Mycobacterium avium subspecies paratuberculosis – DRAFT risk assessment related to exposure via food

Statement of purpose

- To assess the risk to consumers from *Mycobacterium avium* subspecies *paratuberculosis* (MAP) via food.

Hazard Identification

1. MAP is a multi-host pathogen and has been isolated from a variety of wild and domesticated animals (Verma, 2013). MAP commonly infects dairy cattle, leading to Johne's disease, which is also known as paratuberculosis. The infection is chronic, progressive, and incurable (Collins, 1997). Paratuberculosis affects domesticated and wild ruminants including those used in food production mainly cattle, but also sheep and goats. There are few published cases of MAP infection in pigs but the first such report was by Miranda *et al*., (2014) where MAP infection occurred in four domestic pigs. Although successful experimental infection with MAP in poultry has been reported, there have been no cases to date where MAP has been associated with avian tuberculosis (Dhama *et al*., 2011).

2. Some strains of MAP preferentially infect specific hosts and two main types have been reported. Type II primarily infect cattle (C type strains) and Type I primarily infect sheep (S type strains) and they have been distinguished by restriction fragment length polymorphisms. C and S types are named after the host species from which they were originally isolated (The Centre for Food Security and Public Health, 2007).

3. Literature acknowledges that C strains are reported to have a broad host range, including cattle, goats and camelids and both ruminant and non-ruminant wildlife. The S strains have been reported to manly infect sheep and other small ruminants and red deer. Although uncommon, cross-species transmission can occur between sheep and cattle ((The Centre for Food Security and Public Health, 2007).

4. MAP has been hypothesised to be an infectious cause of Crohn's disease in humans However, due to key knowledge gaps, the potential public health impact of MAP remains unclear (Waddell *et al.,* 2015). Debate also continues about the nature of the role of MAP in Crohn's disease; as a causative agent or whether underlying disease has allowed MAP to secondarily infect the bowel and invade the bloodstream. In addition to Crohn's disease, it has been suggested that MAP could be associated with a number of other diseases, though this is also controversial and will be discussed in more detail during the assessment (Grant, 2015).

5. Infected cattle appear to be the most important source of human exposure to MAP and the associated food vehicles are suspected to be milk, dairy products and beef (Mihajlovic *et al*., 2011).

6. Transmission of MAP to humans via food, most likely would occur via (i) consumption of milk from herds that include infected animals and/or products prepared from such milk, (ii) consumption of meat and organ tissues from infected animals or animals contaminated by faeces of infected animals, and (iii) drinking water contaminated with MAP from faeces of infected animals implying that environmental exposure to MAP from food animals could also be important. (Gill *et al*., 2011).

Exposure assessment

Transmission in animals

7. Distribution of MAP infection in animals is worldwide. Prevalence data reported in literature are variable **(Uncertainty).** The highest published prevalence is in dairy cattle, with 20%–80% of herds infected in many of the major dairy-producing countries. Limited information is available about the prevalence in other food animal species **(Uncertainty).** (Merck veterinary manual 2013).

8. In ruminants, MAP transmission occurs mainly via the faecal oral route, although the infectious dose in animals is unknown. Introduction of the disease into a non-infected herd is usually through herd expansion or replacement purchases; the infection is introduced via subclinically infected carriers (Merck veterinary manual 2013). Infected animals are capable of shedding large numbers of MAP in faeces, even before the onset of clinical symptoms. Asymptomatic carriers may also shed MAP intermittently. MAP has also been isolated from colostrum, milk, udder and male and female reproductive tracts. Transmission can also occur on fomites, and insects may act as mechanical vectors (The centre for food security and public health, 2007).

9. Young animals are most susceptible to infection but clinical signs rarely develop in cattle less than 2 years old, because progression to clinical disease occurs slowly. Resistance to infection increases with age, and cattle exposed as adults are much less likely to become infected (Merck veterinary manual, 2013). It is reported that animals usually become infected when nursing from an udder soiled with faeces or when housed in contaminated pens, but can also become infected by drinking contaminated milk or colostrum, solid feed, or contaminated water, or licking and grooming behaviour in a contaminated environment (Merck veterinary manual, 2013). The Centre for Food Security and Public Health carried out a review of paratuberculosis in animals in 2007, and reported that in one study, MAP was shed in milk by 3-19% of asymptomatic cows and for colostrum, this figure was 9-36%. The study also reports that up to 35% of symptomatic cows shed MAP in milk. *In utero* transmission of MAP

has also been reported in this review; the estimated risk of foetal transmission from culture positive cows was 26% but this figure may be much lower in asymptomatic cows.

10. Paragraph 5 discussed host specificity of MAP strains and highlights that cross-species transmission of C and S strains can occur but is relatively uncommon, highlighting that the greatest risk of infection for food-producing animals such as cattle is from other cattle and for sheep from other sheep.

11. A review carried out by Rhodes *et al.* (2014), highlighted that subclinical MAP infection is widespread in domestic livestock, especially cattle, sheep and goats, with Europe and North America being particularly affected. It was estimated that the herd prevalence for Johne's disease in cattle in the USA is 68% and 32% in U.K. (data originate from 2009). Data on MAP prevalence in sheep and goats are limited but prevalences of up to 20% in European herds have been suggested (Gill *et al.,* 2011). More recently, the Advisory Committee on Dangerous Pathogens (ACDP) was presented with a paper prepared by Dr Irene Grant relating to MAP and Crohn's disease at its meeting in February 2015 https://app.box.com/s/uolyuc1tmjarix61b93xtsd9z731k549#/s/uolyuc1tmjarix61b93xtsd9z731k549/1/3219178817/27066245741/1?&_suid=1476202608830097952574484283 41. This paper acknowledged that herd prevalence of Johne's disease caused by MAP in dairy cattle has reached extremely high levels (>90% of dairy herds in the USA are infected and 40-50% of dairy herds in the UK).

Transmission to humans via food

Milk and dairy products

12. Since the prevalence of paratuberculosis in dairy ruminants is high worldwide, the detection of MAP DNA in raw milk is not surprising, and this has been demonstrated in many studies. However, viable MAP has also been detected in surveys of retail pasteurised milk (Botsaris *et al.,* 2015).

13. MAP can infect lymph nodes associated with the mammary gland and be shed in colostrum and milk from asymptomatic animals and those with clinical signs. Shedding of MAP in milk has been reported to be intermittent as has faecal shedding. Additionally, during milking of both infected and uninfected animals in herds that have some infected animals, milk may sometimes become contaminated with MAP-containing faecal matter (Gill *et al*., 2011).

14. Prevalence of MAP infection in cattle, the rate of shedding in infected animals, the level of contamination of milk and dilution effects, the efficiency of pasteurisation, the organism survival rate in milk and the consumption rate are all factors to consider in assessing exposure (Mihajlovic *et al*., 2011).

15. Mihajilovic *et al*., (2011) drew attention to a modelling approach for a farm with a high prevalence of Johne's disease, it was estimated that the concentration of MAP was $5.4x10^{-3}$ cfu/ml of pasteurised milk. Theoretically, the greatest contribution to contamination originated from clinically affected animals with the contribution from sub-clinically affected cows appearing to be minimal. The authors provided the reasoning that removing clinically affected cattle from the production chain would reduce the point estimate exposure by approx. 99% from $5.4x10^{-3}$cfu/ml to $0.06x 10^{-3}$ cfu/ml, but cautioned that the approach used in the example was based on limited data and a rough estimation. Other authors report $4x10^{-2}$ cfu/ml to $16x10^{-2}$cfu/ml of MAP in milk from asymptomatic cows. However, the authors acknowledged that published MAP concentrations in raw milk were sparse and that pasteurisation studies varied greatly in terms of methodology making comparison difficult. Milk consumption rates in the UK were estimated in a Defra report in 2004 to be 112.3 kg/capita.

16. Over a 17-month period (March 1999 to July 2000), a total of 814 cows' milk samples, 244 bulk raw and 567 commercially pasteurized (228 whole, 179 semi-skimmed , and 160 skimmed), from 241 approved dairy processing establishments throughout the UK were tested for the presence of MAP by immunomagnetic-PCR and culture (Grant *et al.,* 2002). The authors reported that DNA was detected by PCR in 19 (7.8%; 95% confidence interval, 4.3 to 10.8%) and 67 (11.8%; 95% confidence interval, 9.0 to 14.2%) of the raw and pasteurised milk samples, respectively. Confirmed MAP isolates were cultured from 4 (1.6%; 95% confidence interval, 0.04 to 3.1%) and 10 (1.8%; 95% confidence interval, 0.7 to 2.8%) of the raw and pasteurized milk samples, respectively, following chemical decontamination with 0.75% (wt/vol) cetylpyridinium chloride for 5h. The 10 culture-positive pasteurised milk samples were from only 8 (3.3%) of the 241 dairy processing establishments that participated in the survey. Seven of the culture-positive pasteurised milk samples had been heat-treated at 72 to 74°C for 15s; the remainder had been treated at 72 to 75°C for the extended holding time of 25s. MAP counts in the original milk samples were estimated to be about 4 to 20 cfu/50 ml of milk. The authors concluded that viable MAP is occasionally present at low levels in commercially pasteurised cows' milk in the UK.

17. Gill *et al.* 2011, summarised various global surveys relating to the presence of MAP in raw and pasteurised cows' and goat milk and pasteurised cows' milk. With the exception of Switzerland and Italy where higher numbers of MAP were detected in goats' or sheep milk from individual animals and bulk tanks, prevalence of MAP in raw goats' and sheep milk was lower than for cows' milk (in the UK, for goats' milk, 0% of positive milk samples from bulk containers were detected by culture and 1% by PCR; MAP was not detected in any of the 14 bulk milk containers of sheep' milk). Surveys from different parts of the world have reported that MAP can be cultured from approximately 2% of samples of retail pasteurised milk samples (Mihajovic *et al.,* 2011).

18. Interpretation of published information on the presence and levels of MAP in raw milk by the use of traditional culture could be misleading (Grant, 2015). It has previously been presumed that after milk from multiple animals in infected herds is mixed in the bulk tank at farm level, and again at the processing plant, MAP numbers will be reduced as a result of dilution; therefore MAP is unlikely to survive pasteurisation and further processing. Grant 2015 outlines that improved detection methods such as real-time PCR allow quantitation of MAP DNA, and phage amplification-based methods are capable of rapidly detecting and enumerating viable MAP in milk and dairy products. In 2014, it was reported that 28% of approx. 30 retail pasteurised milk samples collected around Nottingham UK were phage assay positive. However, only two samples showed MAP counts >10PFU/50 ml (Grant, 2015).

19. Botsaris *et al.,* (2015) demonstrated that viable MAP could be detected in powdered infant formula (PIF). The paper describes the results of a small survey which showed that a phage-PCR assay detected viable MAP in 13% (4/32) of PIF samples. Culture detected viable MAP in 9% (3/32) PIF samples, all of which were also phage-PCR positive. Direct PCR detected MAP DNA in 22% (7/32) of PIF samples. The presence of viable MAP in PIF indicates that MAP either survived PIF manufacturing or that post-production contamination occurred.

Cheese

20. Gill *et al.*, (2011) summarised the results of seven surveys examining the presence of MAP in dairy products other than milk (largely curd and cheeses). The results of one survey showed that 25% of artisan cheese samples manufactured in Scotland were culture positive for MAP. Cheeses from Greece and Cyprus also showed a relatively large percentage of MAP positive samples via PCR; 50 and 25%, respectively (noting this is not a measurement of viable MAP).

21. Raw and pasteurised milk is used in the manufacture of cheese. As MAP may be present in milk, and is relatively resistant to salt and acid conditions, the extent of survival of MAP during cheese maturation has been studied (Collins 2001).

22. Waddell *et al.*, (2016) carried out a scoping review to investigate sources of human exposure to MAP. Part of the review examined prevalence surveys and cross-sectional studies and used these data to conduct meta-analysis to generate prevalence values. Data from the individual studies used in the meta-analysis are interesting. From 2005–2010, studies examined MAP prevalence by culture for a variety of pasteurised milk cheeses from soft to hard, and report varying levels: Czech Republic (0–4.3%), USA (1%), Scotland (67%). Using PCR, studies examined MAP prevalence in pasteurised milk cheeses between 2005 and 2006 and varying levels of prevalence were observed: Czech Republic (3–20%), Greece (50%) and USA (5%).

23. Waddell *et al.,* (2016) in the same scoping review reported MAP prevalence studies on unpasteurised cheese. While the authors carried out meta-analysis to determine prevalence using data from a number of different surveys, the paper provides some information on data from specific studies which were used in the meta-analysis. Between 2007 and 2010, MAP prevalence in a variety of cheeses using culture as a detection method was as follows: Switzerland (0%), Scotland (0–36%), Cyprus (0%).

24. A review of MAP was also carried out by the Food Safety Authority, Ireland (FSAI) in 2009. The Irish MAP review though older than Waddell *et al.,* (2016) also details some prevalence studies in more specific terms than the more recent review. Though some of the information in both reviews is duplicated, it is useful to consider. The Irish review states that viable MAP was found in 4.7% (2/42) of samples of five brands of feta cheese (made from a mixture of sheep and goats' milk) available on the Greek market, although using PCR, MAP DNA was found in 50% (21/42) of the same samples. In the same survey, cows' milk cheese available for sale in the Czech Republic was studied. In this survey, viable MAP was detected in 4.3% (1/23) of samples of a hard cheese but not detected in five samples of a semi-hard cheese or 14 samples of a soft cheese. In contrast, MAP DNA was detected by PCR in 17.4% (4/23) of the same samples of hard cheese, 20% (1/5) of the same samples of semi-hard cheese but was not found in any of 14 samples of soft cheese examined. The Irish review also draws attention to a publication which showed a significant association (P=0.0018) between MAP infection in humans and the consumption of Artisan cheese directly from farms in Sardinia (FSAI, 2009).

25. The review carried out by FSAI in 2009 also drew attention to MAP being detected after 30 days of ripening in soft Hispanic-style cheese made from milk to which MAP had been added. Viable MAP was also found after 120 days maturation in semi-hard and hard cheese made from raw milk to which MAP had been added. In a study by Donaghy *et al*., (2004), viable MAP was detected at the end of a 27 week maturation period in cheddar cheese prepared from pasteurised milk to which MAP had been added prior to ripening. This study suggested that because mild cheddar is usually ripened for up to 16 weeks, a higher margin of safety (with respect to MAP) may be provided by medium or mature cheddar as they are ripened over a longer time period (FSAI, 2009).

Raw beef (meat)

26. Eltholth *et al*., (2009), reported that various studies indicate that beef can be contaminated with MAP via dissemination of the pathogen in the tissues of infected animals or by contamination of the carcass with contaminated faeces.

27. In cattle, MAP infection is disseminated in the later stages and has been detected in blood and a variety of internal organs, including lymph nodes that are distant from the gastrointestinal tract, liver, spleen, lung, kidney, uterus, mammary gland and epididymis (Collins, 1997). MAP has also

been isolated from short ribs, shin shank, tenderloin and lymph nodes associated with muscle (Mihajlovic *et al.,* 2011).

28. Reports concerning MAP detection in retail meat are conflicting. In a survey of 133 minced beef samples obtained from a meat processing plant in the Republic of Ireland, no viable MAP was detected (FSAI, 2009). Jaravata *et al.* (2007) reported on a survey of 200 retail ground beef samples which were analysed in the USA; MAP was not detected using PCR and culture methods.

29. However, Waddell *et al.,* (2016) provided meta-analysis data from a number of reviews (non-UK) showing that the prevalence of MAP on meat (mainly beef but also mutton) was similar to that of commercial dairy products (3.3% overall prevalence using culture to detect MAP; the reviews were carried out in Spain, USA, Denmark and Australia). PCR-based detection of raw meats in Canada, Denmark and Czech Republic did reveal a higher prevalence (25.5% overall prevalence), though does not provide an indication of viable MAP present.

30. Several studies reported an increased likelihood of MAP detection on meat, in animals clinically suspected of Johne's disease and/or MAP-positive by ELISA, PCR or culture, even though the pathogen load in muscle was low (Waddell *et al*., 2016). Waddell *et al*., (2016) drew attention to one study, which demonstrated that 80% of beef burger samples containing mesenteric lymph nodes from a cow diagnosed with clinical Johne's disease were MAP positive.

31. Savi *et al.,* (2015), confirmed that few studies on MAP in ground beef are currently available. During the period November 2013–March 2014 these authors carried out a MAP survey of ground beef produced in an industrial meat processing plant in Italy. One-hundred and forty samples of ground meat were analysed by PCR and culture. The limit of detection of qPCR was 630 MAP cells/g (107 CFU/g) while the limit of detection for culture was 170–230 MAP cells/g (62–115 CFU/g). No samples were positive by PCR, while two samples were positive by liquid culture. The authors concluded that data suggest that the presence of viable MAP in raw minced meat is possible and stated that thorough cooking is recommended to avoid MAP exposure for humans through the consumption of contaminated meat.

32. A 2005 Defra-funded case-control study investigating drinking water and dairy products in the aetiology of Crohn's disease did not provide any evidence of an increased risk of Crohn's disease in association with exposure to drinking water and milk but meat intake (beef, canned meat) was associated with an increased risk of developing Crohn's disease (Defra, 2005).

Drinking water

33. Given that there is evidence suggesting that MAP is able to survive chlorine disinfection, there is a possibility of human exposure to MAP via water consumption (Mihajlovic *et al*., 2011).

34. In 2005, a Defra funded study investigated the role of drinking water potentially contaminated with MAP in the aetiology of Crohn's disease (Defra, 2005). The findings of this study did not support a role for drinking water in the transmission of MAP from animals to humans in the aetiology of Crohn's disease.

35. Research carried out by the Health Protection Agency in 2002 concluded that there is no conclusive evidence for the presence of MAP in drinking water in the UK (HPA, 2002).

36. A review by FSAI in 2009, drew attention to a survey carried out by Hunter *et al*., (2001) of treated and untreated water in the UK where MAP was not detected in any samples, but other *Mycobacterium* spp. were detected in 11% (19/170) of samples.

37. In Northern Ireland, MAP was detected by culture and/or PCR in 7.8% (15/192) of one litre samples of water entering a water treatment plant (FSAI, 2009). Treated water was not tested, so it was not possible to determine the effects of water treatment. The FSAI review also noted that, previously, investigations in the same laboratory demonstrated that MAP was not destroyed by chlorine at levels as high as 2.0 µg/ml with a contact time of 30 minutes. However, it was noted that MAP had been added to the water used at initial concentrations higher than would be expected in the natural environment (106 cfu/ml) in order to ensure that the number of surviving cells after chlorination were above the sensitivity of the detection method used (FSAI, 2009).

38. Waddell *et al.,* 2016 acknowledged a number of MAP and drinking water prevalence surveys, including one which showed 2% prevalence in South Wales via PCR detection. One study from the USA reported a high prevalence (76-88%) with PCR detection. The authors carried out meta-analysis to investigate prevalence using 10 studies/surveys of MAP and drinking water using either culture or PCR detection methods. From these surveys, prevalence via meta-analysis was determined to be 2.3% (culture) and 35.7% (PCR).

39. Other dairy products, fresh produce and shellfish were not included in the assessment due to the lack of information available associated with MAP.

Ability to survive pasteurisation in milk

40. There is conflicting evidence regarding the ability of MAP to survive in milk during pasteurisation. The heat resistance of MAP in milk at pasteurisation

temperatures has been investigated (ACM/486). Pasteurisation, introduced into the UK around 1920, was designed to kill the most heat resistant, non-spore forming bacteria likely to be present in milk (*Mycobacterium tuberculosis* and *Coxiella burnetti*). Destruction of these bacteria is achieved by either of the following time/temperature regimes: 63°C for 30 mins (holder pasteurisation) or 72°C for 15 secs (High temperature short time (HTST) pasteurisation).

41. The thermal inactivation curve for MAP heated in milk at holder pasteurisation temperature (63°C) was found to be non-linear and demonstrated tailing. From 0-10 min, rapid cell inactivation occurred but in the later stages of heating, low numbers of MAP survived (10-30 mins). *M. bovis*, in contrast exhibited linear thermal inactivation kinetics when heated in milk at 63°C (ACM/486).

42. The tailing phenomenon described above has been documented to be attributed to the natural tendency of MAP to aggregate into clumps due to the hydrophobic nature of the cell wall thereby encouraging heat resistance. Viability staining showed that viable MAP cells were only observed within clumps of predominantly heat-killed cells at heating times which corresponded to the tail region. Additionally, clumped MAP cells have been shown to be twice as heat resistant as single MAP cells (ACM/486).

43. A longer holding period at 72°C has been proven to be more effective in inactivating MAP than a higher pasteurisation temperature. In work carried out with milk, artificially spiked with three strains of MAP, only one strain survived following a treatment of 20 secs and none of the strains survived at the 25 secs treatment. Results from this work showed that MAP may survive HTST pasteurisation (72°C for 15 secs) if present in milk at levels of 100 cfu/ml or greater (ACM/468).

44. Surveys of commercially pasteurised cow's milk have demonstrated that low levels of viable MAP are sometimes detected. During a large-scale UK survey, viable MAP was cultured from 1.8% (10/567) of samples (Grant *et al.*, 2002). All ten MAP positive samples were pasteurised using conditions that met or exceeded EU minimum requirements; seven samples had been pasteurised at 72-74°C for 15 secs and three had been pasteurised at 72-75°C for 25 secs.

Ability to survive in cheese

45. The effect of pH, salt and heat on MAP viability in soft, white, Hispanic cheese was investigated. Salt appeared to have little or no effect on MAP deactivation rates, though a decreasing D value was associated with decreasing pH. Heat-treated MAP cells were inactivated faster than non-heat treated cells (FSAI, 2009).

46. Viable MAP was also detected in cheddar cheese prepared from pasteurised milk to which MAP had been added at the end of a 27-week

maturation process. This study suggested that because mild cheddar is usually ripened for up to 16 weeks, there may be a higher margin of safety (with respect to MAP) for medium or mature cheddar as they are ripened over a longer time period (FSAI, 2009).

Ability to survive in meat

47. Mihajlovic *et al.,* (2011) drew attention to a study which concluded that small numbers of MAP cells may survive meat cooked to a medium-rare condition (63°C) but there is a low probability of MAP survival for well-done meat (75°C).

Ability to survive in drinking water

48. Mihajilovic *et al*., (2011) highlight that the efficacy of water treatment facilities in removing or inactivating MAP in water destined for human consumption have not been thoroughly investigated. A number of Mycobacteria have been shown to be resistant to chlorine or chloramine concentrations used in water treatment. The contact time values for the effect of chlorine on MAP have been estimated to be up to 580-2300 greater than those for *E. coli*. In one study, complete inactivation of MAP by chlorine did not occur at the equivalent concentration of 2ppm or 2 mg/l for 30 mins contact time with initial inoculum levels of 10^6 cfu/ml. (Mihajilovic *et al*., 2011).

49. The World Health Organisation has set a health based guideline maximum value of 5 mg/l for chlorine as a residual disinfectant in drinking water. The levels in tap water in England and Wales are well below this guideline and most water companies aim to keep the level below 1 mg/l (Defra).

Hazard Characterisation

Microbiology

50. MAP is a Gram positive, acid-fast, aerobic, rod shaped (1-2mm) bacterium, previously known as *Mycobacterium paratuberculosis* and *Mycobacterium johnei* and a member of the *M. avium* complex of the Mycobacteriaceae family. MAP is unable to synthesise the iron chelating siderophore mycobactin, therefore, MAP cultivation media must be supplemented accordingly (Verma, 2013).

51. Most mycobacteria grow slowly, with MAP being the slowest growing member of this genus. MAP-inoculated media are incubated at 37°C for at least 6 weeks (incubation period of 12-16 weeks is most common, (Gill *et al*., 2011).

52. In common with other mycobacteria, MAP possesses a thick waxy cell wall responsible for acid fastness, hydrophobicity and resistance to chemicals and tolerance to pasteurisation (Verma, 2013).

Possible association with human diseases and infectious dose

53. There is much controversy relating to the possibility that MAP is a causative agent of Crohn's disease in humans. There have been reports of detection of MAP in the blood and tissues of patients with Crohn's disease or irritable bowel syndrome more frequently than in control patients. In other studies, MAP was not detected in patients with Crohn's disease (FSAI, 2009). However, in most studies, MAP was more often detected in Crohn's disease patients than control subjects (Grant *et al.,* 2015).

54. MAP has also been potentially associated with a number of other chronic diseases (irritable bowel syndrome, Type 1 diabetes melitus, multiple sclerosis, Parkinson's disease, autism and other autoimmune conditions (Grant, 2015). Grant (2015) summarised that genetic susceptibility would appear to play a role in MAP-associated diseases and increasing evidence in the literature indicates that susceptibility genes associated with the inability to handle usual gut bacteria effectively or diminished ability to eradicate intracellular infections or dysfunctional macrophages appear common in affected individuals (Grant, 2015). MAP therefore may be only associated with some, not all Crohn's disease cases and certain genetic susceptibility genes are often demonstrated in MAP positive individuals (Grant, 2015).

55. Given the controversy relating to the role of MAP and various human diseases, it is difficult to establish what (if any) an infectious dose via ingestion may be for humans **(Uncertainty).** Whilst an infectious dose for MAP could not be established there is some limited evidence to suggest that lower doses of MAP could be associated with ulcerative colitis and higher doses with Crohn's disease (Pierce, 2010).

56. The public health consequences, if any, of low numbers of viable MAP being periodically consumed by susceptible individuals are uncertain and the prevalence of MAP in various foods appears to be highly variable and dependent on prevalence within animal herds, often remaining undetected **(Uncertainty).**

57. Waddell *et al*., (2016) reported on nine case-control studies examining risk factors for Crohn's disease which highlighted significant and positive associations with the consumption of processed meats and cheese, while direct contact with ruminants, high risk occupations such as farming, or working in veterinary practice, milk consumption and water source were factors not associated with the disease and/or MAP exposure status.

58. The prevalence of Crohn's disease in the UK is estimated to be 157 per 100,000 with an annual incidence of 9.56 per 100,000 per year. The onset has two age peaks. The first, larger peak is at 15-30 years of age; the second is at 60-80 (Rana and Dawson, 2015).

Risk characterisation

59. *The following factors have been considered in evaluating the risk of MAP infection via the food chain.*

- There is likely to be a high prevalence of MAP in dairy cattle in the UK (40-50% has been reported), though literature provides variable data on prevalence. Much of this infection is likely to be asymptomatic and therefore undetected. Prevalence data in other animals are sketchier and cattle are regarded as probably the most significant animals to consider. **(Uncertainty).**

- MAP has been detected in milk, including raw milk, pasteurised milk (including from goats) and cheese. In terms of meat, data are sketchy where some surveys have not detected MAP in beef burgers and other researchers have reported MAP in raw meat **(Uncertainty)**. It is likely that thorough cooking of meat products will destroy any MAP present. Information on drinking water shows varying levels of prevalence with one major survey not detecting MAP in UK drinking water at the time of the study, but other surveys (not UK except one in S. Wales) have detected MAP in drinking water. MAP has been reported to be relatively resistant to chlorine.

- A 2005 Defra-funded case-control study investigating drinking water and dairy products in the aetiology of Crohn's disease did not provide any evidence of an increased risk of Crohn's disease in association with exposure to drinking water and milk but meat intake was associated with an increased risk of developing Crohn's disease. Consumption of processed meats and cheese has also been reported to have significant associations in developing Crohn's disease **(Uncertainty).**

- Despite the vast amount of literature covering the subject, a causative link between MAP and Crohn's disease in humans has not been established and this is also the case for other diseases where MAP involvement is suspected **(Uncertainty).** Genetic susceptibility appears to play a role in suspected MAP induced disease though the extent of this remains unclear **(Uncertainty).** As a result, the infectious dose (if any) of MAP via ingestion could not be determined **(Uncertainty),** though it could be lower in genetically susceptible individuals than the general population **(Uncertainty).** Given the uncertainty around the pathogenic capability of MAP, detection in powdered infant formulae might perhaps be a concern **(Uncertainty).**

60. Taking into account all the information listed in the assessment and the absence at present of a causative link between MAP and any human disease, it is not possible to derive a risk estimation of MAP infection via the food chain. There are also many uncertainties that have been flagged in the assessment. More data relating to contamination of foods with MAP

and more robust information relating to the pathogenic capability of MAP would help to reduce uncertainty.

Uncertainties

Key uncertainties associated with this assessment are outlined in Appendix 1.

Overall risk

Given the lack of certainty around whether MAP is a causative agent of Crohn's disease and other diseases in humans and the apparent lack of information relating to possible infectious dose, it was not possible at present to assign a risk level for MAP infection via food. Additionally, a high degree of uncertainty is associated with the assessment.

Appendix 1: Key uncertainties

1. Exposure assessment- Animal prevalence data reported in the literature are variable for cattle and very limited data are available for other animals.

2. Exposure assessment - Prevalence data relating to MAP in various foods appear to be highly variable and dependent on prevalence within animal herds, often remaining undetected.

3. Exposure assessment - It is likely that recent advances in detection methods for MAP highlight that MAP prevalence levels in foods such as milk have been underestimated in the past and consumer exposure may have been higher than previously anticipated.

4. Hazard characterisation - A causative link between MAP and Crohn's disease in humans has not been established and this is also the case for other diseases where MAP involvement is suspected.

5. Hazard characterisation - Genetic susceptibility appears to play a role in suspected MAP induced disease though the extent of this remains unclear.

6. Hazard characterisation - As a result of uncertainty 4 and 5, the infectious dose (if any) of MAP via ingestion could not be determined, though it could be lower in genetically susceptible individuals than the general population.

7. Hazard characterisation - Given the uncertainty around the pathogenic capability of MAP, detection in powdered infant formulae might perhaps be a concern.

References

1. ACM/486: Heat resistance of Mycobacterium avium subsp. paratuberculosis (MAP) in milk at pasteurisation temperatures.

   https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/ACM486.PDF

2. Crohn's disease - Clinical review by Dr Abdullah Rana and Mr Joseph Dawson on the 9 December 2015.

   http://www.gponline.com/crohns-disease-clinical-review/gi-inflammatory-bowel-disease/crohns-disease/article/1213250

3. Botsaris, G., Swift, B.M.C., Slana, I., Liapi, M., Christodoulou,M., Hatzitofi, M., Christodoulou, V., Rees, C.E.D. (2016) Detection of viable MAP in powdered infant formula by phage-PCR and confirmed by culture International Journal of Food Microbiology 216: 91-94.

4. Collins, M.T. (1997) MAP: A potential food-borne pathogen? Journal of Dairy Science 80: 3445-3448.

5. Defra, 2005. A Case Control Study Investigating Drinking Water & Dairy Products in the Aetiology of Crohn's Disease - A Possible Role for Mycobacterium avium paratuberculosis – The CMAW Study.

   http://www.dwi.gov.uk/research/completed-research/reports/Crohns_Report.pdf

6. Dhama, K., Mahendran, M., Tiwari, R., Dayal Singh, S., Kumar, D., Singh, S., and Mahadev Sawant, P. (2011) Tuberculosis in Birds: Insights into the Mycobacterium avium Infections Veterinary Medicine International712369.

   https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3135220/citedby/

7. Donaghy J., Totton NL., Rowe MT. (2004) Persistence of Mycobacterium paratuberculosis during manufacture and ripening of cheddar cheese. Applied and Environmental Microbiology 70(8):4899- 905.

8. Eltholth, M.M., Marsh, V.R., Winden, S.V., Guitian, F.J., (2009) Contamination of food products with MAP: a systematic review Journal of Applied Microbiology ISSN 1364-5072: 1-10.

9. Report of the Scientific Committee of the Food Safety Authority of Ireland, 2009. MAP and possible links to Crohn's disease.

10. Gill, C.O., Saucier, L., Meadus, W.J., (2011) MAP in dairy products, milk and drinking water Journal of Food Protection 74(3):480-499.

11. Grant IR., Ball HJ., Rowe MT. (2002) Incidence of Mycobacterium paratuberculosis in Bulk Raw and Commercially Pasteurized Cows' Milk from Approved Dairy Processing Establishments in the United Kingdom. Applied and Environmental Microbiology 68:(5):2428- 35.

12. Grant, I. (2015) Review of the latest research on a possible link between MAP and Crohn's disease. Paper presented the Advisory Committee on Dangerous Pathogens, February 2015. https://app.box.com/s/uolyuc1tmjarix61b93xtsd9z731k549#/s/uolyuc1tmjarix61b93xtsd9z731k549/1/3219178817/27066245741/1?&_suid=1476202608830097952574484283 41

13. Health Protection Agency, 2002 Further studies on the Incidence of *Mycobacterium avium* Complex (MAC) in drinking water supplies (Including the detection of *Helicobacter pylori* in water and biofilm samples) http://dwi.defra.gov.uk/research/completed-research/reports/DWI70-2-146_mac1.pdf

14. Jaravata, CV., Smith WL.,Rensen GJ., Ruzante J., & Cullor JS. (2007) Survey of ground beef for the detection of Mycobacterium avium paratuberculosis. Foodborne Pathogens and Disease 4(1):103-106

15. Merck Veterinary Manual 2013: An overview of paratuberculosis http://www.merckvetmanual.com/mvm/generalized_conditions/paratuberculosis/overview_of_paratuberculosis.html

16. Mihajlovic, B., Klassen, M., Springthorpe, S., Couture, H., Farber, J. (2011) Assessment of sources of exposure for MAP in food and water International Food Risk Analysis Journal 1 (2) 1-22.

17. Miranda, C., Matos. M., Pires, I., Ribeiro, P., Alvares, S., Vieira-Pinto, M., Coelho, A.C (2011) MAP infection in slaughtered domestic pigs for consumption detected by molecular methods. Food Research International 44(10):3276-3277

18. Pierce, E.S. (2010) Ulcerative colitis and Crohn's disease: Is MAP the common villain? Gut Pathology 2(21):1-17.

19. Rhodes, G., Richardson, H., Hermon-Taylor, J., ,Weightman, A., Higham, A., and Pickup, R. (2014) Human exposure through environmental aerosols Pathogens 3(3) 577-595.

20. Savvi, R., Ricchia, M., Cammia, G., Garbarinoa, C., Leoa, S., Pongolinib, S., Arrigonia, N. (2015) Survey on the presence of MAP in ground beef from an industrial meat plant Veterinary Microbiology, 177(12):403–408.

21. The Centre for Food Security and Public Health (2007) Paratuberculosis 1-6. Last updated April 2007.

22. Verma, D.K. (2013) MAP: an emerging animal pathogen of global concern. Advances in Bioreseach December 4(4): 1-8.

23. Waddell, L., Rajic, A., Stark, K., McEwen, S.A., (2016) MAP detection in animals, food, water and other sources or vehicles of human exposure: A scoping review of the existing evidence. Preventative Veterinary Medicine 132: 32-48.