

Introduction

Johne's disease is a bacterial disease of ruminants and other (mainly) mammals, for which there is no cure. The organism has been found in wildlife as well as domesticated animals. It is caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis*, (MAP for short). The bacterium is closely related to organisms that cause tuberculosis and leprosy. MAP was first discovered in Germany at the end of the 1800's in a cow with severe intestinal disease.

The disease has a worldwide prevalence and has important economic implications for farmed livestock. It is estimated that over one third of UK herds are infected at some level, but within herd prevalence is difficult to ascertain, due to low sensitivity of most available tests. Johne's disease results in financial loss to the farmer in terms of lower milk yields, higher somatic cell counts, increased mastitis, reduced fertility, increased susceptibility to other diseases, increase culling rate for all the aforementioned reasons, lower cull cow value, and eventually death in animals that are in the final stages of the disease.

MAP has often been associated with Crohn's disease in humans, because of some common pathological symptoms, although there are differences. There is evidence that MAP is connected in some way to Crohn's disease, but may not be the causal organism. Most studies published since 2000 indicate higher detection rates of MAP in Crohn's patients compared with control subjects. A review paper in 2007 concluded that the current epidemiological evidence strongly supports the conjecture that Crohn's disease is caused by MAP, but this is not proven. Uncertainty remains over the role Johne's disease plays, if any, in Crohn's disease and the current recommendations from the Food Standards Agency is to adopt a precautionary approach to limit the exposure of people to the organism.

MAP has been found in pasteurised milk and milk products, including formula milk, but this begs the question as to whether the pasteurisation process was carried out correctly, whether there was re-contamination post pasteurisation and also the validity of the PCR test for this analysis, given that the PCR test does not distinguish between living and dead DNA from MAP. However, the fact that MAP DNA has been isolated from dairy products is a major concern for the industry and particularly in relation to the possible, but unconfirmed connection with Crohn's disease. There could be a major market shock, particularly in relation to high value products, such as formula milk, milk powder and other constituents. The repercussions of this would far outweigh the on-farm costs of disease control/eradication programmes.

Herd infection with Johne's

The usual entry point of MAP onto a farm is through the importation of an infected animal(s), which may not be exhibiting any signs of the disease, irrespective of what test(s) may have been carried out on it before arrival, or in quarantine post arrival, particularly if the animal(s) was young at the time of importation, (which is usually the situation). The disease can consequently spread silently in the herd to the point where the imported animal(s) and infected cohorts start to show clinical signs, which could be years later. Hence, maintaining a closed herd should be a high priority and/or only purchasing animals from herd with low-risk status, which is rare. Johne's disease status of the exporting herd is usually not available. Johne's disease is not always recognised in a herd, which is often because of high culling rates which are associated with other factors, such as high SCC, poor production, infertility, lameness etc. and because no tests have been carried out.

Data from risk assessments completed on over 3,000 UK dairy herds as part of the NJMP, (National Johne's Management Plan) indicate that only a quarter of them had no cattle introduced

on to the farm over a 10 year period and so could claim to be 'closed herds'. Many of these herds had only introduced one animal, but others had introduced batches of cattle of unknown disease status over a 10 year timeframe. The situation in Northern Ireland is unlikely to be better and may be worse, with expanding herds and bought in replacements for animals culled for TB.

Animal infection with Johne's

The oral ingestion of faeces is considered to be the main entry point for the organism, with young calves thought to be more susceptible than older animals. Older cattle may get infected from the shedding of MAP by other infectious animals in the herd, but the consensus is that the level of exposure must be very high for this to happen.

A calf may get infected in utero, which is largely driven by how advanced disease is in the dam. If the disease is at an advanced stage in the dam, the estimate is ~ 1 in 3 calves get infected this way, if earlier stage infection in the dam, around 1 in 10 will get infected in utero. Ingestion via the consumption of colostrum/milk from an infected dam is a primary source of infection, but faecal contamination of colostrum/milk post collection is likely to be just as important, if not more so, as a route of infection to newborn and young calves. Hence, hygiene of milking and storage containers is very important. Calves should only be fed milk from their dam and/or powdered milk. Whole milk should not be pooled for feeding to calves, especially to replacement heifers. Heifers that get infected in utero, via drinking infected colostrum/milk, or via the ingestion of faeces in their environment can subsequently pass the bacterium on to their herd mates through faecal shedding and by so doing, ensure that the disease persists in a herd. MAP can survive in dust particles and soil and has been found in water used for human consumption. Wildlife such as rabbits can on occasion become infected with MAP, but it is uncertain what impact if any they have as a reservoir of infection for potential spread to cattle and sheep.

Diagnosing the disease

The main problem with the disease diagnosis is that most infected animals are subclinical, i.e. they do not show clinical signs of the disease, unless they have progressed to the terminal stage, when they start to waste away. Euthanasia is really the only option at this stage. However, most infected animals will be asymptomatic for the disease and are culled for other reasons before the final stages of the disease manifests itself. Subclinical animals can spread the disease via faeces to other animals in their vicinity, which may subsequently become infected and/or via colostrum/milk to young calves. Available tests lack sensitivity particularly in the early stages of infection, i.e. the ability to show true positive results.

Three stages of the disease have been described; the infected, the infectious and the affected. Infected animals have contacted the disease at some point in their lives and may have initiated an immune response, but most diagnostic tests will not show a positive result for the disease at this stage and hence, these animals cannot be differentiated from those that are not infected. It has been postulated that the age of onset of faecal shedding is related to the age of the animal when infected and the infection dose. It is generally accepted that faecal shedding precedes the onset of measurable antibody circulation, although this is not always the case. The affected are in the later stages of the disease, where clinical signs become obvious, weight loss, diarrhoea and reduced milk yield. Infectious animals are those that are shedding MAP in their faeces and are therefore at risk of infecting herd mates. Shedding is often intermittent and therefore MAP is not always detected in faeces, (faecal sensitivity is relatively low, particularly in earlier stages of the disease), but this does not mean that the animal is disease free. Such animals can also be infectious in utero to their offspring and in colostrum/milk to their calves and potentially other

calves, if colostrum/milk is pooled for feeding, although not all researchers agree on the importance of milk pooling as a transmission route for the disease.

Testing for Johne's disease

Diagnosis of the disease is difficult because animals that are subclinical don't show measurable immune response levels and don't shed measurable levels of MAP in their faeces. Animals may be infected and showing no signs of the disease but potentially spreading large amounts of MAP in their faeces to their cohorts and also potentially to their offspring in utero and at calving via contaminated colostrum and faeces. Most diagnostic tests lack sensitivity, i.e. the ability to correctly diagnose a positive result for the presence of MAP and this is a source of frustration to the industry. The 'iceberg' concept states that for every clinical case, there are ~15-20 animals infected, (some of whom may also be infectious), but less than half of these animals will be detected by faecal culture as they have not progressed to that stage yet. Different tests for diagnosing the disease are outlined below.

1: Enzyme linked immunosorbent assay (ELISA)

Indirect measures of the immune response of the animal are often used as diagnostic tools, because tests are cheap and quick, but the tests lack sensitivity. Blood serum or milk can be analysed by ELISA. Repeated testing is required to build a 'true' picture of the animal disease status, as sensitivity (true positives) is low in the early stages of infection, but increases with time, as infection progresses. ELISA results are measured by optical density, (OD) and are sometimes expressed as optical density of sample/optical density of control sample. The result may be reported as positive, inconclusive or negative, depending on the cut-off point used in the analysis. The cut-off point for milk ELISA is usually taken as lower than that for blood ELISA.

The sensitivity of ELISA in blood serum of animals is ~ 7% in the silent stage, (infected but no symptoms), 15% in the subclinical stage, (infected and may or may not be excreting MAP) and between 85% to 98% in the clinical stage, (animal failing away, excreting large numbers of MAP, profuse diarrhoea). A Danish study estimated the sensitivity of the ELISA test as 0.27 at 2 years old, 0.54 at 3 years old, 0.68 at 4 years old and a maximum of 0.79 by 10 years old.

There is a 'moderate' correlation between blood serum and milk antibody results, with the level in milk dependant on the circulating level in blood, the days in milk, the milk yield of the cow and the lactation number. Research findings indicate R2 correlations between blood serum and milk ELISA tests of between 0.69 and 0.92, (an R2 of 1.00 indicates complete correlation). Some research has shown a strong association between faecal shedding, faecal PCR and milk ELISA, with all ELISA positive cows having positive faecal PCR results.

Stage of lactation and milk yield also affect ELISA results. High yielding cows can dilute MAP antibodies in their milk, changing what might have been a positive result to a negative result. Equally, 1st lactation animals may not test positive, because of low MAP antibody excretion in their milk, even though they may be subclinical. Generally, younger animals are less likely to test positive for MAP, with the odds of testing positive increasing with age/lactation number. When milk ELISA becomes positive, serum ELISA usually confirms the result and in most cases the cow will stay positive with repeated tests. However, with an ELISA specificity of ~98%, this means that there will be roughly one or two false positives in every 100 tests carried out.

It is recommended that at least 2 tests are carried out at different stages of lactation to determine not only the level of antibodies, but the stability of the result, or in other words, how likely the animal is to be positive. When ELISA milk or blood serum yields positive results in apparently

healthy or low-prevalence herds, it is recommended that a faecal test should be carried out to confirm the stage of infection. If the results are negative, the positive ELISA should be re-examined in 6-12 months, since it may be a false positive, or it may be that at the time of sampling, the animal was not shedding MAP in the faeces in detectable amounts. Cows can change status between samples, hence the need for repeat testing and/or faecal testing to confirm ELISA results.

TB testing can generate false positives from milk or blood ELISA. Blood should not be taken at the second day of the TB test and blood or milk should not be taken for MAP testing for 3 months after a TB test. For TB restricted herds, MAP sampling should be done as late as possible after the last TB test and just before the next test.

2. Bacterial culture and PCR

Faecal testing (bacterial culture or PCR) is considered the gold standard for detection of the disease. These tests are usually carried out subsequent to a positive result from blood or milk ELISA, as a confirmation of disease status. Milk or intestinal scrappings may also be used for culture or PCR to confirm presence of the disease. However, animals may be only shedding the organism intermittently or may have recently ingested contaminated faeces and not actually be diseased but be transitory positive for faecal MAP. (Even if an animal is only transitory positive for faecal culture, it indicates presence of MAP in a herd and probably at a high level, if animals are ingesting faecal MAP from their cohorts). **Culturing MAP is time consuming and expensive, taking many weeks for the organism to grow.** For animals that are showing clinical signs, the faecal test has a sensitivity of ~0.70 – 0.74, or in other words, 70-74% of infected animals will be detected by this test. However, in animals that are also infectious, (potentially spreading MAP in their faeces either intermittently, or in low numbers), but showing no clinical signs, the sensitivity of faecal testing is much lower, ranging from 0.23 – 0.49. In infected animals, but not infectious, (not excreting MAP), the sensitivity of faecal testing is less than 0.1. It is thought that the dose of MAP and the age of the animal when infected impacts on when faecal shedding starts. Most studies report that light faecal shedders are seronegative, i.e. they do not have circulating antibodies to the bacterium and hence, will not test positive by ELISA.

3. Other tests

Agar gel immunodiffusion (AGID) is based on the precipitation of immune complexes formed by the antibodies of infected animals with a soluble antigen from a protoplasmic extract of MAP in a gel matrix of agar. It is a simple, fast and relatively inexpensive method, but has low sensitivity in the early stages of Johne's and therefore it is considered a good diagnostic method in animals in advanced clinical stages. It can be used as a rapid confirmatory test of suspected cases. The sensitivity is good in the advanced clinical stage (90 % - 95 %), but low in subclinical stages, (30 % - 18.9 %).

Complement fixation test (CFT) can be used in the later stages of disease when reduced milk yield and major faecal shedding are apparent. It is difficult to perform and only carried out in reference laboratories.

Interferon gamma (IFN- γ) can be used in younger animals (1-2 yrs old) to detect the initial cellular immune response to the MAP organism. Test sensitivity is higher than for other tests such as ELISA and faecal culture at this stage of disease development, as the test is picking up the initial immune response, but still low, (~40% true positives) and can be even lower in herds with active TB. High cost is a disadvantage, as well as possible cross reactivity with TB and the need for live cells at time of analysis and low specificity.

None of these three (alternative) tests are used to any significant extent and are more of historical interest. The main tests available and their associated pros/cons and costs are outlined in Table 1 below.

Table 1. Tests for Johne's disease

Test	Material tested	Sensitivity / specificity/ issues	Cost (£/cow)
Bacterial culture	Faeces, milk, intestinal scrappings	Gold standard test, relatively sensitive at clinical stage, quantitative, (colony forming units/ml). Difficult procedure and takes many weeks to perform.	High
Polymerase chain reaction (PCR)	Blood, milk, faeces, tissues	Qualitative only, high-cost test, can be false positives or negatives due to contamination and/or presence of inhibitory substances. Sensitivity is considered as high, if not higher than bacterial culture.	High
Enzyme linked immunosorbent assay (ELISA)	Blood	Sensitivity increases as stage of disease progresses.	4.70
Enzyme linked immunosorbent assay (ELISA)	Milk	Level of MAP antibodies in milk dependent on levels in blood, so lower usually lower cut-off point.	2.30
Agar gel immunodiffusion (AGID)	Blood	Low cost, rapid test with good sensitivity in clinical stages, low sensitivity in early stages.	Low
Complement fixation test (CFT)	Blood	Has been widely used to identify positive animals in high prevalence herds, but not sensitive enough for use in low prevalence herds	High
Interferon gamma (IFN- γ)	Blood	Can be used in younger animals in early stage of disease. Sensitivity moderate, specificity moderate	High

Testing Summary

The advice from Animal Health N.I. for a herd of unknown MAP status is to test the entire herd with either blood or milk ELISA, to establish prevalence. Several pooled faecal samples from cows in similar parity, tested for bacterial culture and/or PCR will give some indication of the prevalence in a herd, but is a poor substitute for whole herd individual animal testing. Tests should be repeated in 3-6 months to aid confirmation of results. For non-milk recorded herds, a blood sample can be taken from each cow at the annual TB test and screened for presence of MAP. Animals that test positive by this method should be further tested before being considered for culling, (repeat blood test, milk sample, PCR, or faecal culture). For herds that milk record, a quarterly individual cow test for Johne's can be undertaken to build up a picture of herd prevalence and infection status of individual cows. Cows should be at least 2 months post TB test, as the TB test can interfere with the MAP test results, (false positives). Cows should not necessarily be culled on the basis of one positive result. However, this depends on the history of infection in the herd and the magnitude of the test result. Repeat testing and use of metrics such as likelihood ratios, where age/parity are considered in relation to current test result and previous

test history are useful in deciding what animals to earmark for culling. Animals that are showing clinical signs of the disease should be culled immediately, as they are likely to be shedding large numbers of MAP into their environment and will not improve over time. The interpretation of test results and the actions to take should always be done in conjunction with your veterinary practitioner.

Calves

Offspring from a MAP positive dam are more likely to test positive compared to those from a non-positive MAP dam and offspring are more likely to test positive, even if the dam was not test positive at time of calf birth but tested positive later. Calves born to dams that were MAP positive or confirmed positive within one year of birth, were 3.6 times more likely to go positive themselves and even if the dam's positive test result was beyond a year later, the odds of the offspring testing positive was 2.8 times that of calves born to ELISA negative dams. A review of research studies into possible infection in utero found that on average, 9% of foetuses were infected where the dam was sub-clinical and 39% of foetuses were infected when the dam status was clinical.

Another review of research studies found that calves less than 6 months old had a 74% chance of becoming infected on exposure to MAP, decreasing to 50% between 6 and 12 months old and to 19.3% in animals older than 12 months. There was a strong correlation between MAP disease status of the dam and shedding of MAP into colostrum and milk, (which may subsequently be consumed by the calf).

There is good evidence that calves infected either in utero or via colostrum can spread Johne's to other calves in their cohort and this possible route of transmission should not be overlooked, when considering control measures. Hence rearing baby calves in individual pens, (within sight and sound of other calves), or limiting the size of groups should be considered as part of a herd health plan, which also incorporates Johne's disease control strategies.

Research findings from milk sampling

Milk samples from 281,558 cows monthly milk recorded in the UK, were analysed on a quarterly basis for MAP by ELISA. 82.9% of these cows never had a positive result and were considered negative for the disease. 10.7% of cows only had one positive test result and were also considered negative. 3% of the cows had 2 positive tests, but nearly half of these were not consecutive and were considered not infected. 3% of cows had 3-7 positive tests, with any cow having 2 consecutive positive tests considered as positive. 0.4 % had 8 or more positive tests and were considered infected, even though the last 2 positive results may not have been consecutive. The authors concluded that culling should be based on 2 or more positive results in the last 4 tests, or if the last 3 tests were positive. However, this should be tempered by the history of the disease on the farm and whether there is evidence of disease spread from dam to daughter and/or from calf to calf.

Impact of Johne's disease on cow performance

A UK study used data from milk recorded herds that had quarterly testing by ELISA for MAP antibodies. Animals were classified as; low risk, (all tests negative or one positive, but not last test), medium risk, (last test positive; at least 2 tests positive but not consecutive tests), high risk, (at least 2 consecutive tests positive, even if a negative test followed). Based on this classification the authors found that high risk cows produced 904 kg milk less than low risk cows over 3 lactations. High risk cows also had significantly higher SCC's and mastitis risk compared to low-risk cows.

A Portuguese study of cows over 5 lactations found that MAP positive cows, (serum ELISA), produced 1,285 litres less milk than their non-infected herd mates and also had higher SCC's.

A US study found that faecal positive cows produced 1,355 litres less milk in a lactation, compared to those that were faecal negative for MAP and were at a much greater odds of being culled during the lactation.

Vaccination

Vaccines are available and have been used in many countries as a control measure. They are effective in limiting signs of the disease and are considered cost effective in this circumstance. However, vaccines do not prevent disease establishment. More generally, vaccination will lead animals testing positive by ELISA and so it becomes impossible to distinguish vaccinated from infected animals and so is not a viable option for a herd owner wanting to eradicate the disease from their herd/flock. Another big drawback with the MAP vaccines is that they can interfere with TB test results and therefore vaccination is not permitted in many countries that have a bovine TB testing programme.

Disease prevention

Maintain a closed herd and/or only import animals known to be free from the disease. (Animal movements onto farm are the main source of disease introduction). However, as already outlined it is almost impossible to be sure that bought in animals are not carriers for the disease, particularly if purchased when young.

Disease control/eradication

- a. If high risk animals are in-calf, (as determined by testing), they should be calved in a separate isolated calving pen, with the calf snatched immediately post calving and fed colostrum from a known MAP negative cow. (Ideally all calves should be snatched at calving to minimise faecal ingestion).
- b. Replacement offspring of cows testing positive should be put on a repeat testing regime, as they are more likely to be MAP positive, or to become MAP positive with time.
- c. Pooling of colostrum should not be practiced, as it has the potential to infect many calves from one infected dam
- d. Do not import colostrum from other farms, (should not be necessary), or fodder, unless absolutely necessary, as it could be contaminated with MAP from manure spread on silage fields.
- e. Do not feed waste milk to calves and definitely not to replacement heifers. (They should be reared on their own mothers' milk only initially and then on powdered milk). Pasteurisation of colostrum and milk does kill MAP but may not eliminate it completely if the contamination load is high. Pasteurisation is a helpful tool to further reduce risk in low-risk milk, but it should not be relied upon for high-risk milk, (from test positive animals).
- f. Do not breed replacement heifers from high-risk animals, (identified using one or more of the tests mentioned above). Ideally, high risk animals should be culled as soon as they are identified. (They may be what is called super shedders because of the larger number of MAP organisms excreted in their faeces).
- g. Test animals at least yearly and preferably 3 monthly for the presence of antibodies to MAP (serum or milk). Classify animals into risk categories, particularly for the high-risk category;

animals with 3 consecutive positive tests, or 2 out of the last 4 tests. Consider further testing these animals, if they have already had a positive milk test for MAP and/or faecal sampling for organism growth/PCR analysis.

h. Culling decisions based on only one positive result has serious financial implications, compared to results based on repeated measurements. To achieve a reduction in the prevalence of MAP in a herd, it is commonly recommended to cull animals that repeatedly test positive, (by whatever test), as they are more likely to be infected and potentially excreting MAP into their environment.

i. Waterways should be fenced off so that animals do not have access to them, as water can be infected with MAP via slurry runoff from neighbouring farms.

j. Slurry should not be imported from other farms due to the bio-security risk of importing MAP in the slurry, (although dairy farmers are more likely to be exporting slurry to beef/sheep farms, rather than importing it).

k. Slurry/manure particularly from cows, should not be spread on fields where youngstock graze.

l. Cattle trailers and manure spreading equipment should not be shared between farms.

References

A longitudinal study of factors influencing the result of a *Mycobacterium avium* ssp. paratuberculosis antibody ELISA in milk of dairy cows. S. W. F. Eisenburg, E Veldman, V. P. M. G. Rutten, A. P. Koets. *J. Dairy Sci.* 98 :2345–2355

A Review; Zoonotic potential of *Mycobacterium avium* ssp. paratuberculosis: the current position. I.R. Grant, *Journal of Applied Microbiology* 2005, 98, 1282–1293

Association between dam status and offspring *Mycobacterium avium* ssp paratuberculosis infection in a long-term longitudinal study. S.Patterson, K.Bond, M.Green, S.Vanwinden and, J.Guitian, Proceedings of a meeting held in Tallinn, Estonia 21st – 23rd March 2018

Association of paratuberculosis sero-status with milk production and somatic cell counts across 5 lactations, using multilevel mixed models, in dairy cows. E. G. Martins, P. Oliveira, B. M. Oliveira, D. Mendonça, and J. Niza-Ribeiro. *J. Dairy Sci.* 101:7638–7649

Clinical disease and stage of lactation influence shedding of *Mycobacterium avium* subspecies paratuberculosis into milk and colostrum of naturally infected dairy cows. J. R. Stabel, L. Bradner, S. Robbe-Austerman, D.C. Beitz. *J. Dairy Sci.* 97 :6296–6304

Control of paratuberculosis: who, why and how. A review of 48 countries. *BMC Veterinary Research* (2019) 15:198

Diagnostic performance of the Pourquier ELISA for detection of antibodies against *Mycobacterium avium* subspecies paratuberculosis in individual milk and bulk milk samples of dairy herds. H van Weering, G van Schaik, A van der Meulen, M. Waal, P. Franken, K. van Maanen. *Veterinary Microbiology* 125 (2007) 49–58

Dynamics of Specific Anti-*Mycobacterium avium* Subsp.paratuberculosis Antibody Response through Age. Søren Saxmose Nielsen, Nils Toft, Hisako Okura. *Plos one* April 2013 Vol 8 Issue 4

ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. R.H. Whitlocka, S.J. Wells, R.W. Sweeney, J. Van Tiem. *Veterinary Microbiology* 77 (2000) 387-398

Epidemiology of John's Disease, a Review. Ruhdi Keci, Xhelil Koleci, *Albanian J. Agric. Sci.* 2014;13(3):1-8

Faecal shedding and tissue infections demonstrate transmission of *Mycobacterium avium* subsp. paratuberculosis in group housed dairy calves. Caroline S. Corbett, Jeroen De Buck, Karin Orsel and Herman W. Barkema. *Vet Res* (2017) 48:27

Faecal shedding and tissue infections demonstrate transmission of *Mycobacterium avium* subsp. paratuberculosis in group housed dairy calves. Caroline S. Corbett, Jeroen De Buck, Karin Orsel and Herman W. Barkema. *Vet Res* (2017) 48:27

In utero infection of cattle with subsp. *Mycobacterium avium* paratuberculosis: A critical review and meta-analysis. Richard J. Whittington, Peter A. Windsor. *The Veterinary Journal* 179 (2009) 60–69

Johne's disease in dairy herds 1. Understanding the disease. Pete Orpin, Dick Sibley, Karen Bond. *In Practice* Jan 2020

Longitudinal relationship between faecal culture, faecal quantitative PCR and milk ELISA in *Mycobacterium avium* ssp. paratuberculosis-infected cows from low-prevalence dairy herds. A. Beaver, R. W. Sweeney, E. Hovingh, D. R. Wolfgang, Y. T. Gröhn, and Y. H. Schukken. *J. Dairy Sci.* 100:7507–7521

Longitudinal study of ELISA seroreactivity to *Mycobacterium avium* subsp. paratuberculosis in infected cattle and culture-negative herd mates Raymond W. Sweeney, Robert H. Whitlock, Susan McAdams, Terry Fyock. *J Vet Diagn Invest* 18:2–6 (2006)

Loss of income from cows shedding *Mycobacterium avium* subspecies paratuberculosis prior to calving compared with cows not shedding the organism on two Minnesota dairy farms. E. A. Raizman, J.P. Fetrow, S. J. Wells. *J. Dairy Sci.* 92: 4929–4936

Mycobacterium avium paratuberculosis infection of calves – The impact of dam infection status. S. Patterson, K. Bonda, M. Green, S. van Winden, J. Guitian. *Preventive Veterinary Medicine* 2019

Pasteurization of milk and the heat resistance of *Mycobacterium avium* subsp. paratuberculosis: a critical review of the data. Barbara M. Lunda, Grahame W. Gould, Anita M. Rampling. *International Journal of Food Microbiology* 77 (2002) 135– 145

Paratuberculosis control: a review with a focus on vaccination. Felix Bastida and Ramon A Juste. *Journal of Immune Based Therapies and Vaccines* 2011, 9:8

Review Article Epidemiological evidence for *Mycobacterium avium* subspecies paratuberculosis as a cause of Crohn's disease. J. C. UZOIGWE, M. L. KHAITSA and P. S. GIBBS. *Epidemiol. Infect.* (2007), 135, 1057–1068

Phenotypic effects of subclinical paratuberculosis (Johne's disease) in dairy cattle. *J. Dairy Sci.* 100:679–690