously associated with a high prevalence of paratuberculosis (Tiwari et al., 2009). The immediate neonatal environment is a key element in MAP transmission since young animals are more susceptible to infection (Windsor and Whittington, 2010). Group housing of calving cows leads to exposure of newborn calves to the manure of multiple cows. One might hypothesize that the management of calving pens may be different depending on the number of cows housed in the maternity pen. For example, when only 1 cow is housed in the maternity pen, it may be easier to clean after each use, decreasing calf exposure to feces and limiting the level and duration of MAP transmission (Vilar et al., 2015). In the same way, in smaller herds, maternity pens could be more often emptied, allowing better cleaning and disinfection between animals.

The current study design allowed the identification of risk factors strongly associated with MAP herd status. Other risk factors with a lower impact may not have been detected because the study's power was lower than 80%. In our study sample, the herds in which more than 10% of calves were allowed to nurse their mother or other cows tended to have higher odds of being MAP positive. A low power reduces the chance of identifying a potential risk factor associated with the outcome (Dohoo et al., 2014). The only way to increase the power would be to include more herds (Dohoo et al., 2014); it would have been necessary to include 88 case herds to detect an odds ratio of 2.0 with a power of 0.8. In our study, including more herds was impossible, since all positive herds available at the time were used. Because the number of case herds was limited, more control herds were included to obtain a ratio of 3 controls per case herd.

A directed acyclic graph was constructed and paths proposed based on prior knowledge and biological plausibility as a way to facilitate model building (Dohoo et al., 2014). As the diagram could not present cyclic paths, herd prevalence was included as two latent variables (historic and current), as described in Fig. 1.

Herd size and proportion of cows purchased per year in the last 5 years were associated with a positive MAP herd status in our study. This finding is consistent with what has been found in the rest of the world using different diagnostic approaches and different study populations. These are important risk factors for MAP regardless of management system or geographic area.

One of the unique features of the present study is the diagnostic test used to determine MAP herd status. Environmental sampling is the most cost-effective MAP detection method for dairies to define infection status (Sweeney et al., 2012). The advantage of detecting live MAP by culture is that a positive result confirms the presence of viable MAP on the farm (Sweeney et al., 2012). Given the high specificity of fecal culture (Collins et al., 2006), a minimum of just 1 positive sample was used as the cut-off to declare a herd infected in the present study. Including 2 consecutive negative tests as the criteria to select control herds increased the likelihood that MAP was absent in those herds. The potential misclassification bias was investigated. Using conservative estimates of environmental sampling specificity and sensitivity (Specificity = 1.0 and Sensitivity = 0.32) (Arango-Sabogal et al., 2016) and a herd prevalence of 0.07 determined by environmental sampling (MAPAQ, 2012), the negative predictive value (NPV) was estimated to be 95.1% and the positive predictive value (PPV) 100%. These estimates would translate into 4 control herds misclassified, although in control herds, the potential for misclassification is further decreased in this study by testing two consecutive years. In a previous study, herds with 2 consecutive negative environmental culture results were more likely to have no cows shedding MAP (Arango-Sabogal et al., 2016). We do not suspect the misclassification bias to have significantly impacted our results.

For all herds enrolled, there was an average of 1 year delay between the questionnaire and the first environmental culture. The questionnaire still likely reflects the management practices associated with the status of the herd because of the long incubation period of the disease. Also, control measures are implemented on average 6 months after the enrollment into a program (Collins et al., 2010). Thus, changes implemented by producers at enrollment in the voluntary program are expected to have an impact on the status of the herd a few years later.

One might think that disease spread would be minimized in tie-stalled herds. However, in our sample, housing type (tie-stall vs free-stall) was not associated with MAP herd status.

Our study was performed in a population with particular characteristics. Québec dairy herds are relatively small, mostly tie-stalled and subjected to long, cold winters. Our results can be extrapolated to similar conditions, such as Eastern Canada (Ontario, Canadian Maritimes) or North-west United States, which are comparable to Québec dairy herds to some extent. It is encouraging that some risk factors found significant in our study were previously reported in different geographic areas with different production systems and using different analyses.

4.5 Conclusions

The association between risk factors and MAP herd status in Québec dairy herds was evaluated. Herd size and proportion of cows purchased per year in the last 5 years were significantly associated with a positive MAP herd status. These risk factors are consistent with the literature and should be prioritized in control programs.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding sources

This work was supported by the Programme de soutien à l'innovation agroalimentaire (grant number 807091)

Figure 4.1. Proposed directed acyclic graph for the introduction and within-herd transmission of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in Québec dairy herds. Variables in grey are latent variables not measured in this study. The variable "Management practices" includes all answers to the risk assessment questionnaire. Herd size and proportion of purchased animals were included as they are important herd characteristics. Herd prevalence is found twice in the diagram; one as "historical" and one as "current." This unusual presentation scheme attempts to explain the continuous process of MAP transmission. Shedding in adult cattle infects susceptible young stock which in turn become infectious adults.

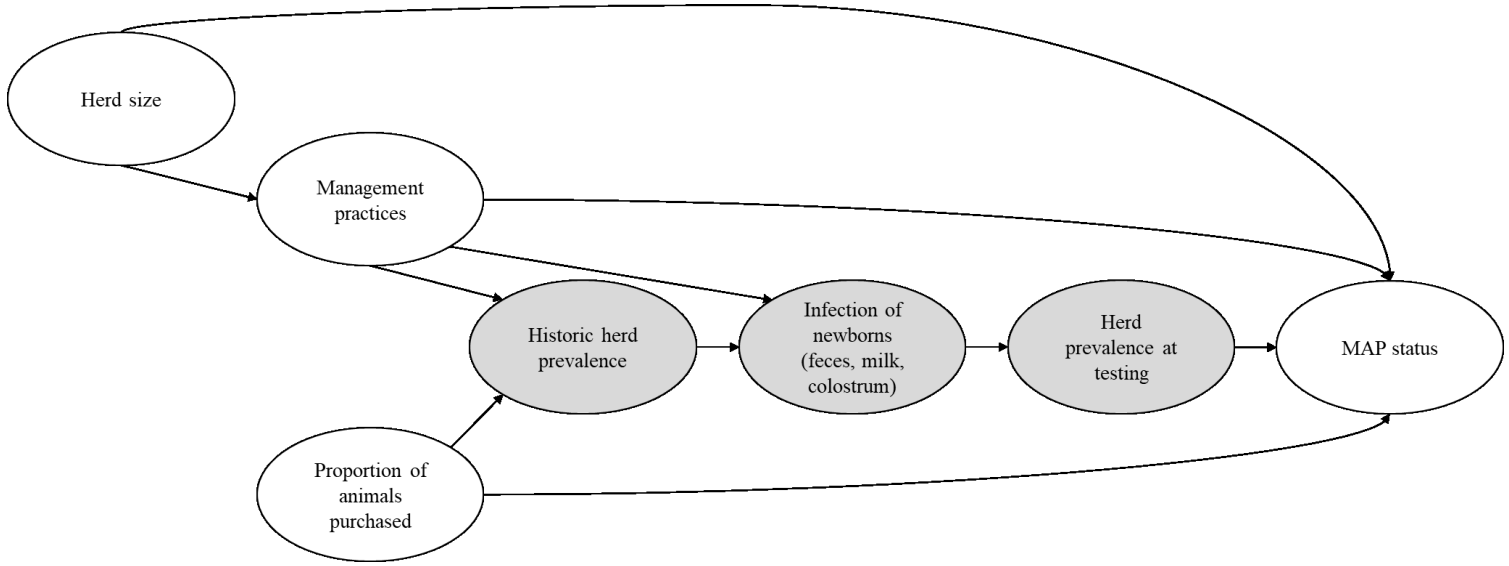


Table 4.1 Total number and distribution of positive samples of 26 *Mycobacterium avium* subsp. *paratuberculosis* positive dairy herds enrolled in the Québec Voluntary Paratuberculosis Prevention and Control Program.

ID	Total number of positive samples*	Sampling Date	Number of positive samples per site		
			Site A**	Site B**	Site C**
1	6	2011	2	2	2
2	5	2010	1	2	2
3	5	2010	2	2	1
4	4	2010	1	2	1
5	3	2010	0	2	1
6	3	2010	1	1	1
7	2	2010	0	1	1
8	2	2010	0	2	0
9	2	2010	0	0	2
10	2	2010	0	2	0
11	1	2009	0	1	0
12	1	2009	0	0	1
13	1	2009	1	0	0
14	1	2009	0	1	0
15	1	2009	1	0	0
16	1	2009	0	0	1
17	1	2010	1	0	0
18	1	2010	0	1	0
19	1	2010	0	0	1
20	1	2010	0	1	0
21	1	2010	0	1	0
22	1	2011	1	0	0
23	1	2011	0	1	0
24	1	2011	0	0	1
25	1	2011	1	0	0
26	1	2011	1	0	0

* All samplings included 6 samples

**A: areas where feces from adult cows accumulate

**B: areas of manure storage, and

**C: areas other than site A where feces accumulate. (This site could be a calving area, pathways where cows often circulate, an area near sick cows or near cows recently calved).

Table 4.2 Herd characteristics considered to be risk factors for case dairy herds (*Mycobacterium avium* subsp. *paratuberculosis* had been isolated from at least 1 environmental sample) and control dairy herds (negative on 2 consecutive yearly environmental samplings and no history of paratuberculosis) enrolled in the Québec Voluntary Paratuberculosis Prevention and Control Program in 2012.

Herd characteristics	Categories	Number of case herds	Number of control herds	N*	Chi-square	P**
Breed	Holstein	18	75	93		
	Holstein-Other	3	10	13	2.18	0.274†
	Other	3	4	7		
Breeding	Artificial Insemination	21	78	99	1.69	0.504†
	Natural	0	2	2		
	Both	5	10	15		
Housing	Free-stall	2	3	5		
	Tie-stall	18	56	74	2.01	0.281†
	Both	6	32	38		
Access to pasture during summer	No	13	28	41	3.01	0.083
	Yes	13	61	74		
Other ruminants housed with cattle	No	24	86	110	1.74	0.223†
	Yes	2	2	4		
Farm equipment shared with neighbors	No	12	55	67	1.24	0.265
	Yes	13	36	49		
Use of monensin	No	2	33	39	2.06	0.151
	Yes	20	53	73		
Type of calving area	Tie-stall	13	42	55		
	Pen	10	32	42	0.57	0.871†
	Both	3	16	19		
Access to watercourse or pond	No	23	82	105	0.04	1.000†
	Yes	2	6	8		
Manure spread by a private company	No	18	53	71	1.56	0.211
	Yes	7	38	45		
Quarantine for new animals	No	22	60	82	2.15	0.330†
	Yes	0	6	6		
Inquired about herd PTB status when buying* **	No	20	59	79	0.04	1.000†
	Yes	2	7	9		
Inquired about animal PTB status when buying* **	No	21	56	77	0.61	0.724†
	Yes	2	10	12		

*N: number of herds with information available.

** Pearson's chi-square test. Fisher's exact test if † is indicated.

***: Required response regardless of if animals had been purchased or not.

Table 4.3 Herd management continuous variables univariable analysis of data collected from case dairy herds (MAP had been isolated from at least 1 environmental sample) and control dairy herds (negative on 2 consecutive yearly environmental samplings and no history of paratuberculosis) enrolled in the Québec Voluntary Paratuberculosis in 2012.

Variable	**Herd**	**N***	**Mean**	**Median**	**Min**	**Max**	**P value ****
Number of dairy cows	Control	89	64	55	19	170	0.05
	Case	26	89	63	27	240	
Proportion of purchased cows in the last year	Control	88	2.9	0.0	0	30	0.02
	Case	26	9.3	2.0	0	100	
Proportion of purchased cows per year in the last 5 years	Control	86	2.5	1.2	0	24	0.01
	Case	26	4.4	3.8	0	20	
Proportion of purchased animals per year in the last 5 years	Control	86	3.2	1.9	0	24	0.02
	Case	26	4.7	4.2	0	20	

*Number of herds with the information available
** Wilcoxon rank-sum test

Table 4.4 Final multivariable logistic regression model for identifying the association of management practices and a positive herd status for *Mycobacterium avium* subsp. *paratuberculosis* based on 24 case dairy herds (MAP had been isolated from at least 1 environmental sample) and 82 control dairy herds (negative on 2 consecutive yearly environmental samplings and no history of paratuberculosis) enrolled in the Québec Voluntary Paratuberculosis Prevention and Control Program in 2012.

Risk factor	**OR**	**95% CI**	**Wald**	**P**
Proportion of purchased cows per year in the last 5 years:				
0%	1.00	-	-	-
>0% - ≤4%	1.57	0.39-6.25	0.64	0.524
>4%	5.44	1.23-23.98	2.24	0.025
Herd size (per 10 cows)*	1.17	1.02-1.33	2.33	0.020
Housing more than 1 cow in the maternity pen in more than 10% of calving events	3.16	0.94-10.65	1.86	0.063
More than 10% of calves are allowed to nurse their dam or another cow	2.60	0.85-7.93	1.67	0.094
Frozen colostrum from another dam was fed to calves at least once in the last year	0.46	0.13-1.68	-1.18	0.239
Intercept	0.03	0.01-0.16	-4.15	0.000

*Included as confounder

4.5 References

Ansari-Lari, M., Haghkhah, M., Bahramy, A., Novin Baheran, A.M., 2009. Risk factors for *Mycobacterium avium* subspecies *paratuberculosis* in Fars province (Southern Iran) dairy herds. Trop Anim Health Prod 41, 553-557 http://dx.doi.org/10.1007/s11250-008-9221-7.

Arango-Sabogal, J.C., Cote, G., Pare, J., Labrecque, O., Roy, J.P., Buczinski, S., Dore, E., Fairbrother, J.H., Bissonnette, N., Wellemans, V., Fecteau, G., 2016. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in tie-stall dairy herds using a standardized environmental sampling technique and targeted pooled samples. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 80, 175-182

Barkema, H.W., Hesselink, J.W., McKenna, S.L.B., Benedictus, G., Groenendaal, H., 2010. Global prevalence and economics of infection with *Mycobacterium avium* subsp. *paratuberculosis* in ruminants. In: Behr, M.A., Collins, D.M. (Eds.), Paratuberculosis : Organism, Disease, Control. 10-21 http://dx.doi.org/10.1079/9781845936136.0010.

Benedictus, A., Mitchell, R.M., Linde-Widmann, M., Sweeney, R., Fyock, T., Schukken, Y.H., Whitlock, R.H., 2008. Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program. Preventive Veterinary Medicine 83, 215-227 http://dx.doi.org/10.1016/j.prevetmed.2007.07.008.

Bennett, D.A., 2001. How can I deal with missing data in my study? Australian and New Zealand Journal of Public Health 25, 464-469 http://dx.doi.org/10.1111/j.1467-842X.2001.tb00294.x.

Berghaus, R.D., Lombard, J.E., Gardner, I.A., Farver, T.B., 2005. Factor analysis of a Johne's disease risk assessment questionnaire with evaluation of factor scores and a subset of original questions as predictors of observed clinical paratuberculosis. Preventive Veterinary Medicine 72, 291-309 http://dx.doi.org/10.1016/j.prevetmed.2005.07.011.

Bolton, M.W., Pillars, R.B., Kaneene, J.B., Mauer, W.A., Grooms, D.L., 2011. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in naturally exposed dairy heifers and associated risk factors. Journal of Dairy Science 94, 4669-4675 http://dx.doi.org/10.3168/jds.2011-4158.

Collins, M.T., Eggleston, V., Manning, E.J.B., 2010. Successful control of Johne's disease in nine dairy herds: Results of a six-year field trial. Journal of dairy science 93, 1638-1643 http://dx.doi.org/10.3168/jds.2009-2664.

Collins, M.T., Gardner, I.A., Garry, F.B., Roussel, A.J., Wells, S.J., 2006. Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. Journal of the American Veterinary Medical Association 229, 1912-1919 http://dx.doi.org/10.2460/javma.229.12.1912.

Correia-Gomes, C., Mendonca, D., Niza-Ribeiro, J., 2010. Risk associations to milk ELISA result for paratuberculosis in dairy cows in northern Portugal using a multilevel regression model. Revue de Medecine Veterinaire 161, 295-301

Dieguez, F.J., Arnaiz, I., Sanjuan, M.L., Vilar, M.J., Yus, E., 2008. Management practices associated with *Mycobacterium avium* subspecies *paratuberculosis* infection and the effects of the infection on dairy herds. Veterinary Record 162, 614-617 http://dx.doi.org/10.1136/vr.162.19.614.

Dohoo, I., Martin, W., Stryhn, H., 2014. Veterinary Epidemiologic Research. VER Inc Charlottetown, PEI, Canada

Doré, E., Paré, J., Cote, G., Buczinski, S., Labrecque, O., Roy, J.P., Fecteau, G., 2012. Risk factors associated with transmission of *Mycobacterium avium* subsp. *paratuberculosis* to calves within dairy herd: a systematic review. Journal of veterinary internal medicine 26, 32-45 http://dx.doi.org/10.1111/j.1939-1676.2011.00854.x.

Eamens, G.J., Whittington, R.J., Marsh, I.B., Turner, M.J., Saunders, V., Kemsley, P.D., Rayward, D., 2000. Comparative sensitivity of various faecal culture methods and ELISA in dairy cattle herds with endemic Johne's disease. Vet Microbiol 77, 357-367

Garry, F., 2011. Control of paratuberculosis in dairy herds. The Veterinary clinics of North America. Food animal practice 27, 599-607, vii http://dx.doi.org/10.1016/j.cvfa.2011.07.006.

Groupe AGÉCO, 2013. Nombre moyen de vaches laitières par ferme et par province au 1er janvier, Canada. Canada.

Hendrick, S.H., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M., Duffield, T.F., 2005. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. Journal of the American Veterinary Medical Association 227, 1302-1308 http://dx.doi.org/10.2460/javma.2005.227.1302.

Hirst, H.L., Garry, F.B., Morley, P.S., Salman, M.D., Dinsmore, R.P., Wagner, B.A., McSweeney, K.D., Goodell, G.M., 2004. Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity. Journal of the American Veterinary Medical Association 225, 97-101

Kudahl, A.B., Nielsen, S.S., 2009. Effect of paratuberculosis on slaughter weight and slaughter value of dairy cows. J. Dairy Sci. 92, 4340-4346 http://dx.doi.org/10.3168/jds.2009-2039.

Kunzler, R., Torgerson, P., Keller, S., Wittenbrink, M., Stephan, R., Knubben-Schweizer, G., Berchtold, B., Meylan, M., 2014. Observed management practices in relation to the risk of infection with paratuberculosis and to the spread of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss dairy and beef herds. BMC veterinary research 10, 132 http://dx.doi.org/10.1186/1746-6148-10-132.

Lavers, C.J., McKenna, S.L., Dohoo, I.R., Barkema, H.W., Keefe, G.P., 2013. Evaluation of environmental fecal culture for *Mycobacterium avium* subspecies *paratuberculosis* detection in dairy herds and association with apparent within-herd prevalence. The Canadian veterinary journal = La revue veterinaire canadienne 54, 1053-1060

Lewallen, S., Courtright, P., 1998. Epidemiology in practice: case-control studies. Community eye health 11, 57-58

Lombard, J.E., Garry, F.B., McCluskey, B.J., Wagner, B.A., 2005. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. Journal of the American Veterinary Medical Association 227, 1975-1981 http://dx.doi.org/10.2460/javma.2005.227.1975.

Manning, E.J.B., Collins, M.T., 2010. Epidemiology of paratuberculosis. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, Wallingford, Oxfordshire, 22-28 http://dx.doi.org/10.1079/9781845936136.0022.

MAPAQ, 2012. Revue d'épidémiosurveillance animale du RAIZO, bilan 2011. Secteur bovin / Programme volontaire de prévention et de contrôlede la paratuberculose au Québec.

Québec, Canada. website: http://www.mapaq.gouv.qc.ca/SiteCollectionDocuments/Santeanimale/RevueRAIZO/BilanRAIZO2011.pdf

Nielsen, S.S., Toft, N., 2011. Effect of management practices on paratuberculosis prevalence in Danish dairy herds. Journal of Dairy Science 94, 1849-1857 http://dx.doi.org/10.3168/jds.2010-3817.

Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. Prev Vet Med 32, 243-251

Pieper, L., Sorge, U.S., DeVries, T., Godkin, A., Lissemore, K., Kelton, D., 2015. Comparing ELISA test-positive prevalence, risk factors and management recommendations for Johne's disease prevention between organic and conventional dairy farms in Ontario, Canada. Prev Vet Med 122, 83-91 http://dx.doi.org/10.1016/j.prevetmed.2015.09.004.

Pillars, R.B., Grooms, D.L., Gardiner, J.C., Kaneene, J.B., 2011. Association between risk-assessment scores and individual-cow Johne's disease-test status over time on seven Michigan, USA dairy herds. Prev Vet Med 98, 10-18 http://dx.doi.org/10.1016/j.prevetmed.2010.10.001.

Pillars, R.B., Grooms, D.L., Woltanski, J.A., Blair, E., 2009. Prevalence of Michigan dairy herds infected with *Mycobacterium avium* subspecies *paratuberculosis* as determined by environmental sampling. Prev Vet Med 89, 191-196 http://dx.doi.org/10.1016/j.prevetmed.2009.02.022.

Ridge, S.E., Heuer, C., Cogger, N., Heck, A., Moor, S., Baker, I.M., Vaughan, S., 2010. Herd management practices and the transmission of Johne's disease within infected dairy herds in Victoria, Australia. Preventive Veterinary Medicine 95, 186-197 http://dx.doi.org/10.1016/j.prevetmed.2010.05.001.

Shin, S.J., Han, J.H., Manning, E.J., Collins, M.T., 2007. Rapid and reliable method for quantification of *Mycobacterium paratuberculosis* by use of the BACTEC MGIT 960 system. Journal of clinical microbiology 45, 1941-1948 http://dx.doi.org/10.1128/jcm.02616-06.

Shrier, I., Platt, R.W., 2008. Reducing bias through directed acyclic graphs. BMC medical research methodology 8, 70 http://dx.doi.org/10.1186/1471-2288-8-70.

Sorge, U.S., Lissemore, K., Godkin, A., Jansen, J., Hendrick, S., Wells, S., Kelton, D.F., 2012. Risk factors for herds to test positive for *Mycobacterium avium* ssp. *paratuberculosis*-antibodies with a commercial milk enzyme-linked immunosorbent assay (ELISA) in Ontario and western Canada. Canadian Veterinary Journal 53, 963-970

Sweeney, R.W., 1996. Transmission of paratuberculosis. The Veterinary clinics of North America. Food animal practice 12, 305-312

Sweeney, R.W., Collins, M.T., Koets, A.P., McGuirk, S.M., Roussel, A.J., 2012. Paratuberculosis (Johne's disease) in cattle and other susceptible species. Journal of veterinary internal medicine 26, 1239-1250 http://dx.doi.org/10.1111/j.1939-1676.2012.01019.x.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., Scott, H.M., Whiting, T., 2009. Risk factors associated with *Mycobacterium avium* subspecies *paratuberculosis* seropositivity in Canadian dairy cows and herds. Preventive Veterinary Medicine 88, 32-41 http://dx.doi.org/10.1016/j.prevetmed.2008.06.019.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Stryhn, H., Keefe, G.P., Haddad, J.P., 2005. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, *Mycobacterium avium* subspecies *paratuberculosis,* and Neospora caninum on culling in dairy cattle in four Canadian provinces. Veterinary Microbiology 109, 147-158 http://dx.doi.org/10.1016/j.vetmic.2005.05.011.

Vilar, A.L.T., Santos, C.S.A.B., Pimenta, C.L.R.M., Freitas, T.D., Brasil, A.W.L., Clementino, I.J., Alves, C.J., Bezerra, C.S., Riet-Correa, F., Oliveira, T.S., Azevedo, S.S., 2015. Herd-level prevalence and associated risk factors for *Mycobacterium avium* subsp. *paratuberculosis* in cattle in the State of Paraiba, Northeastern Brazil. Preventive Veterinary Medicine 121, 49-55 http://dx.doi.org/10.1016/j.prevetmed.2015.06.003.

Waddell, L.A., Rajic, A., Stark, K.D., Mc, E.S., 2015. The zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: a systematic review and meta-analyses of the evidence. Epidemiology and infection 143, 3135-3157 http://dx.doi.org/10.1017/s095026881500076x.

Wells, S.J., Wagner, B.A., 2000. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. Journal of the American Veterinary Medical Association 216, 1450-1457 http://dx.doi.org/10.2460/javma.2000.216.1450.

Williamson, E.J., Aitken, Z., Lawrie, J., Dharmage, S.C., Burgess, J.A., Forbes, A.B., 2014. Introduction to causal diagrams for confounder selection. Respirology 19, 303-311 http://dx.doi.org/10.1111/resp.12238.

Windsor, P.A., Whittington, R.J., 2010. Evidence for age susceptibility of cattle to Johne's disease. Vet. J. 184, 37-44 http://dx.doi.org/10.1016/j.tvjl.2009.01.007.

Wolf, R., Barkema, H.W., De Buck, J., Orsel, K., 2016. Dairy farms testing positive for *Mycobacterium avium* ssp. *paratuberculosis* have poorer hygiene practices and are less cautious when purchasing cattle than test-negative herds. J Dairy Sci 99, 4526-4536 http://dx.doi.org/10.3168/jds.2015-10478.

5. Discussion générale

Dans cette section nous allons discuter des facteurs de risque (évalués à l'aide d'un questionnaire) associées à un statut de troupeau positif à MAP. Pour chaque facteur de risque nous allons comparer ce qui est rapporté dans la littérature (article 1) et les facteurs de risque associés à un statut positif à MAP dans les troupeaux laitiers du Québec (article 2). Par la suite, les caractéristiques générales des études recensés dans la revue systématique seront comparées à celles de l'étude cas-témoins du Québec. Pour chacune des études qui composent ce mémoire (la revue de la littérature et l'étude cas-témoins du Québec), les points forts, les retombées et les limites seront également discutés. Finalement, les perspectives futures seront présentées.

Plusieurs études ont utilisé un questionnaire d'analyse de risque (QAR) pour évaluer les facteurs de risque associés au statut du troupeau à MAP. Parmi les études recensées, il y a une variabilité quant au devis de l'étude, les tests diagnostiques utilisés et la méthodologie statistique. Cela ne permet pas de faire une analyse statistique incluant les données de toutes les études (méta-analyse). Toutefois, l'analyse descriptive demeure intéressante pour mieux connaître les facteurs de risque les plus importants et cibler les efforts pour le contrôle et la prévention de la paratuberculose.

Dans la révision systématique de la littérature et dans l'étude cas-témoins du Québec, la mesure d'exposition était un QAR. Les prédicteurs (variables indépendantes) sont alors les pratiques de gestion évaluées par le QAR. Toutefois, selon la formulation de la question, la définition du même facteur de risque pouvait varier d'une étude à l'autre. D'un autre côté, bien que toutes les études mesurent le statut de troupeau à MAP (variable dépendante), la définition d'un troupeau positif varie d'une étude à l'autre.

5.1 Facteurs de risque

Malgré l'hétérogénéité liée aux caractéristiques de chaque étude (discutées plus bas, section 5.3), certains facteurs de risque sont plus souvent étudiés et identifiés comme importants.

5.1.1 Taille du troupeau

Dans la littérature, la taille du troupeau est souvent associée au statut du troupeau positif à MAP. À mesure que le nombre d'animaux augmente, la possibilité d'être infecté par MAP augmente. Dans l'étude cas-témoins au Québec, les troupeaux les plus grands avaient aussi plus de chances d'être positifs.

Cependant, qualifier la taille d'un troupeau dépend du contexte de la production dans une région. Selon les études, la définition d'un troupeau qualifié grand ou petit variait considérablement. Par exemple, une étude a trouvé que les troupeaux de plus de 12 vaches avaient plus de chances d'être positifs (Vilar et al., 2015). Une deuxième étude a trouvé que les troupeaux de >345 vaches étaient plus à risque d'être positifs (Ridge et al., 2010). Également, la variable est étudiée comme variable continue ou catégorique selon les études. De plus, la catégorisation varie d'une étude à l'autre. Dans l'étude cas-témoins du Québec, le troupeau le plus grand avait 240 vaches. Pour le contexte du Québec, il est considéré comme un grand troupeau, tandis qu'ailleurs il pourrait être considéré comme un troupeau de petite taille. Ainsi, une bonne façon d'étudier cette variable est l'analyser comme une variable continue.

Dans les troupeaux plus grands, la propagation de la maladie pourrait être plus efficiente, puisque la gestion des vêlages et de l'élevage devient plus difficile (Ridge et al., 2010). Il y aura plus de vaches qui vêlent en même temps. Il serait plus difficile de les loger individuellement pour le vêlage; et l'aire de vêlage est moins souvent vide entre les vêlages pour le nettoyage et la désinfection. Il est suggéré que dans les plus grands troupeaux le contact entre les jeunes veaux et les vaches adultes ou leur fèces peut être plus fréquent (Vilar et al., 2015). Aussi, on pourrait penser que les troupeaux plus grands ont aussi un taux de remplacement plus élevé, ou qu'ils sont le résultat d'une expansion récente du troupeau, via l'achat et le mélange d'animaux provenant de sources multiples (Barkema et al., 2015). Finalement, les troupeaux plus grands pourraient avoir plus de mouvements d'animaux à l'intérieur et à l'extérieur de la ferme, augmentant le risque d'introduction de la maladie (Daniels et al., 2002).

5.1.2 Introduction d'animaux au troupeau

Dans la littérature, l'introduction d'animaux dans le troupeau (mesurée de plusieurs façons) est une pratique souvent associée à un statut du troupeau positif à MAP, peu importe la région, le type de production, le test diagnostique ou l'analyse statistique utilisés. De la même façon, dans l'étude cas-témoins du Québec, les troupeaux qui avaient acheté en moyenne plus d'une vache par chaque 25 vaches (>4% des vaches adultes) dans le troupeau par année, avaient 5 fois plus de chances d'être positifs que les troupeaux fermés.

Lors de l'achat, les vaches peuvent être apparemment saines, mais elles pourraient être infectés dans le stade 1 ou 2 de la maladie. Les animaux achetés peuvent contaminer d'autres animaux du troupeau si les pratiques de gestion en place sont favorables à la transmission. Quand finalement cette vache achetée sera diagnostiquée infectée par le MAP, elle aura probablement déjà transmis la maladie à plusieurs autres animaux du troupeau. Aussi, il est fort possible qu'elle soit éliminée sans avoir été diagnostiquée avec la maladie.

Un autre élément souvent étudié est la provenance des animaux achetés. Dans la littérature, deux études ont trouvé une association entre la source des animaux et la paratuberculose. Dans l'une de ces études (Norton et al., 2009), acheter des vaches de plusieurs troupeaux était associé avec une incidence plus élevée de paratuberculose dans le troupeau. Dans la deuxième étude (Cetinkaya et al., 1997) la provenance des animaux achetés était associée avec le statut positif à MAP. Par contre, le lien était contre-intuitif, acheter d'une source privée et connue augmentait le risque comparé à acheter des animaux dans les encans ou les foires agricoles (Cetinkaya et al., 1997). En général, l'achat de génisses de remplacement provenant de sources connues est considéré comme une méthode de réduction du risque d'introduction d'une maladie (Weber et al., 2004; Weber et al., 2006; Sweeney et al., 2012). Dans ce cas, les auteurs attribuent cette trouvaille à une haute prévalence de paratuberculose dans les Shorthorn ou Ayrshire, dont les remplacements sont achetés en privé en raison du petit nombre d'éleveurs (Cetinkaya et al., 1997).

Dans l'étude cas-témoins du Québec, deux questions étaient posées au sujet des mesures en place lors de l'achat d'animaux. Malheureusement, très peu de producteurs semblaient se renseigner sur le statut (du troupeau ou individuel) avant l'achat d'un animal. Ces questions,

ayant plus de 10% de données manquantes et une valeur $P<0.20$ à l'analyse univariée, n'ont pas été considérées dans l'analyse multivariée.

Selon un groupe d'experts, la recommandation générale à ce sujet est de ne pas introduire d'animaux et de maintenir le troupeau fermé, si possible (Sweeney et al., 2012). Dans le cas où acheter des animaux est inévitable, il est recommandé d'acheter d'un troupeau à faible risque (déterminé par les pratiques de gestion et les mesures de biosécurité) et acheter si possible d'un seul troupeau source, qui n'a pas d'antécédents de paratuberculose (Weber et al., 2004; Weber et al., 2006).

5.1.3 Histoire de cas de paratuberculose

Dans la littérature, un sujet fréquemment investigué dans les QAR est l'historique de la maladie et les antécédents du troupeau relativement à la paratuberculose. Cette information était variable dans les études recensées et pourrait être définie comme l'historique d'avoir eu des animaux avec signes cliniques ou d'avoir un test diagnostique positif. Dans l'étude cas-témoins du Québec, cette information n'était pas demandée dans le questionnaire.

Les animaux qui montrent des signes cliniques de paratuberculose ne sont que le pic de l'iceberg et il y a généralement, plusieurs autres cas sous-cliniques non détectés (Obasanjo et al., 1997). Les animaux avec des signes cliniques ou un test positif à MAP sont susceptibles d'excréter le MAP perpétuant la transmission de la maladie dans le troupeau. Alors, plus il y a de vaches positives ou avec des signes cliniques, plus la probabilité de transmission dans le troupeau est grande (Sorge et al., 2012).

Ceci est probablement une information utile pour les acheteurs potentiels. Idéalement, on souhaiterait acheter de troupeaux avec un historique négatif pour le MAP, parce que ceux-là sont moins susceptibles d'être infectés (Kobayashi et al., 2007). Cependant, cette information n'est pas toujours facile à obtenir.

5.1.4 Pratiques de gestion autour de l'élevage des veaux

5.1.4.1 Aire de vêlage

Plusieurs facteurs de risque rapportés dans la littérature sont liés à l'aire de vêlage. Il y avait différentes questions qui évaluaient le risque lié à l'aire de vêlage. Par exemple, on posait la question sur l'existence ou non d'une aire de vêlage, de l'hygiène et la densité dans l'aire de vêlage et la présence de vaches suspectes de paratuberculose dans l'aire de vêlage. Dans l'étude cas-témoins du Québec, les troupeaux qui gardaient en même temps plus d'une vache dans l'aire de vêlage et où les veaux tétaient leur mère ou une autre vache avaient une tendance à être positifs à MAP. La valeur *P* était de 0.06 et 0.09 respectivement. On peut penser qu'avec une plus grande taille d'échantillon, la puissance de l'étude aurait été suffisante pour trouver ces deux variables significatives. Pour ces deux facteurs la puissance était 69% et 29% respectivement.

Comme les jeunes veaux sont les plus susceptibles à l'infection (Windsor and Whittington, 2010), et les vaches adultes sont plus susceptibles d'excréter MAP dans les fèces (Fecteau and Whitlock, 2010; Koets et al., 2015), l'aire de vêlage est un point critique pour le contrôle de la maladie. Par exemple, le logement collectif des vaches en période peripartum expose des veaux nouveau-nés aux fèces de plusieurs vaches. Avoir une seule vache à la fois dans l'aire de vêlage, facilite la gestion car il est possible de nettoyer après chaque utilisation et de maintenir une meilleure hygiène (Vilar et al., 2015). On pourrait croire que l'aire de vêlage représente un plus grand défi dans les grands troupeaux. Dans les petits troupeaux l'aire de vêlage pourrait être plus souvent vidée, permettant un meilleur nettoyage et désinfection entre les utilisations. Par contre, dans les petits troupeaux, l'espace est souvent limité et l'aire de vêlage pourrait devenir aussi l'aire de vaches malades. Dans le QAR de l'étude cas-témoins du Québec une question demandait si l'aire de vêlage était utilisée à d'autres fins. Mais, cette question avait plus de 10% de données manquantes. Pour cette raison, elle n'a pas été retenue pour l'analyse multivariée.

5.1.4.2 Gestion du colostrum et du lait

Dans la littérature, la gestion du colostrum et du lait sont deux facteurs souvent associés à un statut de troupeau positif à MAP. Leur importance est telle, qu'il y a une étude (Nielsen et

al., 2008) qui se concentre uniquement sur ces deux facteurs et utilisait un questionnaire de 4 questions pour l'évaluation spécifique du colostrum et du lait comme facteurs de risque pour MAP. Dans la littérature, l'hétérogénéité des prédicteurs. Dans l'étude cas-témoins du Québec, nous avons observé une tendance pour les troupeaux positifs à laisser les veaux téter leur mère ou une autre vache quand ils se trouvent dans l'aire de vêlage.

Le colostrum et le lait sont potentiellement contaminés par le MAP en raison des deux aspects suivants : 1) la contamination du colostrum ou du lait par des matières fécales de vaches infectées par le MAP. 2) l'excrétion du MAP dans le lait et le colostrum a aussi été démontrée (Taylor et al., 1981; Sweeney et al., 1992b; Streeter et al., 1995; Giese and Ahrens, 2000).

5.1.4.3 Contact des veaux avec adultes ou fèces des adultes

Dans la littérature, le contact des veaux avec les vaches adultes ou les fèces des adultes est rapporté comme un facteur de risque dans plusieurs études. On pourrait supposer que ce facteur de risque est en association avec l'aire de vêlage et la gestion du colostrum et du lait. Mais ce facteur est important non seulement au moment de la naissance, mais à différents âges, de la naissance à 6 mois, d'après les résultats de la revue systématique. Les pratiques liées à l'élevage des veaux avant et après le sevrage peuvent être étudiées et mesurées avec des questions indépendantes. Dans l'étude cas-témoins du Québec, le questionnaire avait une section pour les veaux avant sevrage et une autre pour les génisses sevrées. Aucun des prédicteurs de ces 2 sections n'était significativement associé au statut du troupeau à MAP.

5.1.5 Contact des vaches adultes avec du MAP

Même si les jeunes veaux sont plus susceptibles, les vaches adultes peuvent aussi s'infecter (Espejo et al., 2013). Ainsi, l'étude des pratiques de gestion des vaches adultes demeure intéressante. De ce fait, dans la littérature, des facteurs de risque portant sur les taures et les vaches adultes sont associés au statut de troupeau positif à MAP. Par exemple, parmi les études recensées, avoir une aire d'exercice pour les vaches adultes (Johnson-Ifearulundu and Kaneene, 1998; Johnson-Ifearulundu and Kaneene, 1999), et le contact des vaches adultes avec des animaux d'autres troupeaux (Fredriksen et al., 2004; Correia-Gomes et al., 2010) ou des animaux non-domestiques (Cetinkaya et al., 1997; Daniels et al., 2002; Fredriksen et al., 2004),

sont des facteurs associés au statut de troupeau positif à MAP. Dans l'étude cas-témoins du Québec, aucun facteur de risque portant sur les vaches adultes était associé au statut du troupeau MAP.

5.1.6 Autres pratiques de gestion et caractéristiques des troupeaux

D'autres facteurs ont été moins souvent associées au statut du troupeau à MAP, par conséquent ils ne seront pas discutés en détail. Par exemple, une étude (Cetinkaya et al., 1997) rapporte que les troupeaux où les races « *Channel Islands* » (Jersey et Guernsey) prédominaient étaient plus susceptibles d'avoir des cas de paratuberculose que les troupeaux ou la race Holstein ou autre race prédominait. La variabilité génétique de la susceptibilité aux infections bactériennes a été estimée pour certaines maladies. Certaines études ont estimé l'héritabilité de la susceptibilité à la paratuberculose (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006). Ainsi, la sélection d'animaux pour augmenter la résistance à la paratuberculose devrait être possible. Les races des « *Channel Islands* » étaient plus susceptibles de présenter un test ELISA positif pour le lait que les autres races dans une étude canadienne (Sorge et al., 2011).

5.2 Facteurs protecteurs

Selon la mesure d'association étudiée dans chaque étude (qui dépend du type d'étude et de l'approche statistique qui seront discutés plus bas), certains facteurs étudiés étaient associés avec un moindre risque d'être positif à MAP. Les facteurs protecteurs pouvaient avoir un sens biologique (être en accord avec les connaissances actuelles de l'épidémiologie de la maladie) ou être contre-intuitifs (avoir un effet inattendu). Le facteur protecteur le plus souvent rapporté dans la littérature était d'avoir des aires de vêlage individuelles (Cetinkaya et al., 1997; Barrett et al., 2008; Cashman et al., 2008). Ce résultat est en accord avec les facteurs de risque discutés antérieurement et confirme l'importance d'interrompre le cycle de la transmission de la maladie en évitant le contact des animaux susceptibles avec les animaux excréteurs. Dans l'étude cas-témoins du Québec, cette question n'était pas formulée de la même façon. La question qui s'y rapproche le plus est de garder plus d'une vache dans l'aire de vêlage, discuté précédemment.

Certaines pratiques de gestion, considérées comme des facteurs de risque, se sont avérées être des facteurs protecteurs, par exemple nourrir les veaux avec du lait qui ne convient pas à la vente

(Kunzler et al., 2014), le contact des jeunes animaux avec les adultes ou leurs fèces (Johnson-Ifearulundu and Kaneene, 1999; Berghaus et al., 2005; Barrett et al., 2008), la mauvaise hygiène dans l'aire de vêlage et la contamination de l'équipement (McNab et al., 1992; Kinsel et al., 2010). Ce genre d'association peut survenir dans les études transversales car on ne sait pas si les producteurs conscients d'un résultat positifs, ont instauré ces pratiques en essayant de contrôler la maladie. Il est impossible de déterminer si la pratique de gestion était antécédente à la maladie.

5.3 Caractéristiques des études

Les études recensées qui évaluaient les facteurs de risque associés au statut de troupeau positif à MAP à l'aide d'un QAR, variaient considérablement. Les unités d'échantillonnage, et les test diagnostiques variaient selon le devis de l'étude, de même que l'approche statistique. Cette hétérogénéité a comme conséquence que les mesures d'association sont aussi différentes. Cette variabilité rend difficile voire impossible la comparaison et l'analyse quantitative (méta-analyse).

Les études transversales étaient les plus nombreuses dans la revue globale de la littérature. Ce résultat était attendu, car réaliser une étude transversale ou cas-témoins est plus facile et moins coûteuse que de réaliser une étude longitudinale (Dohoo et al., 2014). Les études cas-témoins ont une certitude scientifique plus grande que les études transversales, mais plus faible que les études longitudinales (Dohoo et al., 2014). Les études cas-témoins sont généralement rétrospectives, comme l'étude cas-témoins au Québec. Les cas et les témoins ont été sélectionnés parmi des troupeaux pour lesquels l'information nécessaire était disponible. Les études cas-témoins sont une option intéressante quand la prévalence de la maladie est faible, quand il y a une longue période de latence entre une exposition et la maladie et que les budgets sont limités, ne permettant pas de réaliser une étude de cohortes.

L'unité d'étude était le troupeau et la variable dépendante le statut du troupeau à MAP. Cependant, l'unité d'étude n'était pas nécessairement la même que l'unité d'échantillonnage (exemple plus bas). Aussi, la définition d'un troupeau positif variait d'une étude à l'autre. La plupart définissaient comme troupeau positif un troupeau avec une vache positive ou plus (unité d'échantillonnage et d'étude différentes). Finalement, le test diagnostique utilisé varie selon

l'unité d'échantillonnage et l'échantillon lui-même (fèces, sang, lait). Parmi les études recensées, le test diagnostique le plus souvent utilisé était l'ELISA sur le sérum ou le lait. Même si elles ne sont pas identiques, ces informations nous ont permis de comparer tout ce qui s'est fait autour du monde, avec l'étude cas témoins du Québec. Dans l'étude cas-témoins du Québec, l'unité d'échantillonnage et d'étude étaient la même, le troupeau, et l'issue d'intérêt était le statut du troupeau défini à l'aide de la CPE.

Si l'unité d'échantillonnage est l'individu, la taille de l'échantillon par troupeau doit être calculée en fonction de la prévalence attendue dans le troupeau. Dans ce cas, il faut manipuler les animaux, ce qui exige des ressources économiques et humaines plus importantes. Par contre, si l'unité d'échantillonnage est le troupeau, généralement il ne faut pas manipuler les animaux, il y aura moins d'échantillons à analyser, et généralement une seule personne peut faire les prélèvements. Par rapport aux test diagnostiques, l'ELISA a des avantages comme la facilité, la rapidité et le faible coût (Nielsen, 2010). Grâce à la capacité du MAP à survivre dans l'environnement, l'environnement garde une mémoire de l'infection par MAP dans le troupeau. Par exemple, si une vache positive est vendue juste avant de l'échantillonnage, son fumier ne fera pas partie de l'échantillon si on fait de cultures fécales individuelles. Cependant, si l'échantillon est environnemental, son fumier pourrait se retrouver dans celui-ci. Seulement deux autres études recensées ont utilisé la CPE pour définir le statut du troupeau (Pillars et al., 2009b; Wolf et al., 2016). L'avantage d'utiliser la CPE est discuté plus loin dans les points forts de cette étude.

Les études recensées avaient plusieurs prédicteurs (variables indépendantes) et une issue d'intérêt (variable dépendante). Pour cette raison, l'analyse statistique de la plupart des études incluait une première analyse univariée suivie d'une analyse multivariée. Parmi les approches utilisées pour l'analyse multivariée, la plus utilisée était la régression logistique. L'analyse des facteurs confondants et des interactions entre les prédicteurs, n'était pas toujours réalisé. On pourrait émettre l'hypothèse que les études publiées seront de plus en plus rigoureuses dans le futur et prendront en compte les éléments importants. Afin de diminuer les biais dans les études observationnelles, celles-ci pourraient inclure des diagrammes acycliques dirigés et l'évaluation des facteurs confondants et des interactions entre les prédicteurs. Dans l'étude cas-témoins du Québec, nous avons été le plus strict possible dans le contrôle des biais en nous basant sur un

diagramme acyclique dirigé et en évaluant les facteurs confondants potentiels et les interactions entre les prédicteurs.

5.4 Enjeux des études

5.4.1 Points forts

L'étude cas-témoins du Québec est la première étude évaluant les facteurs de risque associés à un statut positif à MAP dans les troupeaux laitiers du Québec. L'une des caractéristiques uniques de l'étude cas-témoins du Québec est la stratégie de dépistage utilisée pour déterminer le statut de troupeau. Selon certains experts, la CPE est la stratégie de dépistage de MAP la plus économique pour définir le statut à MAP des fermes laitières (Sweeney et al., 2012). L'avantage de détecter le MAP par culture est qu'un résultat positif confirme la présence de MAP viable sur la ferme (Sweeney et al., 2012). Compte tenu de la spécificité élevée de la culture fécale (Collins et al., 2006), seulement 1 échantillon positif (sur 6 échantillons prélevés) a été utilisé comme seuil pour déclarer un troupeau infecté. Inclure 2 tests négatifs consécutifs pour la sélection des troupeaux témoins a augmenté la probabilité que MAP soit absent dans ces troupeaux. Nous considérons que les définitions des cas et des témoins était un des points forts de l'étude cas-témoins du Québec. Un troupeau positif à la culture de l'un des 6 prélèvements de l'environnement démontre que le MAP est présent sur la ferme. Puis, un troupeau avec 2 CPE annuels consécutifs négatifs et qui n'avaient pas d'antécédents de cas cliniques de PTB comme définition pour un troupeau négatif, augmente la certitude d'un statut véritablement négatif. En utilisant des estimations conservatives de la spécificité et de la sensibilité de l'échantillonnage environnemental (Spécificité = 1.0 et Sensibilité = 0.32) (Arango-Sabogal et al., 2016) et une prévalence du troupeau de 0.07 déterminée par échantillonnage environnemental (MAPAQ, 2012a), la valeur prédictive négative a été estimée à 95.1% et la valeur prédictive positive 100%.

Deux autres revues systématiques portant sur les facteurs de risque de paratuberculose ont été réalisées. La première comportait 5 questions spécifiques sur la transmission du MAP aux veaux (Doré et al., 2012). La deuxième a étudié si l'introduction de bovins dans un troupeau, et la présence d'animaux sauvages et domestiques sont des facteurs de risque pour l'introduction de

MAP dans un troupeau. La différence avec celles-ci et le point le plus important de cette revue systématique, est qu'elle englobe les pratiques de gestion d'introduction et de transmission de la maladie.

La cible de la revue globale de la littérature était des études qui utilisaient un QAR pour mesurer l'exposition. Partout dans le monde, des questionnaires sont utilisés pour mieux comprendre et contrôler plusieurs maladies. L'utilisation d'un questionnaire comme outil de collecte de données permet d'enquêter sur des sujets spécifiques liés à la maladie. Un questionnaire bien conçu devrait être objectif et fiable (Dohoo et al., 2014). Certaines études (Hirst et al., 2004) ont montré que les associations n'utilisent que des informations disponibles sur les registres que les producteurs collectent habituellement (par exemple: production de lait, nouveaux animaux, animaux vendus). Un QAR permet de construire une analyse multivariée plus complète et plus précise. Un QAR peut être conçu pour évaluer des pratiques spécifiques liées au sujet étudié (Boynton and Greenhalgh, 2004). Cependant, la qualité des données collectées avec les questionnaires peut varier. Le mode d'administration (par exemple auto-administré versus mode d'interview) a été identifié comme ayant un effet important sur les données (Bowling, 2005). Par exemple, dans l'étude cas-témoins du Québec, le questionnaire était rempli à l'aide du médecin vétérinaire pour être le plus objectif possible. Nous pouvons également supposer que la qualité des données sera meilleure lorsque les producteurs font partie d'un programme volontaire. Le taux de réponse pourrait être plus élevé et plus soigneusement fourni lorsque les producteurs sont motivés.

5.4.2 Difficultés rencontrées et limitations

Dans l'étude cas-témoins du Québec, la plus grande limite était la taille de l'échantillon. Malgré que nous ayons utilisé tous les troupeaux disponibles au moment de la collecte de données, le nombre de troupeaux cas était relativement faible. L'impact le plus important d'une taille d'échantillon faible est une puissance faible (70% de puissance pour détecter un OR supérieur ou égal à 3.0) pour identifier les facteurs de risque significativement associés au statut positif à MAP. En faisant la comparaison avec les études existantes dans la littérature, la puissance des études n'est pas fréquemment rapportée. Par contre, à partir de l'information fournie dans certains articles il était possible de calculer la puissance post hoc. Le calcul de la

puissance post hoc est intéressant pour contextualiser les résultats non significatifs. Par contre, les associations identifiées en dépit d'une faible puissance demeure intéressantes et valides. C'est le risque de biais alors qui détermine la validité des évidences observées. Nous avons calculé la puissance post hoc pour certains facteurs de risque associés au statut du troupeau à MAP quand les données étaient disponibles. Les puissances étaient souvent très faibles, ce qui explique pourquoi les facteurs plus importants n'étaient pas significatifs. Par exemple, pour l'introduction d'animaux au troupeau la puissance post hoc moyenne était de 0.25 (étendue : 0.02-0.48); pour le contact des veaux avec les adultes ou ses fèces la puissance post hoc moyenne était de 0.24 (étendue : 0.06-0.51); pour l'absence d'aire de vêlage la puissance post hoc moyenne était de 0.30 (étendue : 5.0-63.0); et pour la taille du troupeau, une unique étude avait l'information nécessaire et la puissance était de 0.36. Deuxièmement, notre étude a été réalisée dans une population avec des caractéristiques particulières. Les troupeaux laitiers du Québec sont relativement petits, la plupart sont en stabulation entravée et ils sont soumis à des hivers longs et froids. Nos résultats peuvent être extrapolés à des conditions similaires, comme l'est du Canada (Ontario, les Maritimes canadiennes) ou le nord-est des États-Unis, qui sont comparables, dans une certaine mesure, aux troupeaux laitiers du Québec. Quand même, il est rassurant de constater que certains facteurs de risque jugés importants dans l'étude cas-témoins du Québec ont déjà été signalés dans plusieurs régions géographiques avec des systèmes de production différents et en utilisant un devis d'étude différent.

Dans le cas de la revue systématique de la littérature, la plus grande limite est l'impossibilité de faire une analyse quantitative des facteurs de risque. Mais, nous avons évalué les facteurs de risque associés à un statut de troupeau positif à MAP et faire une analyse descriptive détaillée intéressante tenant compte du risque de biais des études. Il faut tenir compte que toutes les études étaient des études observationnelles, alors la qualité de l'évidence est faible (Guyatt et al., 2011). Le risque de biais devient alors une option intéressante pour comparer les études et mettre du poids aux conclusions de chacune. Le risque de biais a été déterminé par trois évaluateurs, diminuant le biais de classification des études. Aussi, l'évaluation de chaque étude de façon indépendante garantie l'objectivité du processus. Selon l'évaluation de qualité faite dans la revue systématique, 13 articles avaient un risque de biais faible. Cela veut dire que nous pouvons être plus certains de leurs conclusions.

5.5 Étapes suivantes

La suite de ce mémoire pourrait comprendre une étude longitudinale avec une taille d'échantillon plus grande, pour mettre en évidence la relation de causalité entre les facteurs de risque et la paratuberculose. Pour aller plus loin une étude de type intervention (e.g. Randomisée contrôlée) pourrait être justifiée à un certain moment afin de valider que l'implantation d'une pratique permettra vraiment de prévenir les nouvelles infections. Cependant, il n'est pas possible d'instaurer une pratique de gestion qui est considérée comme un facteur de risque dans un troupeau pour une période de 4 à 5 ans.

Un autre sujet qui pourrait être intéressant est le développement et la validation d'un score attribué à chaque troupeau selon les réponses au QAR. Chaque réponse va donner un nombre de points qui est plus haut quand le risque est plus haut. La somme du score de toutes les questions donne le score total du questionnaire. Une étude (Arango-Sabogal et al., 2017) a déterminé un seuil pour classer un troupeau étant à faible risque. Le valider dans une banque de données plus large serait intéressant. Des scores similaires sont utilisés dans les programmes des États-Unis et de l'Ontario. Cette approche quantitative est intéressante pour avoir une idée globale du risque de chaque troupeau.

Conclusion

Les facteurs de risque associés au statut du troupeaux à MAP ont été étudiés dans plusieurs études à travers le monde. On trouve dans la littérature des études réalisées dans différentes zones géographiques et différentes populations. Selon les auteurs, le type d'étude, le test diagnostique utilisé et l'approche statistique varient significativement. Toutefois, des facteurs de risque sont rapportés dans plusieurs études. La taille du troupeau et l'achat d'animaux sont les 2 facteurs les plus souvent associés au statut du troupeau à MAP, peu importe la façon de définir le statut.

Les résultats de l'étude cas-témoins du Québec sont en accord avec les évidences scientifiques. La CPE a été utilisée pour déterminer le statut du troupeau à MAP. La taille du troupeau était associée à un statut positif à MAP. Les troupeaux qui achetaient plus de 4% de vaches par année dans les 5 dernières années avaient plus de chances d'être positifs que les troupeaux fermés. Cela confirme que ces pratiques sont des facteurs de risque importants indépendamment du système de gestion ou de la zone géographique, et devraient être priorisées dans les programmes de contrôle au Québec et ailleurs.

Bibliographie

Agri-Traçabilité Québec, 2015. Boucles d'identification des bovins.

Alinovi, C.A., Ward, M.P., Lin, T.L., Moore, G.E., Wu, C.C., 2009. Real-time PCR, compared to liquid and solid culture media and ELISA, for the detection of *Mycobacterium avium* ssp. *paratuberculosis*. Vet Microbiol 136, 177-179.

Aly, S.S., Mangold, B.L., Whitlock, R.H., Sweeney, R.W., Anderson, R.J., Jiang, J., Schukken, Y.H., Hovingh, E., Wolfgang, D., Van Kessel, J.A., Karns, J.S., Lombard, J.E., Smith, J.M., Gardner, I.A., 2010. Correlation between Herrold egg yolk medium culture and real-time quantitative polymerase chain reaction results for *Mycobacterium avium* subspecies *paratuberculosis* in pooled fecal and environmental samples. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 22, 677-683.

Arango-Sabogal, J.C., Cote, G., Pare, J., Labrecque, O., Roy, J.P., Buczinski, S., Dore, E., Fairbrother, J.H., Bissonnette, N., Wellemans, V., Fecteau, G., 2016. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in tie-stall dairy herds using a standardized environmental sampling technique and targeted pooled samples. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 80, 175-182.

Arango-Sabogal, J.C., Pare, J., Labrecque, O., Cote, G., Roy, J.P., Buczinski, S., Wellemans, V., Fecteau, G., 2017. Incidence of fecal excretion of *Mycobacterium avium* subsp. *paratuberculosis* in dairy cows before and after the enrolment in the Quebec voluntary program. Preventive veterinary medicine 148, 94-105.

Bakker, D., 2010. Paratuberculosis control in Europe. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 306-318.

Baldwin, E.W., 1976. Isoniazid therapy in two cases of Johne's disease. Veterinary medicine, small animal clinician : VM, SAC 71, 1359-1362.

Barkema, H.W., von Keyserlingk, M.A., Kastelic, J.P., Lam, T.J., Luby, C., Roy, J.P., LeBlanc, S.J., Keefe, G.P., Kelton, D.F., 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. Journal of dairy science 98, 7426-7445.

Barrett, D., Mee, J., Good, M., Mullowney, P., Clegg, T., More, S., 2008. Risk factors for Johne's disease in Irish dairy herds: a case-control study. Cattle Practice 16, 209-215.

Berghaus, R.D., Farver, T.B., Anderson, R.J., Jaravata, C.C., Gardner, I.A., 2006. Environmental sampling for detection of *Mycobacterium avium* ssp. *paratuberculosis* on large California dairies. Journal of dairy science 89, 963-970.

Berghaus, R.D., Lombard, J.E., Gardner, I.A., Farver, T.B., 2005. Factor analysis of a Johne's disease risk assessment questionnaire with evaluation of factor scores and a subset of original questions as predictors of observed clinical paratuberculosis. Preventive veterinary medicine 72, 291-309.

Bolskë, G., Herthnek, D., 2010. Diagnosis of Paratuberculosis by PCR. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 267-283.

Bosshard, C., Stephan, R., Tasara, T., 2006. Application of an F57 sequence-based real-time PCR assay for *Mycobacterium paratuberculosis* detection in bulk tank raw milk and slaughtered healthy dairy cows. Journal of food protection 69, 1662-1667.
Bowling, A., 2005. Mode of questionnaire administration can have serious effects on data quality. Journal of public health (Oxford, England) 27, 281-291.
Boynton, P.M., Greenhalgh, T., 2004. Selecting, designing, and developing your questionnaire. BMJ 328, 1312-1315.
Buergelt, C.D., Hall, C., McEntee, K., Duncan, J.R., 1978. Pathological evaluation of paratuberculosis in naturally infected cattle. Veterinary pathology 15, 196-207.
Canadian Dairy Information Center, G.o.C., 2016. Dairy Barns by Type in Canada (2016-11-03).
Cashman, W., Buckley, J., Quigley, T., Fanning, S., More, S., Egan, J., Berry, D., Grant, I., O'Farrell, K., 2008. Risk factors for the introduction and within-herd transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection on 59 Irish dairy herds. Irish veterinary journal 61, 464-467.
Cetinkaya, B., Erdogan, H.M., Morgan, K.L., 1997. Relationships between the presence of Johne's disease and farm and management factors in dairy cattle in England. Preventive veterinary medicine 32, 253-266.
Chiodini, R.J., 1989. Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. Clinical microbiology reviews 2, 90-117.
Collins, M.T., 1996. Diagnosis of paratuberculosis. The Veterinary clinics of North America. Food animal practice 12, 357-371.
Collins, M.T., 2011. Diagnosis of paratuberculosis. The Veterinary clinics of North America. Food animal practice 27, 581-591, vi.
Collins, M.T., Gardner, I.A., Garry, F.B., Roussel, A.J., Wells, S.J., 2006. Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. J Am Vet Med Assoc 229, 1912-1919.
Collins, M.T., Kenefick, K.B., Sockett, D.C., Lambrecht, R.S., McDonald, J., Jorgensen, J.B., 1990. Enhanced radiometric detection of *Mycobacterium paratuberculosis* by using filter-concentrated bovine fecal specimens. Journal of clinical microbiology 28, 2514-2519.
Collins, M.T., Wells, S.J., Petrini, K.R., Collins, J.E., Schultz, R.D., Whitlock, R.H., 2005. Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clinical and diagnostic laboratory immunology 12, 685-692.
Correia-Gomes, C., Mendonca, D., Niza-Ribeiro, J., 2010. Risk associations to milk ELISA result for paratuberculosis in dairy cows in northern Portugal using a multilevel regression model. Revue de Medecine Veterinaire 161, 295-301.
Côté, G., Institut national de santé animale, Ministère de l'Agriculture des Pêcheries et l'Alimentation du Québec., 2003. Rapport Final. Enquête de prévalence de la paratuberculose, de la leucose bovine enzootique et des immunotolérants à la diarrhée virale bovine dans les troupeaux laitiers du Québec.,
Coussens, P., Lamont, A., Kabara, E., Sreevatsan, S., 2010. Host-Pathogen Interactions and Intracellular Survival of *Mycobacterium avium* subsp. *paratuberculosis*. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 109-125.

Crossley, B.M., Zagmutt-Vergara, F.J., Fyock, T.L., Whitlock, R.H., Gardner, I.A., 2005. Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* by dairy cows. Vet Microbiol 107, 257-263.

Daniels, M.J., Hutchings, M.R., Allcroft, D.J., McKendrick, J., Greig, A., 2002. Risk factors for Johne's disease in Scotland--the results of a survey of farmers. The Veterinary record 150, 135-139.

Dargatz, D.A., Byrum, B.A., Barber, L.K., Sweeney, R.W., Whitlock, R.H., Shulaw, W.P., Jacobson, R.H., Stabel, J.R., 2001. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. J Am Vet Med Assoc 218, 1163-1166.

Delgado, L., Marin, J.F., Munoz, M., Benavides, J., Juste, R.A., Garcia-Pariente, C., Fuertes, M., Gonzalez, J., Ferreras, M.C., Perez, V., 2013. Pathological findings in young and adult sheep following experimental infection with 2 different doses of *Mycobacterium avium* subspecies *paratuberculosis*. Veterinary pathology 50, 857-866.

Dohoo, I., Martin, W., Stryhn, H., 2014. Veterinary Epidemiologic Research. VER Inc.

Doré, E., Paré, J., Cote, G., Buczinski, S., Labrecque, O., Roy, J.P., Fecteau, G., 2012. Risk factors associated with transmission of *Mycobacterium avium* subsp. *paratuberculosis* to calves within dairy herd: a systematic review. Journal of veterinary internal medicine 26, 32-45.

Eamens, G.J., Whittington, R.J., Marsh, I.B., Turner, M.J., Saunders, V., Kemsley, P.D., Rayward, D., 2000. Comparative sensitivity of various faecal culture methods and ELISA in dairy cattle herds with endemic Johne's disease. Vet Microbiol 77, 357-367.

Espejo, L.A., Kubat, N., Godden, S.M., Wells, S.J., 2013. Effect of delayed exposure of cattle to *Mycobacterium avium* subsp *paratuberculosis* on the development of subclinical and clinical Johne's disease. American journal of veterinary research 74, 1304-1310.

Fecteau, M.E., Whitlock, R.H., 2010. Paratuberculosis in Cattle. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 144-156.

Fecteau, M.E., Whitlock, R.H., 2011. Treatment and chemoprophylaxis for paratuberculosis. The Veterinary clinics of North America. Food animal practice 27, 547-557, v.

Fecteau, M.E., Whitlock, R.H., Fyock, T.L., McAdams, S.C., Boston, R.C., Sweeney, R.W., 2011. Antimicrobial activity of gallium nitrate against *Mycobacterium avium* subsp. *paratuberculosis* in neonatal calves. Journal of veterinary internal medicine 25, 1152-1155.

Fredriksen, B., Djonne, B., Sigurdardottir, O., Tharaldsen, J., Nyberg, O., Jarp, J., 2004. Factors affecting the herd level of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. The Veterinary record 154, 522-526.

Garry, F., 2011. Control of paratuberculosis in dairy herds. The Veterinary clinics of North America. Food animal practice 27, 599-607, vii.

Giese, S.B., Ahrens, P., 2000. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in milk from clinically affected cows by PCR and culture. Vet Microbiol 77, 291-297.

Gonda, M.G., Chang, Y.M., Shook, G.E., Collins, M.T., Kirkpatrick, B.W., 2006. Genetic variation of *Mycobacterium avium* ssp. *paratuberculosis* infection in US Holsteins. Journal of dairy science 89, 1804-1812.

Gonzalez, J., Geijo, M.V., Garcia-Pariente, C., Verna, A., Corpa, J.M., Reyes, L.E., Ferreras, M.C., Juste, R.A., Garcia Marin, J.F., Perez, V., 2005. Histopathological classification of lesions associated with natural paratuberculosis infection in cattle. Journal of comparative pathology 133, 184-196.

Goodell, G., Hirst, H., Garry, F., Dinsmore, P., 2000. Comparison of cull rates and milk production of clinically normal dairy cows grouped by ELISA *Mycobacterium avium paratuberculosis* serum antibody results. Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics.

Groupe AGÉCO, 2016a. Nombre de producteurs de lait par province, Canada, 2010 à 2016.

Groupe AGÉCO, 2016b. Nombre moyen de vaches laitières par ferme et par province au 1er juillet, Canada, 2005 à 2016

Guyatt, G., Oxman, A.D., Akl, E.A., Kunz, R., Vist, G., Brozek, J., Norris, S., Falck-Ytter, Y., Glasziou, P., DeBeer, H., Jaeschke, R., Rind, D., Meerpohl, J., Dahm, P., Schunemann, H.J., 2011. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. Journal of clinical epidemiology 64, 383-394.

Harris, N.B., Barletta, R.G., 2001. *Mycobacterium avium* subsp. *paratuberculosis* in Veterinary Medicine. Clinical microbiology reviews 14, 489-512.

Hendrick, S.H., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M., Bagg, R., Dick, P., Duffield, T.F., 2006. Efficacy of monensin sodium for the reduction of fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in infected dairy cattle. Preventive veterinary medicine 75, 206-220.

Hendrick, S.H., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Archambault, M., Duffield, T.F., 2005. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. Journal of the American Veterinary Medical Association 227, 1302-1308.

Herthnek, D., Bolske, G., 2006. New PCR systems to confirm real-time PCR detection of *Mycobacterium avium* subsp. *paratuberculosis*. BMC microbiology 6, 87.

Hirst, H.L., Garry, F.B., Morley, P.S., Salman, M.D., Dinsmore, R.P., Wagner, B.A., McSweeney, K.D., Goodell, G.M., 2004. Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection among dairy cows in Colorado and herd-level risk factors for seropositivity. Journal of the American Veterinary Medical Association 225, 97-101.

Hostetter, J., Steadham, E., Haynes, J., Bailey, T., Cheville, N., 2003. Phagosomal maturation and intracellular survival of *Mycobacterium avium* subspecies *paratuberculosis* in J774 cells. Comparative immunology, microbiology and infectious diseases 26, 269-283.

Johnson-Ifearulundu, Y., Kaneene, J.B., 1999. Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. American journal of veterinary research 60, 589-596.

Johnson-Ifearulundu, Y.J., Kaneene, J.B., 1998. Management-related risk factors for M. paratuberculosis infection in Michigan, USA, dairy herds. Preventive veterinary medicine 37, 41-54.

Johnson-Ifearulundu, Y.J., Kaneene, J.B., Sprecher, D.J., Gardiner, J.C., Lloyd, J.W., 2000. The effect of subclinical *Mycobacterium paratuberculosis* infection on days open in Michigan, USA, dairy cows. Preventive veterinary medicine 46, 171-181.

Kalis, C.H., Hesselink, J.W., Barkema, H.W., Collins, M.T., 2001. Use of long-term vaccination with a killed vaccine to prevent fecal shedding of *Mycobacterium avium* subsp *paratuberculosis* in dairy herds. American journal of veterinary research 62, 270-274.

Kelton, D., Barkema, H.W., Bauman, C., Pickel, C., 2016. Regional differences in Johne's disease prevalence based on environmental culture and bulk tank milk. In:

Paratuberculosis, I.A.i. (Ed.), 13th International Colloquium on Paratuberculosis, Nantes, France, 114.
Kennedy, D., 2011. International efforts at paratuberculosis control. The Veterinary clinics of North America. Food animal practice 27, 647-654, viii.
Kinsel, M., Mueller, B., Honstead, J., 2010. A retrospective analysis of risk factors associated with Johne's disease in Pacific Northwest dairy herds. Bovine Practitioner 44, 114-122.
Kobayashi, S., Tsutsui, T., Yamamoto, T., Nishiguchi, A., 2007. Epidemiologic indicators associated with within-farm spread of Johne's disease in dairy farms in Japan. The Journal of veterinary medical science 69, 1255-1258.
Koets, A.P., Adugna, G., Janss, L.L., van Weering, H.J., Kalis, C.H., Wentink, G.H., Rutten, V.P., Schukken, Y.H., 2000. Genetic variation of susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle. Journal of dairy science 83, 2702-2708.
Koets, A.P., Eda, S., Sreevatsan, S., 2015. The within host dynamics of *Mycobacterium avium* ssp. *paratuberculosis* infection in cattle: where time and place matter. Veterinary research 46, 61.
Kudahl, A.B., Nielsen, S.S., 2009. Effect of paratuberculosis on slaughter weight and slaughter value of dairy cows. J. Dairy Sci. 92, 4340-4346.
Kunzler, R., Torgerson, P., Keller, S., Wittenbrink, M., Stephan, R., Knubben-Schweizer, G., Berchtold, B., Meylan, M., 2014. Observed management practices in relation to the risk of infection with paratuberculosis and to the spread of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss dairy and beef herds. BMC veterinary research 10, 132.
Lavers, C.J., McKenna, S.L., Dohoo, I.R., Barkema, H.W., Keefe, G.P., 2013. Evaluation of environmental fecal culture for *Mycobacterium avium* subspecies *paratuberculosis* detection in dairy herds and association with apparent within-herd prevalence. The Canadian veterinary journal = La revue veterinaire canadienne 54, 1053-1060.
Les producteurs de lait du Québec, 2017a. Portrait de la production de lait au Québec.
Les producteurs de lait du Québec, 2017b. Sur la ferme.
Lombard, J.E., Garry, F.B., McCluskey, B.J., Wagner, B.A., 2005. Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. J. Am. Vet. Med. Assoc. 227, 1975-1981.
Lombard, J.E., Wagner, B.A., Smith, R.L., McCluskey, B.J., Harris, B.N., Payeur, J.B., Garry, F.B., Salman, M.D., 2006. Evaluation of environmental sampling and culture to determine *Mycobacterium avium* subspecies *paratuberculosis* distribution and herd infection status on US dairy operations. Journal of dairy science 89, 4163-4171.
Manning, E.J.B., Collins, M.T., 2010a. Epidemiology of paratuberculosis. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 22-28.
Manning, E.J.B., Collins, M.T., 2010b. History of paratuberculosis. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 1-21.
MAPAQ, 2012a. Revue d'épidémiosurveillance animale du RAIZO, bilan 2011. Secteur bovin / Programme volontaire de prévention et de contrôlede la paratuberculose au Québec. 74-76
MAPAQ, Ministère de l'Agriculture des Pêcheries et l'Alimentation du Québec, 2012b. Revue d'épidémiosurveillance animale du RAIZO: bilan 2011.,

McKenna, S.L., Keefe, G.P., Tiwari, A., VanLeeuwen, J., Barkema, H.W., 2006. Johne's disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. The Canadian veterinary journal = La revue veterinaire canadienne 47, 1089-1099.

McNab, W.B., Meek, A.H., Martin, S.W., Duncan, J.R., 1992. Associations between lipoarabinomannan enzyme-immuno-assay test results for paratuberculosis and farm-management factors. Preventive veterinary medicine 13, 39-51.

Merkal, R.S., Larsen, A.B., Kopecky, K.E., 1968. Comparison of examination and test methods for early detection of paratuberculous cattle. American journal of veterinary research 29, 1533-1538.

Mitchell, R.M., Schukken, Y., Koets, A., Weber, M., Bakker, D., Stabel, J., Whitlock, R.H., Louzoun, Y., 2015. Differences in intermittent and continuous fecal shedding patterns between natural and experimental *Mycobacterium avium subspecies paratuberculosis* infections in cattle. Veterinary research 46, 66.

Momotani, E., Whipple, D.L., Thiermann, A.B., Cheville, N.F., 1988. Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Veterinary pathology 25, 131-137.

Mortensen, H., Nielsen, S.S., Berg, P., 2004. Genetic variation and heritability of the antibody response to *Mycobacterium avium subspecies paratuberculosis* in Danish Holstein cows. Journal of dairy science 87, 2108-2113.

Mortier, R.A., Barkema, H.W., Bystrom, J.M., Illanes, O., Orsel, K., Wolf, R., Atkins, G., De Buck, J., 2013. Evaluation of age-dependent susceptibility in calves infected with two doses of *Mycobacterium avium subspecies paratuberculosis* using pathology and tissue culture. Veterinary research 44, 94.

Mortier, R.A., Barkema, H.W., De Buck, J., 2015. Susceptibility to and diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection in dairy calves: A review. Preventive veterinary medicine 121, 189-198.

Motiwala, A.S., Strother, M., Theus, N.E., Stich, R.W., Byrum, B., Shulaw, W.P., Kapur, V., Sreevatsan, S., 2005. Rapid detection and typing of strains of *Mycobacterium avium* subsp. *paratuberculosis* from broth cultures. Journal of clinical microbiology 43, 2111-2117.

Nielsen, S.S., 2010. Immune-based diagnosis of Paratuberculosis. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 284-293.

Nielsen, S.S., Bjerre, H., Toft, N., 2008. Colostrum and milk as risk factors for infection with *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. Journal of dairy science 91, 4610-4615.

Nielsen, S.S., Nielsen, K.K., Huda, A., Condron, R., Collins, M.T., 2001. Diagnostic techniques for paratuberculosis. International Dairy Federation. Bulletin., 5-17.

Nielsen, S.S., Toft, N., 2006. Age-specific characteristics of ELISA and fecal culture for purpose-specific testing for paratuberculosis. Journal of dairy science 89, 569-579.

Nielsen, S.S., Toft, N., 2008. Ante mortem diagnosis of paratuberculosis: a review of accuracies of ELISA, interferon-gamma assay and faecal culture techniques. Vet Microbiol 129, 217-235.

Norton, S., Heuer, C., Jackson, R., 2009. A questionnaire-based cross-sectional study of clinical Johne's disease on dairy farms in New Zealand. N Z Vet J 57, 34-43.

Obasanjo, I.O., Grohn, Y.T., Mohammed, H.O., 1997. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. Preventive veterinary medicine 32, 243-251.

Okuni, J.B., Reinacher, M., Loukopoulos, P., Ojok, L., 2013. Prevalence and spectrum of Johne's disease lesions in cattle slaughtered at two abattoirs in Kampala, Uganda. Trop Anim Health Prod 45, 1197-1202.

Okura, H., Nielsen, S.S., Toft, N., 2010. Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection in adult Danish non-dairy cattle sampled at slaughter. Preventive veterinary medicine 94, 185-190.

Pieper, L., Sorge, U.S., DeVries, T.J., Godkin, A., Lissemore, K., Kelton, D.F., 2015. Evaluation of the Johne's disease risk assessment and management plan on dairy farms in Ontario, Canada. Journal of dairy science 98, 6792-6800.

Pillars, R.B., Grooms, D.L., Kaneene, J.B., 2009a. Longitudinal study of the distribution of *Mycobacterium avium* subsp. *paratuberculosis* in the environment of dairy herds in the Michigan Johne's disease control demonstration herd project. The Canadian veterinary journal = La revue veterinaire canadienne 50, 1039-1046.

Pillars, R.B., Grooms, D.L., Woltanski, J.A., Blair, E., 2009b. Prevalence of Michigan dairy herds infected with *Mycobacterium avium* subspecies *paratuberculosis* as determined by environmental sampling. Preventive veterinary medicine 89, 191-196.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., Fitzpatrick, E.S., 2011. Veterinary Microbiology and Microbial Disease. Hoboken : John Wiley & Sons, 2011.

Raizman, E.A., Wells, S.J., Godden, S.M., Bey, R.F., Oakes, M.J., Bentley, D.C., Olsen, K.E., 2004. The distribution of *Mycobacterium avium* ssp. *paratuberculosis* in the environment surrounding Minnesota dairy farms. Journal of dairy science 87, 2959-2966.

Raizman, E.A., Wells, S.J., Godden, S.M., Fetrow, J., Friendshuh, K., Michael Oakes, J., 2006. Characterization of Minnesota dairy herds participating in a Johne's disease control program and evaluation of the program risk assessment tool. Preventive veterinary medicine 75, 22-33.

Ranasinghe, I., Hsu, R., 2017. Crohn Disease. StatPearls. StatPearls Publishing LLC.

Ridge, S.E., Heuer, C., Cogger, N., Heck, A., Moor, S., Baker, I.M., Vaughan, S., 2010. Herd management practices and the transmission of Johne's disease within infected dairy herds in Victoria, Australia. Preventive veterinary medicine 95, 186-197.

Rowe, M.T., Grant, I.R., 2006. *Mycobacterium avium* ssp. *paratuberculosis* and its potential survival tactics. Letters in applied microbiology 42, 305-311.

Schukken, Y.H., Whitlock, R.H., Wolfgang, D., Grohn, Y., Beaver, A., VanKessel, J., Zurakowski, M., Mitchell, R., 2015. Longitudinal data collection of *Mycobacterium avium* subspecies *Paratuberculosis* infections in dairy herds: the value of precise field data. Veterinary research 46, 65.

Shin, S.J., Cho, D., Collins, M.T., 2008. Diagnosis of bovine paratuberculosis by a novel enzyme-linked immunosorbent assay based on early secreted antigens of *Mycobacterium avium* subsp. *paratuberculosis*. Clinical and vaccine immunology : CVI 15, 1277-1281.

Shin, S.J., Han, J.H., Manning, E.J., Collins, M.T., 2007. Rapid and reliable method for quantification of *Mycobacterium paratuberculosis* by use of the BACTEC MGIT 960 system. Journal of clinical microbiology 45, 1941-1948.

Slana, I., Kralik, P., Kralova, A., Babak, V., Pavlik, I., 2012. Short communication: Examination of milk filters by real-time PCR as a herd-level indicator of the presence of *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds. Journal of dairy science 95, 1162-1165.

Slana, I., Liapi, M., Moravkova, M., Kralova, A., Pavlik, I., 2009. *Mycobacterium avium* subsp. *paratuberculosis* in cow bulk tank milk in Cyprus detected by culture and quantitative IS900 and F57 real-time PCR. Preventive veterinary medicine 89, 223-226.

Smith, R.L., Schukken, Y.H., Pradhan, A.K., Smith, J.M., Whitlock, R.H., Van Kessel, J.S., Wolfgang, D.R., Grohn, Y.T., 2011. Environmental contamination with *Mycobacterium avium* subsp. *paratuberculosis* in endemically infected dairy herds. Preventive veterinary medicine 102, 1-9.

Sorge, U.S., Lissemore, K., Godkin, A., Hendrick, S., Wells, S., Kelton, D., 2011. Associations between paratuberculosis milk ELISA result, milk production, and breed in Canadian dairy cows. Journal of dairy science 94, 754-761.

Sorge, U.S., Lissemore, K., Godkin, A., Jansen, J., Hendrick, S., Wells, S., Kelton, D.F., 2012. Risk factors for herds to test positive for *Mycobacterium avium* ssp. *paratuberculosis*-antibodies with a commercial milk enzyme-linked immunosorbent assay (ELISA) in Ontario and western Canada. Canadian Veterinary Journal 53, 963-970.

Stabel, J.R., 2000. Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet Microbiol 77, 465-473.

Stevenson, K., 2010. Comparative Differences between Strains on *Mycobacterium avium* subsp. *paratuberculosis*. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 126-137.

Stevenson, K., 2015. Genetic diversity of *Mycobacterium avium* subspecies *paratuberculosis* and the influence of strain type on infection and pathogenesis: a review. Veterinary research 46, 64.

Streeter, R.N., Hoffsis, G.F., Bech-Nielsen, S., Shulaw, W.P., Rings, D.M., 1995. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. American journal of veterinary research 56, 1322-1324.

Sweeney, R.W., 1996. Transmission of paratuberculosis. The Veterinary clinics of North America. Food animal practice 12, 305-312.

Sweeney, R.W., 2011. Pathogenesis of paratuberculosis. The Veterinary clinics of North America. Food animal practice 27, 537-546, v.

Sweeney, R.W., Collins, M.T., Koets, A.P., McGuirk, S.M., Roussel, A.J., 2012. Paratuberculosis (Johne's disease) in cattle and other susceptible species. Journal of veterinary internal medicine 26, 1239-1250.

Sweeney, R.W., Whitlock, R.H., Bowersock, T.L., Cleary, D.L., Meinert, T.R., Habecker, P.L., Pruitt, G.W., 2009. Effect of subcutaneous administration of a killed *Mycobacterium avium* subsp *paratuberculosis* vaccine on colonization of tissues following oral exposure to the organism in calves. American journal of veterinary research 70, 493-497.

Sweeney, R.W., Whitlock, R.H., Hamir, A.N., Rosenberger, A.E., Herr, S.A., 1992a. Isolation of *Mycobacterium paratuberculosis* after oral inoculation in uninfected cattle. American journal of veterinary research 53, 1312-1314.

Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992b. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. Journal of clinical microbiology 30, 166-171.

Sweeney, R.W., Whitlock, R.H., Rosenberger, A.E., 1992c. *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. American journal of veterinary research 53, 477-480.

Taylor, T.K., Wilks, C.R., McQueen, D.S., 1981. Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. The Veterinary record 109, 532-533.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Keefe, G.P., Weersink, A., 2008. Estimate of the direct production losses in Canadian dairy herds with subclinical *Mycobacterium avium* subspecies *paratuberculosis* infection. The Canadian veterinary journal = La revue veterinaire canadienne 49, 569-576.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Stryhn, H., Keefe, G.P., Haddad, J.P., 2005. Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus, *Mycobacterium avium* subspecies *paratuberculosis,* and Neospora caninum on culling in dairy cattle in four Canadian provinces. Veterinary Microbiology 109, 147-158.

Tiwari, A., VanLeeuwen, J.A., McKenna, S.L., Keefe, G.P., Barkema, H.W., 2006. Johne's disease in Canada Part I: clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. The Canadian veterinary journal = La revue veterinaire canadienne 47, 874-882.

USDA-APHIS-VS, 2010. Uniform program standards for the voluntary bovine Johne's disease control program.

Vilar, A.L.T., Santos, C.S.A.B., Pimenta, C.L.R.M., Freitas, T.D., Brasil, A.W.L., Clementino, I.J., Alves, C.J., Bezerra, C.S., Riet-Correa, F., Oliveira, T.S., Azevedo, S.S., 2015. Herd-level prevalence and associated risk factors for *Mycobacterium avium* subsp. *paratuberculosis* in cattle in the State of Paraiba, Northeastern Brazil. Preventive veterinary medicine 121, 49-55.

Waddell, L.A., Rajic, A., Stark, K.D., Mc, E.S., 2015. The zoonotic potential of *Mycobacterium avium* ssp. *paratuberculosis*: a systematic review and meta-analyses of the evidence. Epidemiology and infection 143, 3135-3157.

Weber, M.F., Groenendaal, H., van Roermund, H.J., Nielen, M., 2004. Simulation of alternatives for the Dutch Johne's disease certification-and-monitoring program. Preventive veterinary medicine 62, 1-17.

Weber, M.F., van Roermund, H.J., Vernooij, J.C., Kalis, C.H., Stegeman, J.A., 2006. Cattle transfers between herds under paratuberculosis surveillance in The Netherlands are not random. Preventive veterinary medicine 76, 222-236.

Whitlock, R.H., 2010. Paratuberculosis control measures in the USA. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 319-329.

Whitlock, R.H., Buergelt, C., 1996. Preclinical and clinical manifestations of paratuberculosis (including pathology). The Veterinary clinics of North America. Food animal practice 12, 345-356.

Whittington, R., 2010. Cultivation of *Mycobacterium avium* subsp. *paratuberculosis*. In: Behr, M.A., Collins, D. (Eds.), Paratuberculosis: Organism, Disease, Control. CAB International, 245-266.

Whittington, R.J., Windsor, P.A., 2009. In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: a critical review and meta-analysis. Veterinary journal (London, England : 1997) 179, 60-69.

Windsor, P.A., Whittington, R.J., 2010. Evidence for age susceptibility of cattle to Johne's disease. Veterinary journal (London, England : 1997) 184, 37-44.

Wolf, R., Barkema, H.W., De Buck, J., Orsel, K., 2016. Dairy farms testing positive for *Mycobacterium avium* ssp. *paratuberculosis* have poorer hygiene practices and are less cautious when purchasing cattle than test-negative herds. Journal of dairy science 99, 4526-4536.

Wolf, R., Barkema, H.W., De Buck, J., Slomp, M., Flaig, J., Haupstein, D., Pickel, C., Orsel, K., 2014. High herd-level prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Western Canadian dairy farms, based on environmental sampling. Journal of dairy science 97, 6250-6259.

Zanetti, S., Molicotti, P., Cannas, S., Ortu, S., Ahmed, N., Sechi, L.A., 2006. "In vitro" activities of antimycobacterial agents against *Mycobacterium avium* subsp. *paratuberculosis* linked to Crohn's disease and paratuberculosis. Annals of clinical microbiology and antimicrobials 5, 27.

Annexe 1 Checklist for appraising the quality of case-control and cross-sectional studies

	Quality item	Coding	Explanation
Objectives and Study Population			
1	**Were the objectives stated adequately?**	Yes (Y)	Yes: Objectives clearly stated
		No (N)	No: Objectives not clearly stated
2	Was the sample size justified?	Yes (Y)	Yes: Use of sample-size formulas, based on desired power or precision and estimate of expected variability to detect differences
		Partial (P)	Partially: Informal guesses of a sample size
		No (N)	No: no details in the text
3	Were the animals housed or grouped in a way tht is representative of field conditions?	Yes (Y)	Yes: animals were housed in densities representatives of field condition
		No (N)	No: conditions of animals were not representative of field conditions.
		Not described (ND)	Not described
4	Was the reason and proportion of non-participents described?	Yes (Y)	Yes: The reason and proportion of non-response are stated clearly
		No (N)	No: The reason and proportion of non-response are not stated clearly
		Not described (ND)	Not described
5	Were the study participants (samples) selected randomly so the sample reflects disease and exposure in the population of interest? OR Were the controls selected from the same source population as the cases?	Yes (Y)	Yes: Random selection of the study participants or samples are stated and described or objective identification of controls in case control stated
		Unclear (U)	Unclear- too few details are available to make a clear judgement
		No (N)	No: Study participants were selected non-randomly or were not Described
		NA (NA)	NA
Exposure (blinding, measure of exposure)			

6	Were the exposure variable and outcome variable measured independently of each other?	Yes (Y)	Yes: The exposure and outcome variables were measured independently
		No (N)	No: The exposure and outcome variables were not measured independently or not clearly stated
7	**Were the methods used to measure the exposure variable standard and adequately described?**	Yes (Y)	Yes: The methods used were standard and adequately described
		No (N)	No: The methods used were not standard or adequately described
Outcome assesment (Disease positive designation and appropriate outcome)			
8	**Was the outcome (disease status) of participants measured by a medical professional in a standard and reliable way?**	Yes (Y)	Yes: The disease status was determined using a standard and reliable technique.
		No (N)	No: The disease status was not determined using a standard and reliable technique
Data Analysis			
9	Were observations excluded from the analysis reported?	Yes (Y)	Yes
		Unclear (U)	Unclear- too few details are available to make a clear judgement
		No (N)	No
10	Does the study appear to have reported all intended outcomes?	Yes (Y)	Yes
		Unclear (U)	Unclear- too few details are available to make a clear judgement
		No (N)	No
11	Was the type of statistical analysis appropriate for the study design?	Yes (Y)	Yes: Analysis fits study design, appropriate analysis of clustered data when required. (percentages and ignoring important confounders are examples of inappropriate analysis.)
		No (N)	No: Analysis is inadequate or does not fit study design
12	**Were the estimates and measures of variability used to address the research question presented adequately or is a sufficient amount of raw data presented for complete data extraction?**	Yes (Y)	Yes: Parameter estimates + measure of variability and/or P value provided, SE, 95%CI.
		Raw Data Presented (RD)	Partial: The estimates were inappropriate, however sufficient raw data is provided for post-hoc corrected analysis.
		No (N)	No: There is NO data to extract from this study.

13	Consider the magnitude and precision of the results for upgrading or down grading?	Upgrade (U)	Upgrade: large magnitude, precise results
		No grade change (NC)	No concern: results are precise
		Downgrade (D)	Downgrade: low power, imprecision, little confidence is the outcome measure.
14	Is there reason to believe that due to the population studied, the magnitude of effect (association) of the intervention (outcome) may be underestimated?	Yes, an underestimation is likely (Y)	You would answer yes ONLY if there was good reason to think that the study underestimated the potential association or effect of an intervention due to the population that was sampled
		No, there is no reason to believe the estimated effect is underestimated (N)	E.g. a drug was only tested on severely disease individuals and not on all diseased individuals, but it is likely that a better success rate would have been found if all diseased individuals were studied
			e.g. The magnitude of association was lower than it likely is in the general population because the comparison group has a similar disease which in also more likely to result in having the exposure of interest
15	Was a dose-response gradient detected for the intervention or exposure being examined?	Yes, dose-response gradient detected. (Y)	If a dose response gradient is demonstrated in some or all of the studies, this increases our confidence in the findings of the study and thus we can consider upgrading the evidence.
		No, no does-response gradient reported. (N)	
16	Was the study free of other problems that could put it at a high risk of bias?	Yes (Y)	Yes, I have no additional concerns about the design and/or conduct and reporting of this study.
	e.g.: non-randomization, clusters, stopping the study early without explanation, sample size intended (these are NOT more likely to have biased results)	Unclear (U)	
	Vs. Obvious imbalance in baseline factors that have an influence on the outcome. Outcome assessment can become biased. Selective reporting of subgroups can be biased (these ARE more likely to have biased results)	No (N)	No, the following are concerns I have that this study is at risk of bias. (list with page#)

17	Are there any concerns that confounders have not been appropriately identified and accounted?	Yes (Y)	Yes: All-important confounding factors were identified, accounted for by exclusion, matching or analysis. (sex, age)
		Raw Data Presented (RD)	Partial: some confounders controlled but not all of them
		No (N)	No: not stated
Conclusions			
18	**Overall, based on the GRADE questions please indicate the risk of bias for this study**	Low RoB (L)	Low risk of bias, no biases were indicated in the assessment. Thus plausible bias is unlikely in all key domains (within this study). (Across studies: most studies indicate low risk)
		Unclear RoB (U)	Unclear risk of bias, there are plausible bias that raises doubt about the results as some key domains are "unclear (within this study). (Across studies: most information is from low or unclear RoB)
		High RoB (H)	High Risk of bias indicates that in one or more of the domains serious plausible bias was identified (within the study). (Across studies: The proportion of studies that are at high risk of bias is sufficient to affect the interpretation of results.)

Adapted from CRD and AHRQ manuals and updated in 2011 to include GRADE criteria for GRADING the evidence.

Annexe 2 Checklist for appraising the quality of cohort studies

	Quality item	Coding	Explanation
Objectives and Study Population			
1	**Were the objectives stated adequately?**	Yes (Y)	Yes: Objectives clearly stated with including population, intervention, outcomes and controls that will be measured.
		No (N)	No: One or more of the components are missing.
2	Was the sample size justified?	Yes (Y)	Yes: Use of sample-size formulas, based on desired power or precision and estimate of expected variability to detect differences
		Partial (P)	Partially: Informal guesses of a sample size
		No (N)	No: no details in the text
3	Was the reason and proportion of non-participents described?	Yes (Y)	Yes: The reason and proportion of non-response are stated clearly
		No (N)	No: The reason and proportion of non-response are not stated clearly
		Not described (ND)	Not described
Exposure (blinding, measure of exposure)			
4	**Was the level of exposure representative of exposure in the population of interest?**	Yes (Y)	Yes: Does the sample reflect the proportion of high risk and low risk people in the population the investigator would like to extrapolate the results to?
		No (N)	No
5	**Was there a clear definition of exposure and detection methods?**	Yes (Y)	Yes
		No (N)	No
6	**Was an appropriate control group used?**	Yes (Y)	Yes- from the population of interest with a representative proportion of exposed and non-exposed people. Were they concurrent?
		No (N)	No
7	Was blinding appropriate? (Patient, doctor, farm hand,	Yes (Y)	Was knowledge of the intervention/ status of the

	outcome assessor, manuscript writer.) Please note if there is a different answer for different outcomes	Unclear (U)	individual or sample adequately prevented during the study?
		No (N)	
8	**Were the methods used to measure the exposure variable standard and adequately described?**	Yes (Y)	Yes: The methods used were standard and adequately described
		No (N)	No: The methods used were not standard or adequately described
Outcome assesment (Disease positive designation and appropriate outcome)			
9	**Was the disease status of participants validated by a medical professional as opposed to being self reported?**	Yes (Y)	Yes
		No (N)	No
		Not applicable (NA)	Not applicable
Withdrawals Assesment (Disease positive designation)			
10	**Was loss to follow-up reported and equal in both groups?**	Yes (Y)	Yes
		Unclear (U)	Unclear, there are too few details to make a judgement
		No (N)	No, there was loss of follow-up and it was not clearly reported
		NA	NA: no loss of follow-up
Data Analysis			
11	Were observations excluded from the analysis reported?	Yes (Y)	Yes
		Unclear (U)	Unclear- too few details are available to make a clear judgement
		No (N)	No
12	Does the study appear to have reported all intended outcomes?	Yes (Y)	Yes
		Unclear (U)	Unclear- too few details are available to make a clear judgement
		No (N)	No
13	Was the type of statistical analysis appropriate for the study design?	Yes (Y)	Yes: Analysis fits study design, appropriate analysis of clustered data when required. (percentages and ignoring important confounders are examples of inappropriate analysis.)
		No (N)	No: Analysis is inadequate or does not fit study design
14	**Were the estimates and measures of variability used to address the research question**	Yes (Y)	Yes: Parameter estimates + measure of variability and/or P value provided, SE, 95%CI.

	presented adequately or is a sufficient amount of raw data presented for complete data extraction?	Raw Data Presented (RD)	Partial: The estimates were inappropriate, however sufficient raw data is provided for post-hoc corrected analysis.
		No (N)	No: There is NO data to extract from this study.
15	Consider the magnitude and precision of the results for upgrading or down grading?	Upgrade (U)	Upgrade: large magnitude, precise results
		No grade change (NC)	No concern: results are precise
		Downgrade (D)	Downgrade: low power, imprecision, little confidence is the outcome measure.
16	Is there reason to believe that due to the population studied, the magnitude of effect (association) of the intervention (outcome) may be underestimated?	Yes, an underestimation is likely (Y)	You would answer yes ONLY if there was good reason to think that the study underestimated the potential association or effect of an intervention due to the population that was sampled
		No, there is no reason to believe the estimated effect is underestimated. (N)	E.g. a drug was only tested on severely disease individuals and not on all diseased individuals, but it is likely that a better success rate would have been found if all diseased individuals were studied
			e.g. The magnitude of association was lower than it likely is in the general population because the comparison group has a similar disease which in also more likely to result in having the exposure of interest
17	Was a dose-response gradient detected for the intervention or exposure being examined?	Yes, dose-response gradient detected. (Y)	If a dose response gradient is demonstrated in some or all of the studies, this increases our confidence in the findings of the study and thus we can consider upgrading the evidence.
		No, no does-response gradient reported. (N)	
18	Was the study free of other problems that could put it at a high risk of bias?	Yes (Y)	Yes, I have no additional concerns about the design and/or conduct and reporting of this study.
	e.g.: non-randomization, clusters, stopping the study early without explanation, sample size intended (these are NOT more likely to have biased results)	Unclear (U)	

	Vs. Obvious imbalance in baseline factors that have an influence on the outcome. Outcome assessment can become biased. Selective reporting of subgroups can be biased (these ARE more likely to have biased results)	No (N)	No, the following are concerns I have that this study is at risk of bias. (list with page#)
19	Are there any concerns that confounders have not been appropriately identified and accounted?	Yes (Y)	Yes: All-important confounding factors were identified, accounted for by exclusion, matching or analysis. (sex, age)
		Raw Data Presented (RD)	Partial: some confounders controlled but not all of them
		No (N)	No: not stated
Conclusions			
20	**Overall, based on the GRADE questions please indicate the risk of bias for this study**	Low RoB (L)	Low risk of bias, no biases were indicated in the assessment. Thus plausible bias is unlikely in all key domains (within this study). (Across studies: most studies indicate low risk)
		Unclear RoB (U)	Unclear risk of bias, there are plausible bias that raises doubt about the results as some key domains are "unclear (within this study). (Across studies: most information is from low or unclear RoB)
		High RoB (H)	High Risk of bias indicates that in one or more of the domains serious plausible bias was identified (within the study). (Across studies: The proportion of studies that are at high risk of bias is sufficient to affect the interpretation of results.)

Adapted from CRD and AHRQ manuals and updated in 2011 to include GRADE criteria for GRADING the evidence.

Annexe 3 Data extraction list of collected items

Study ID
Author
Year of publication
Title
Journal
Country
If not eligible, the reason
Study design
Target population
Source population
Study sample (description)
Sample size (animals and/or herds)
Case definition
Outcome
Herd status case definition
Unit of study
Sample used
Diagnostic tests used
If ELISA, kit used
Culture media
Descriptive statistic (yes/no)
Univariable analysis (yes/no)
Univariable analysis method
Univariable analysis cutoff
Multivariable analysis (yes/no)
Multivariable analysis method
Interactions assessed (yes/no)
Confounding assessed (yes/no)
Causal web (yes/no)
Questionnaire available (yes/no)
Risk factors significantly associated
Protective factors
Useful references
Notes

Annexe 4 Type of diagnostic test used in 29 studies evaluating the association between risk factors (identified using a risk assessment questionnaire) and *Mycobacterium avium* subsp. *paratuberculosis* herd status

Type of test	Diagnostic test	Number of studies	References
Immune response detection	Individual Serum ELISA	15	McNab et al., 1992 Johnson-Ifearulundu and Kaneene, 1998 Johnson-Ifearulundu and Kaneene, 1999 Wells and Wagner, 2000 Muskens et al., 2003 Fredriksen et al., 2004 Ridge et al., 2005 Weering et al., 2005 Pozzato et al., 2007 Dieguez et al., 2008 Kinsel et al., 2010 Erume and Mutebi, 2012 Kunzler et al., 2014 Sun et al., 2015 Vilar et al., 2015
	Individual Milk ELISA	2	Correia-Gomes et al., 2010 Sorge et al., 2012

	Bulk tank ELISA	1	Cazer et al., 2013
	ELISA (no sample specified)	1	Kobayashi et al., 2007
	Complement fixation	1	Kobayashi et al., 2007
	Johnin intradermal hypersensitivity	1	Kobayashi et al., 2007
Organism detection	Individual fecal culture	6	Kunzler et al., 2014 [a, b]
			Barrett et al., 2011
			Pozzato et al., 2007
			Barrett et al., 2008
			Kinsel et al., 2010
			Obasanjo et al., 1997 [d]
	Environmental culture	2	Wolf et al., 2016 [c]
			Pillars et al., 2009 [c]
	Milk sock filter residue culture	1	Cashman et al., 2008 [d]
	Individual fecal PCR	2	Kunzler et al., 2014 [a, b]
			Kinsel et al., 2010
	Bulk tank milk PCR	1	Ansari-Lari et al., 2009 [e]
	Bacterioscopy	1	Kobayashi et al., 2007
	Culture (no sample specified)	1	Kobayashi et al., 2007
Clinical disease detection	Clinical signs	4	Cetinkaya et al., 1997
			Daniels et al., 2002
			Berghaus et al., 2005

	Ridge et al., 2005

ELISA: Enzyme linked immunosorbent assay; PCR: polymerase chain reaction.

[a] Loewenstein-Jensen medium (Enclit, Oelzschau, Germany)

[b] F57 RT-PCR

[c] ESP II, ESP Culture System II, Trek Diagnostic Systems, Cleveland, OH, USA

[d] Herrold's Egg Yolk

[e] IS*900* PCR test

Annexe 5 *Mycobacterium avium* subsp *paratuberculosis* ELISA kits used in studies evaluating the association between risk factors and *Mycobacterium avium* subsp. *paratuberculosis* herd status.

MAP ELISA kit	Cut-off	Sensitivity**	Specificity**	Reference
Paratuberculosis	Sample considered positive if S:P≥0.03	NR		Muskens et al., 2003 [a]
Screening Ab Test,	Sample considered positive if S:P≥0.15	NR		Fredriksen et al., 2004 [a]
IDEXX	Sample considered positive if S:P≥55%	89.0	100.0	Correia-Gomes et al., 2010
Laboratories, Inc	Sample considered positive if S:P≥55%	NR		Erume and Mutebi, 2012
	Sample considered positive if S:P≥70%	73.6	98.0	Vilar et al., 2015
	Sample considered positive if S:P ≥90-110%	NR		Weering et al., 2005
	NR	48.5-50.0	98.9-99.4	Dieguez et al., 2008 [a]
	NR	50.0	99.0	Wells and Wagner, 2000
	NR	64.0	96.0	Johnson-Ifearulundu and Kaneene, 1999
				Johnson-Ifearulundu and Kaneene, 1998
	NR	NR		Sun et al., 2015
				Kunzler et al., 2014

				Pozzato et al., 2007
Paracheck ELISA, Prionics	Sample considered positive or negative in relation to the average negative control plus 0.01	21.0-83.0	NR	Cazer et al., 2013
	NR	61.0	95.0	Sorge et al., 2012
In-house ELISA, Eurofins Steins Laboratory	Sample considered positive if corrected OD ≥0.3	NR		
Lipoarabinomannan antigen ELISA	NR	49.0	87.0	McNab et al., 1992
Johne's Absorbed enzyme immonuassay Kit, Commonwealth Serum Laboratories	NR	NR		Ridge et al., 2005

NR: Not reported; S:P: Sample to positive ratio; OD: optical density.

[a] Herdchek ELISA; IDEXX Laboratories.

* Studies which reported the ELISA kit used.

** Reported in each study.

Annexe 6 Risk Assessment Questionnaire

Québec Voluntary Paratuberculosis Prevention and Control Program

General herd characteristics (GHC)

GHC1 – Current herd size, number of dairy cows: _____

GHC2 – Number of cows purchased in the last year: _____

GHC3 – Number of cows purchased in the last 5 years: _____

GHC4 – Number of animals purchased in the last 5 years (cows, calves, heifers, reproduction bulls): _____

GHC5 – Do you inquire about origin herd Paratuberculosis status when buying an animal?

Yes

No

GHC6 – Do you inquire about animal Paratuberculosis status when buying?

Yes

No

GHC7 – Do you have the possibility of isolating new animals (recently acquired) from the herd?

Yes

No

GHC8 – Breed: _____

GHC9 – Breeding:

Artificial insemination

Natural

Both

GHC10 – Housing:

Tie-stall

Free-stall

Both

GHC11 – Do animals have access to pasture during summer?

Yes

No

GHC12 – Are there other ruminant species housed with cattle?

Yes

No

GHC13 – Do you share agricultural material (tractor, manure spreaders, other equipment) with your neighbours?

Yes

No

GHC14 – Do you use the ionophore monensin for any age group on your farm?

Yes

No

GHC15 – Calving area location:

Tie-stall

Pen

Both

GHC16 – Do cattle have access to water courses or ponds?

Yes

No

GHC17 – Do you entrust the spreading of manure to a farming company?

Yes

No

Risk Factors

Calving area (CA)

CA1 – How often is there more than 1 cow in the maternity pen at the same time?

Risk level	Risk-level description
None	Never occurs
Low	Occurs <10% of calving events
Moderate	Occurs 10 to 40% of calving events
High	Occurs ≥50% of calving events
Very high	Always occurs

CA2 – Is there manure build-up in the maternity pen? How often do you add new bedding to the pen and how does the bedding look today?

Risk level	Risk-level description
None	No manure accumulation
Low	Manure cleaned and new bedding added daily, very little visible manure accumulation
Moderate	New bedding added 1 to 2 times per week. Manure-free area larger than contaminated area
High	New bedding added 1 to 2 times per month. Contaminated area larger than manure-free area
Very high	New bedding added <1 time per month, extensive manure accumulation

CA3 – If you kneel down on the bedding in the calving pen today for 25 seconds, are your knees wet?

Risk level	Risk-level description
Low	No
High	Yes

CA4 – How often are maternity pens / calving areas used for other purposes (e.g. for ill or lame cows or cows with special needs)?

Risk level	Risk-level description
None	Never
Very high	Always shared

CA5 – How often are clinical JD or known MAP infected animals calving in the same area as non-infected cows?

Risk level	Risk-level description
None	Never occurs, JD cows and suspected cows calve in a specific area
Very high	Always

CA6 – Do you have specific strategies for clinical JD or known MAP infected cows at calving?

Risk level	Risk-level description
Low	Yes (e.g. specific calving pens) Specify:
High	No

CA7 – How often are calves born in tie-stalls, free-stalls, pasture, or other cow contact areas?

Risk level	Risk-level description
None	Never occurs
Low	Occurs <10% of calving events
Moderate	Occurs 10 to 20% of calving events
High	Occurs 30 to 40% of calving events
Very high	Occurs ≥50% of calving events

CA8 – Is there manure soiling the majority of cows' udders and hindlegs in the maternity area?

Risk level	Risk-level description
None	No manure contamination
Low	Teats clean, slight to moderate manure on udders and hindlegs (below dewclaws)
Moderate	Teats clean, moderate to heavy manure on udder and hindlegs (to mid-tibia)
High	Small amounts of manure on teats and udders and hindlegs covered with manure (above hock)
Very high	Teats, udders and hindlegs covered with manure

CA9 – Are udders clipped and cleaned prior to calving?

Risk level	Risk-level description
None	Always
Very high	Never

CA10 – How often are calves separated from their dam within 30 minutes of birth?

Risk level	Risk-level description
None	Always
Low	Occurs ≥50% of calving events
Moderate	Occurs 10 to 40% of calving events
High	Occurs <10% of calving events
Very high	Never occurs

CA11 – How often are calves allowed to nurse their dam or another cow?

Risk level	Risk-level description
None	Never occurs
Low	Occurs <10% of calving events
Moderate	Occurs 10 to 40% of calving events
High	Occurs ≥50% of calving events
Very high	Always

CA12 – How old are calves when separated from cows?

Risk level	Risk-level description
None	Immediately, cow licks dry, no nursing
Low	After nursing, but <3 hours
Moderate	After nursing, but <12 hours
High	12-24 hours
Very high	More than 24 hours

CA13 – How often are calves fed at least 4L of colostrum within 6 hours of birth?

Risk level	Risk-level description
None	Always
Low	Occurs ≥50% of calving events
Moderate	Occurs 10 to 30% of calving events
High	Occurs <10% of calving events

Very high	Never occurs

CA14 – How often are female calves fed colostrum from more than one dam (pooled colostrum)?

Risk level	Risk-level description
None	Never fed pooled colostrum (calf receives own dam's colostrum)
Low	Colostrum from 1 cow fed to many calves
Moderate	Pooled colostrum fed 1-2 times per month
High	Pooled colostrum fed most of the time
Very high	Always fed pooled colostrum

CA15 – How often were female calves fed with frozen colostrum from another cow in the last year?

Risk level	Risk-level description
None	Never occurs
Low	1-2 times per year
Moderate	1-2 times per month
High	At least 1 time per week

CA16 – Is the colostrum fed to calves from low risk cows?

Risk level	Risk-level description
None	Fed artificial colostrum
Low	Colostrum from test-negative cows
Moderate	Colostrum from unknown status heifers
High	Colostrum from unknown status cows
Very high	Colostrum from test-positive cows

Preweaned heifer calves (i.e: milk fed) (PW)

PW1 – Is the milk fed to calves from low risk cows?

Risk level	Risk-level description

None	Calves fed milk replacer
Low	Milk from test-negative cows
Moderate	Milk from unknown status heifers
High	Milk from unknown status cows
Very high	Milk from test-positive cows

PW2 – How often is pooled milk fed to calves?

Risk level	Risk-level description
None	Never fed pooled milk
Low	Pooled milk fed 1-2 time per year
Moderate	Pooled milk fed 1-2 times per month
High	Pooled milk fed 1-2 times per week
Very high	Always fed pooled milk

PW3 – How often is non-saleable (waste) milk fed to calves?

Risk level	Risk-level description
None	Never fed non-saleable milk
Low	Non-saleable milk fed 1-2 time per year
Moderate	Non-saleable milk fed 1-2 times per month
High	Non-saleable milk fed every week
Very high	Always fed non-sellable pooled milk

PW4 – Is non-saleable (waste) milk fed to calves pasteurized?

Risk level	Risk-level description
Low	Yes
High	No

PW5 – Do you wash calves' bottles and pails with soap and water daily?

Risk level	Risk-level description	Bottle	Pail
Low	Yes		

Moderate	40-60% of the time		
High	No		

PW6 – Is the same bottle or pail used for many calves each day?

Risk level	Risk-level description	Bottle	Pail
Low	No		
Moderate	40-60% of the time		
High	Yes		

PW7 – Do calves have contact with cows or cow manure after 1 day of age?

Risk level	Risk-level description
None	Preweaned calves never housed near cows and equipment never shared
Low	Housed near cows only when necessary, only for short periods of time, no run-off possible and minimal direct contact
Moderate	Housed near cows only for short periods of time, where run-off and minimal direct contact is possible
High	Housed next to cows for short periods of time, where run-off is possible, and direct contact probable
Very high	Always housed near cows

PW8 – Is there contamination of milk, feed, calf water or calf pen with cow manure?

Risk level	Risk-level description
None	No cow manure contamination
Low	Trace amounts of manure visible, waterers and feeders cleaned more than once a month
Moderate	Some manure visible, waterers and feeders cleaned less than once a month
High	Large amounts of manure visible, waterers and feeders not cleaned regularly
Very high	Extensive manure contamination

PW9 – Do people from this farm walk among calves after contact with cow manure without cleaning or changing boots?

Risk level	Risk-level description
Low	No
Moderate	40-60% of the time
High	Yes (routinely and daily)

Weaned heifer calves (W)

W1 – Do heifers have contact with cows or cow manure?

Risk level	Risk-level description
None	Weaned heifers never housed near cows
Low	Housed near cows only when necessary, only for short periods of time, no run-off possible, and minimal direct contact
Moderate	Housed near cows only for short periods of time where run-off is possible and direct contact minimal
High	Housed next to cows for short periods of time where run-off is possible and direct contact probable
Very high	Always housed near cows

W2 – Are feed, water or housing areas contaminated with cow manure?

Risk level	Risk-level description
None	No cow manure contamination
Low	Trace amounts of manure visible, waterers and feeders cleaned more than once a month
Moderate	Some manure visible, waterers and feeders cleaned less than once a month
High	Large amounts of manure visible, waterers and feeders not cleaned regularly
Very high	Extensive manure contamination

W3 – Is there shared feed (including leftover feed), water or housing with cows?

Risk level	Risk-level description
None	Never share feed, water or housing
Low	Shared feed, water or housing only when necessary or by mistake and less than once a month
Moderate	Shared feed, water or housing 2-5 times per month
High	Shared feed, water or housing more often than not
Very high	Always share feed, water, or housing

W4 – Is there manure contamination of the feeding equipment used to feed heifers?

Risk level	Risk-level description
None	No cow manure contamination
Low	Trace amounts of manure visible, feeding equipment cleaned more than once a month
Moderate	Some manure visible, feeding equipment cleaned less than once a month
High	Large amounts of manure visible, feeding equipment not cleaned regularly
Very high	Extensive manure contamination

W5 – Do heifers share (at the same time) or graze (not at the same time) the same pasture with cows (dry or milking)?

Risk level	Risk-level description
None	Never share pasture or graze the same pasture
Low	Share pasture only when heifers escape
Moderate	Share pasture or graze less than 25% of the time
High	Share pasture or graze more than 25% of the time but less than 100% of the time
Very high	Always share pasture or graze pasture

W6 – Is manure spread on forage grazed by or harvested for heifers (in the same season)?

Risk level	Risk-level description

None	Never spread manure on pasture for heifers
Low	Manure spread on pasture only when no other option, more than 2 months before grazed or harvested
Moderate	Manure spread on pasture to be grazed or harvested between 0 and 2 months after spreading
High	Manure spread routinely on pasture to be grazed or harvested when forage matures, regardless of time
Very high	Manure always spread on pasture

Breeding age heifers (B)

BH1 – Do breeding heifers have contact with cows or cow manure?

Risk level	Risk-level description
None	Never housed near cows
Low	Housed near cows only when necessary, only for short periods of time, no run-off possible, and minimal direct contact
Moderate	Housed near cows only for short periods of time where run-off is possible and direct contact minimal
High	Housed next to cows for short periods of time where run-off is possible and direct contact probable
Very high	Always housed near cows

BH2 – Is feed, water, or housing area contaminated with cow manure?

Risk level	Risk-level description
None	No cow manure contamination
Low	Trace amounts of manure visible, waterers and feeders cleaned more than once a month
Moderate	Some manure visible, waterers and feeders cleaned less than once a month
High	Large amounts of manure visible, waterers and feeders not cleaned regularly
Very high	Extensive manure contamination

BH3 – Is there manure contamination of feeding equipment used to feed breeding age heifers?

Risk level	Risk-level description
None	No cow manure contamination
Low	Trace amounts of manure visible, feeding equipment cleaned more than once a month
Moderate	Some manure visible, feeding equipment cleaned less than once a month
High	Large amounts of manure visible, feeding equipment not cleaned regularly
Very high	Extensive manure contamination

BH4 – Do heifers share pasture with cows?

Risk level	Risk-level description
None	Never share pasture or graze the same pasture
Low	Share pasture only when heifers escape
Moderate	Share pasture or graze less than 25% of the time
High	Share pasture or graze more than 25% of the time but less than 100% of the time
Very high	Always share pasture or graze pasture

BH5 – Is manure spread on forage grazed by or harvested for breeding age heifers (in the same season)?

Risk level	Risk-level description
None	Never spread manure on pasture
Low	Manure spread on pasture only when no other option
Moderate	Manure spread on pasture to be grazed or harvested between 0 and 2 months after spreading
High	Manure spread routinely on pasture to be grazed or harvested when forage matures, regardless of time
Very high	Manure always spread on pasture

Cows (C)

C1 – Is there manure contamination of feeders or waterers?

Risk level	Risk-level description
None	No manure contamination
Low	Trace amounts of manure visible, waterers and feeders cleaned more than once a month
Moderate	Some manure visible, waterers and feeders cleaned less than once a month
High	Large amounts of manure visible, waterers and feeders not cleaned regularly
Very high	Extensive manure contamination

C2 – Is there manure contamination of feeding equipment or feed storage areas?

Risk level	Risk-level description
None	No cow manure contamination
Low	Trace amounts of manure visible, feeding equipment cleaned more than once a month
Moderate	Some manure visible, feeding equipment cleaned less than once a month
High	Large amounts of manure visible, feeding equipment not cleaned regularly
Very high	Extensive manure contamination

C3 – Do cows have access to manure storage areas or run-off?

Risk level	Risk-level description
None	No access to manure storage or run-off
Low	Access to manure storage or run-off occurs by mistake, less than once a month
Moderate	Access to manure storage or run-off occurs 2-5 times per month
High	Access to manure storage or run-off occurs more often than not
Very high	Always have access to manure storage or run-off

C4 – Is manure spread on forage grazed by or harvested for cows (in the same season)?

Risk level	Risk-level description
None	Manure never spread on pasture
Low	Manure spread on pasture or forage only when no other option
Moderate	Manure spread on pasture or forage to be grazed or harvested between 0 and 2 months after spreading
High	Manure spread routinely on pasture or forage to be grazed or harvested when forage matures, regardless of time
Very high	Manure always spread on pasture or forage

C5 – Milking cows' hygiene score**

Risk level	Risk-level description
Low	Manure up to fetlock, no manure on teats and/or udder
Moderate	Manure up to hock, slight amount of manure on teats and/or udder
High	Manure above hock, significant amount of manure on teats and/or udder

**Score half the milking cows if ≤50 cows

**Score 10% of milking cows if >50 cows

C6 – Close-up dry cows (within 3 weeks of calving) hygiene score

Risk level	Risk-level description
Low	Manure up to fetlock, no manure on teats and/or udder
Moderate	Manure up to hock, slight amount of manure on teats and/or udder
High	Manure above hock, significant amount of manure on teats and/or udder

Annexe 7 Univariable analysis of risk factors of case dairy herds (*Mycobacterium avium* ssp *paratuberculosis* had been isolated from at least 1 environmental sample) and control dairy herds (negative on 2 consecutive yearly environmental samplings and no history of paratuberculosis) enrolled in the Québec Voluntary Paratuberculosis Prevention and Control Program in 2012.

Variable	Number of herds	Dichotomic category*	Number of case herds	Number of control herds	OR	95% CI	P-value**
CA1 – How often is there more than 1 cow in the maternity pen at the same time?							
Never occurs	78	Low	15	75			
Occurs <10% of calving events	12						
Occurs 10 to 40% of calving events	5	High	9	12	3.75	1.34-10.47	0.016†
Occurs ≥50% of calving events	5						
Always occurs	11						
CA2 – Is there manure build-up in the maternity pen? How often do you add new bedding to the pen and how does the bedding look today?							
No manure accumulation	23	Low	21	70			
Manure cleaned and new bedding added daily, very little visible manure accumulation	68						
New bedding added 1 to 2 times per week. More manure-free area than manure contaminated area	18	High	3	19	0.53	0.14-1.95	0.399†
New bedding added 1 to 2 times per month. More manure contaminated area than manure-free area	4						
New bedding added <1 time per month, extensive manure accumulation	0						
CA3 – If you kneel down on the bedding in the calving pen today for 25 seconds, are your knees wet?							
No	69	Low	11	58			
Yes	35	High	11	24	2.42	0.92-6.32	0.068

CA4 – How often are maternity pens / calving areas used for other purposes (e.g. for ill or lame cows or cows with special needs)?							
Never	73	Low	13	60			
Always shared	27	High	9	18	2.31	0.85-6.27	0.096
CA5 – How often are clinical JD or known MAP infected animals calving in the same area as non-infected cows?							
Never occurs, JD cows and suspected cows calve in a specific area	22	Low	5	17			
Always	3	High	2	1	6.8	0.51-91.49	0.180†
CA6 – Do you have specific strategies for clinical JD or known MAP infected cows at calving?							
Yes	16	Low	6	10			
No	65	High	17	48	0.59	0.19-1.87	0.371†
CA7 – How often are calves born in tie-stalls, free-stalls, pasture, or other cow contact areas?							
Never occurs	16	Low	11	31			
Occurs <10% of calving events	26						
Occurs 10 to 20% of calving events	8	High	15	60	0.70	0.29-1.72	0.440
Occurs 30 to 40% of calving events	5						
Occurs ≥50% of calving events	62						
CA8 – Is there manure soiling the majority of cows' udders and hind legs in the maternity area?							
No manure contamination	33	Low	18	75			
Teats clean, slight to moderate manure on udders and hind legs (below dewclaws)	60						
Teats clean, moderate to heavy manure on udder and hind legs (to mid-tibia)	20	High	7	16	1.82	0.65-5.09	0.247
Small amounts of manure on teats and udders and hind legs covered with manure (above hock)	3						
Teats, udders and hind legs covered with manure	0						
CA9 – Are udders clipped and cleaned prior to calving?							
Always	32	Low	5	27			
Never	85	High	21	64	1.77	0.61-5.19	0.292
CA10 – How often are calves separated from their dam within 30 minutes of birth?							
Always	40	Low	16	49			
Occurs ≥50% of calving events	25						
Occurs 10 to 40% of calving events	7	High	10	42	0.73	0.30-1.78	0.486

Occurs <10% of calving events	3						
Never occurs	42						
CA11 – How often are calves allowed to nurse their dam or another cow?							
Never occurs	58	Low	17	71			
Occurs <10% of calving events	30						
Occurs 10 to 40% of calving events	14	High	9	20	1.88	0.73-4.85	0.188
Occurs ≥50% of calving events	5						
Always	10						
CA12 – How old are calves when separated from cows?							
Immediately, cow licks dry, no nursing	58	Low	15	43			
After nursing, but <3 hours	19	High	11	48	0.66	0.27-1.58	0.348
After nursing, but <12 hours	21						
12-24 hours	10						
More than 24 hours	9						
CA13 – How often are calves fed at least 4L of colostrum within 6 hours of birth?							
Always	29	Low	19	55			
Occurs ≥50% of calving events	45						
Occurs 10 to 30% of calving events	14	High	7	36	0.56	0.21-1.47	0.239
Occurs <10% of calving events	8						
Never occurs	21						
CA14 – How often are female calves fed colostrum from more than one dam (pooled colostrum)?							
Never fed pooled colostrum (calf receives own dam's colostrum)	103	Low	23	80			
Colostrum from 1 cow fed to many calves	9	High	3	11	0.95	0.24-3.69	1.000†
Pooled colostrum fed 1-2 times per month	5						
Pooled colostrum fed most of the time	0						
Always fed pooled colostrum	0						
CA15 – How often were female calves fed with frozen colostrum from another cow in the last year?							
Never occurs	78	Low	21	57			
1-2 times per year	33	High	5	34	0.40	0.14-1.16	0.084
1-2 times per month	4						
At least 1 time per week	2						
CA16 – Is the colostrum fed to calves from low risk cows?							

Fed artificial colostrum	0	Low	0	0			
Colostrum from test-negative cows	0						
Colostrum from unknown status heifers	3	High	26	91	1.00	--	--
Colostrum from unknown status cows	114						
Colostrum from test-positive cows	0						
PW1 – Is the milk fed to calves from low risk cows?							
Calves fed milk replacer	22	Low	3	19			
Milk from test-negative cows	0						
Milk from unknown status heifers	2	High	23	71	2.05	0.56-7.57	0.397†
Milk from unknown status cows	92						
Milk from test-positive cows	0						
PW2 – How often is pooled milk fed to calves?							
Never fed pooled milk	28	Low	6	25			
Pooled milk fed 1-2 time per year	3						
Pooled milk fed 1-2 times per month	3	High	20	65	1.28	0.46-3.56	0.633
Pooled milk fed 1-2 times per week	4						
Always fed pooled milk	78						
PW3 – How often is non-saleable (waste) milk fed to calves?							
Never fed non-saleable milk	48	Low	12	49			
Non-saleable milk fed 1-2 time per year	13						
Non-saleable milk fed 1-2 times per month	39	High	14	41	1.39	0.58-3.35	0.456
Non-saleable milk fed every week	14						
Always fed pooled milk	2						
PW4 – Is non-saleable (waste) milk fed to calves pasteurized?							
Yes	3	Low	1	2			
No	77	High	17	60	0.57	0.05-6.63	0.540†
PW5 – Do you wash calves' bottles and pails with soap and water daily?							
Yes	55	Low	11	44			
40-60% of the time	14	High	15	46	1.30	0.54-3.15	0.554
No	47						
PW6 – Is the same bottle or pail used for many calves each day?							
No	45	Low	12	33			
40-60% of the time	23	High	14	57	0.68	0.28-1.63	0.382

Yes	48						
PW7 – Do calves have contact with cows or cow manure after 1 day of age?							
Pre-weaned calves never housed near cows and equipment never shared	74	Low	19	71			
Housed near cows only when necessary, only for short periods of time, no run-off possible and minimal direct contact	16						
Housed near cows only for short periods of time, where run-off is possible, and minimal direct contact	9	High	7	19	1.38	0.50-3.76	0.531
Housed next to cows for short periods of time, where run-off is possible, and direct contact probable	4						
Always housed near cows	13						
PW8 – Is there contamination of milk, feed, calf water or calf pen with cow manure?							
No cow manure contamination	98	Low	24	88			
Trace amounts of manure visible, waterers and feeders cleaned more than once a month	14						
Some manure visible, waterers and feeders cleaned less than once a month	3	High	2	2	3.67	0.49-27.40	0.217†
Large amounts of manure visible, waterers and feeders not cleaned regularly	1						
Extensive manure contamination	0						
PW9 – Do people from this farm walk among calves after contact with cow manure without cleaning or changing boots?							
No	29	Low	8	21			
40-60% of the time	34	High	18	69	0.68	0.26-1.80	0.441
Yes (routinely and daily)	53						
W1 – Do heifers have contact with cows or cow manure?							
Weaned heifers never housed near cows	84	Low	25	77			
Housed near cows only when necessary, only for short periods of time, no run-off possible, and minimal direct contact	18						
Housed near cows only for short periods of time, where run-off is possible, and minimal direct contact	6	High	1	13	0.24	0.03-1.90	0.187†
Housed next to cows for short periods of time, where run-off is possible, and direct contact probable	0						
Always housed near cows	8						
W2 – Are feed, water or housing areas contaminated with cow manure?							

No cow manure contamination	96	Low	26	89			
Trace amounts of manure visible, waterers and feeders cleaned more than once a month	19						
Some manure visible, waterers and feeders cleaned less than once a month	1	High	0	1	1.00	--	1.000†
Large amounts of manure visible, waterers and feeders not cleaned regularly	0						
Extensive manure contamination	0						
W3 – Is there shared feed (including leftover feed), water or housing with cows?							
Never share feed, water or housing	90	Low	21	78			
Shared feed, water or housing only when necessary or by mistake and less than once a month	9						
Shared feed, water or housing 2-5 times per month	2	High	4	12	1.24	0.36-4.24	0.748†
Shared, water or housing more often than not	5						
Always share feed, water, or housing	9						
W4 – Is there manure contamination of the feeding equipment used to feed heifers?							
No cow manure contamination	97	Low	26	84			
Trace amounts of manure visible, feeding equipment cleaned more than once a month	13						
Some manure visible, feeding equipment cleaned less than once a month	3	High	0	6	1.00	--	0.335†
Large amounts of manure visible, feeding equipment not cleaned regularly	3						
Extensive manure contamination	0						
W5 – Do heifers share (at the same time) or graze (not at the same time) the same pasture with cows (dry or milking)?							
Never share pasture or graze the same pasture	109	Low	23	86			
Share pasture only when heifers escape	0						
Share pasture or graze less than 25% of the time	3	High	3	4	2.80	0.59-13.43	0.186†
Share pasture or graze more than 25% of the time but less than 100% of the time	2						
Always share pasture or graze pasture	2						
W6 – Is manure spread on forage grazed by or harvested for heifers (in the same season)?							
Never spread manure on pasture for heifers	43	Low	11	40			
Manure spread on pasture only when no other option, more than 2 months before grazed or harvested	8						

Manure spread on pasture to be grazed or harvested between 0 and 2 months after spreading	59	High	15	50	1.10	0.45-2.64	0.847
Manure spread routinely on pasture to be grazed or harvested when forage matures, regardless of time	3						
Manure always spread on pasture	3						
BH1 – Do breeding heifers have contact with cows or cow manure?							
Never housed near cows	56	Low	19	64			
Housed near cows only when necessary, only for short periods of time, no run-off possible, and minimal direct contact	27						
Housed near cows only for short periods of time, where run-off is possible, and minimal direct contact	12	High	7	26	0.91	0.34-2.41	0.845
Housed next to cows for short periods of time, where run-off is possible, and direct contact probable	7						
Always housed near cows	14						
BH2 – Is feed, water, or housing area contaminated with cow manure?							
No cow manure contamination	96	Low	25	89			
Trace amounts of manure visible, waterers and feeders cleaned more than once a month	18						
Some manure visible, waterers and feeders cleaned less than once a month	2	High	1	1	3.56	0.21-58.96	0.400†
Large amounts of manure visible, waterers and feeders not cleaned regularly	0						
Extensive manure contamination	0						
BH3 – Is there manure contamination of feeding equipment used to feed breeding age heifers?							
No cow manure contamination	102	Low	26	88			
Trace amounts of manure visible, feeding equipment cleaned more than once a month	12						
Some manure visible, feeding equipment cleaned less than once a month	2	High	0	2	1.00	--	1.000†
Large amounts of manure visible, feeding equipment not cleaned regularly	0						
Extensive manure contamination	0						
BH4 – Do heifers share pasture with cows?							
Never share pasture or graze the same pasture	81	Low	17	65			
Share pasture only when heifers escape	1						

Share pasture or graze less than 25% of the time	10	High	9	25	1.38	0.54-3.49	0.500
Share pasture or graze more than 25% of the time but less than 100% of the time	10						
Always share pasture or graze pasture	14						
BH5 – Is manure spread on forage grazed by or harvested for breeding age heifers (in the same season)?							
Never spread manure on pasture	38	Low	11	39			
Manure spread on pasture only when no other option	12						
Manure spread on pasture to be grazed or harvested between 0 and 2 months after spreading	58	High	15	51	1.04	0.43-2.52	0.926
Manure spread routinely on pasture to be grazed or harvested when forage matures, regardless of time	4						
Manure always spread on pasture	4						
C1 – Is there manure contamination of feeders or waterers?							
No manure contamination	96	Low	25	91			
Trace amounts of manure visible, waterers and feeders cleaned more than once a month	20						
Some manure visible, waterers and feeders cleaned less than once a month	1	High	1	0	1.00	--	0.222†
Large amounts of manure visible, waterers and feeders not cleaned regularly	0						
Extensive manure contamination	0						
C2 – Is there manure contamination of feeding equipment or feed storage areas?							
No cow manure contamination	102	Low	23	88			
Trace amounts of manure visible, feeding equipment cleaned more than once a month	9						
Some manure visible, feeding equipment cleaned less than once a month	2	High	1	1	3.83	0.23-63.52	0.381†
Large amounts of manure visible, feeding equipment not cleaned regularly	0						
Extensive manure contamination	0						
C3 – Do cows have access to manure storage areas or run-off?							
No access to manure storage or run-off	109	Low	26	87			
Access to manure storage or run-off occurs by mistake, less than once a month	4						

Access to manure storage or run-off occurs 2-5 times per month	2	High	0	4	1.00	--	0.574†
Access to manure storage or run-off occurs more often than not	0						
Always have access to manure storage or run-off	2						
C4 – Is manure spread on forage grazed by or harvested for cows (in the same season)?							
Manure never spread on pasture	34	Low	10	37			
Manure spread on pasture or forage only when no other option	13						
Manure spread on pasture or forage to be grazed or harvested between 0 and 2 months after spreading	61	High	16	54	1.10	0.45-2.68	0.840
Manure spread routinely on pasture or forage to be grazed or harvested when forage matures, regardless of time	5						
Manure always spread on pasture or forage	4						
C5 – Milking cows' hygiene score							
Manure up to fetlock, no manure on teats and/or udder	70	Low	14	56			
Manure up to hock, slight amount of manure on teats and/or udder	36	High	12	34	1.41	0.59-3.41	0.442
Manure above hock, significant amount of manure on teats and/or udder	10						
C6 – Close-up dry cows (within 3 weeks of calving) hygiene score							
Manure up to fetlock, no manure on teats and/or udder	79	Low	16	63			
Manure up to hock, slight amount of manure on teats and/or udder	31	High	10	25	1.58	0.63-3.94	0.329
Manure above hock, significant amount of manure on teats and/or udder	4						

* Multiple answer variables were dichotomized according to risk and distribution to perform the multivariable analysis.

** Pearson's chi-square test. Fisher's exact test if † is indicated.