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Exploration of the prevalence and production impact of *Mycobacterium avium subsp. paratuberculosis* (MAP) infection in the sheep populations of Great Britain and New Zealand

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ABSTRACT

Mycobacterium avium subspecies paratuberculosis (MAP) is a prevalent, obligate pathogen of many wild and domestic species with a global distribution. MAP prevalence, epidemiology and production impact has been extensively studied in cattle whilst comparatively little research has been conducted on sheep. The aim of this project was to assess the prevalence of MAP in Great Britain (GB) and New Zealand (NZ) using faecal PCR and describe the relationship between MAP faecal shedding, reproductive failure (as determined by ultrasound pregnancy diagnosis) and longevity. A total of 162 farms in GB and 76 in NZ were sampled using a combination of individual and pooled faecal sampling frames of which 70 % of GB flocks and 88 % of NZ flocks tested positive for MAP. MAP shedding was associated with a 3 times increased risk of reproductive failure and significantly higher flock replacement rates in infected flocks. Reproductive failure is an important cause of forced culling in commercial flocks, leading to higher replacement rates and reduced lifetime output per breeding ewe. Excess culling and replacement costs due to preventable infectious disease reduce flock productivity, profitability and negatively impact sustainable lamb production. Effective control measures are available for MAP infected flocks but adoption is hampered primarily, but not exclusively, by poor recognition and underdiagnosis of the disease coupled with a poor awareness of the production limiting nature of the pathogen at the flock level.

Introduction

Mycobacterium avium subspecies *paratuberculosis* (MAP) is an obligate pathogen with a global distribution and wide range of mammalian host species. Ruminant species are the most severely affected by MAP infection, frequently referred to as either paratuberculosis or Johne's Disease. The vast majority of the epidemiologic research into MAP globally has focused on cattle for a variety of clinical and economic reasons, while the prevalence and pathogenicity in sheep are less well understood. It is generally recognised that clinical symptoms of MAP infection in sheep are more subtle and non-specific, compared to cattle, with fewer overt clinical symptoms (Idris et al., 2021). Whilst the early stages of infection are extremely challenging to detect in any host species, as the disease progresses in cattle to the later, clinical phase, profuse diarrhoea and concurrent wasting make Johne's disease a readily recognisable and regularly diagnosed disease of cattle. This is not the case with sheep, contributing to poor awareness of the disease among sheep farmers (Lima et al., 2019). This lack of awareness of MAP as a differential diagnosis for common abnormalities such as; nonspecific low body condition, poor growth rate or poor reproductive performance (Dennis et al., 2011; McGregor et al., 2015; Morris et al., 2006) may cause underdiagnosis due to lack of appropriate investigation and misattribution to other causes.

In Australia (De Silva et al., 2018) showed merino sheep experimentally infected with MAP can develop several different post-infection phenotypes, ranging from resistant and resilient sub-clinical animals which showed no shedding or only transient shedding respectively, through to paucibacillary and multibacilliary clinically diseased phenotypes. Annual mortality due to clinical Johne's disease has been estimated by two studies in Australian and New Zealand flocks (Bush et al., 2006; Gautam et al., 2018) with an average of 6.2 % (range 2.1 – 17.5 %) in Australia and a range of 0.7–1.8 % in New Zealand. However,

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this data was drawn from relatively small samples of mainly merino flocks which appear to be more susceptible to the most overtly severe clinical manifestation of the disease than native breeds in Great Britain, as shown by (Bush et al., 2006). Morbidity losses associated with sub-clinical infection are far less well understood.

In Greece (Kostoulas et al., 2006) described a negative relationship between reproductive failure and sub-clinical infection in dairy sheep. In addition to estimating the mortality directly attributed to clinical infection, understanding the potential impact of sub-clinical infection on reproductive performance of lamb producing (meat) flocks is important since reproductive failure is a primary reason for premature culling of breeding ewes in these flocks globally (Abebe et al., 2020; Flay et al., 2021; Ridler et al., 2025). If MAP infection is a significant factor in poor reproductive performance, this would lead to higher forced replacement rates and reduced average ewe longevity, which in turn negatively impacts farm economics and carbon footprint. In this way, morbidity due to sub-clinical infection and disease may be more detrimental than the mortality associated with the manifestation of overt clinical disease. For clarity it is important to define the terminology used. The authors use the term reproductive failure and reproductive performance as defined by ultrasound diagnosis of pregnancy in mid-gestation (pregnant vs non-pregnant ewes). This encompasses failure to conceive (infertility) and failure to maintain an early pregnancy (failure to implant, early embryonic loss). The authors do not include late embryonic loss (abortion) or impaired fecundity.

The relative commercial and economic importance of one livestock disease compared to another depends upon its prevalence and severity. These parameters can be further subdivided into the within farm and between farm prevalence, and the clinical versus sub-clinical severity. In this context severity should be understood not just as a welfare issue for the individual but also as the production losses associated with increased mortality and/or morbidity.

In this paper the authors describe studies conducted in Great Britain (GB) and New Zealand (NZ) to explore the prevalence of MAP and its relationship with reproductive failure and longevity as key performance determinants in sheep.

Methods

Flocks

Ethical approval was obtained from The University of Nottingham, University of Liverpool and Massey University research ethics committees [VREC833, VREC833a, VREC919, VREC1442 & RIS125811, AEC 23/20]. Prevalence data was estimated from five study populations, four in GB and one in NZ as described in summary below.

Study 1 comprised data analysed from a convenience sample of 53 flocks from England, Wales and Scotland previously recruited as part of Animal Health Development Board (AHDB) funded 'Ram Compare' and 'Demonstrator Farms' programmes in addition to flocks participating in an active surveillance programme funded by Innovis Breeding Sheep Ltd. Study 2 comprised a convenience sample of 40 flocks recruited from the Hybu Cig Cymru (HCC) 'Stoc+ ' flock health improvement programme for the 'Iceberg Diseases Project'. Study 3 comprised a purposive sample of 74 commercial farms across England, Wales and Scotland selected from a pool of 220 farms with cattle, sheep and mixed species cattle and sheep, who had expressed an interest in being involved in a Johne's research study funded by Virbac Animal Health and University of Liverpool. Study 4 comprised data analysed from a convenience sample of Welsh sheep flocks participating in a HCC 'Stoc+ Fertility Partnership Initiative between the farmers, private veterinary practices and the sector levy body to improve flock reproductive health and productivity. Flock level summary physical performance data and historic flock health status screening data and farmer reported management practices was available from 64 flocks with detailed age structure data available for 25 flocks and detailed individual animal reproductive, health status and associated metadata was from 10 flocks. Study 5 comprised a purposive sample of 83 flocks (mobs) from 76 commercial farms from across New Zealand in collaboration between Massey University and University of Liverpool. Summary details of the number of farms, animals sampled and sampling frame to select animals for sampling are included in Table 1. Full details of the recruitment and sampling frames, sample collection along with description of the sample processing and analysis are provided for each study in the supplementary material document 1.

Laboratory testing

MAP status was determined by faecal PCR on individual and pooled samples in each study. MAP PCR assays were performed by the Animal and Plant Health Agency (APHA Starcross) according to their standard operating procedures as detailed in the supplementary materials with additional and cross validation MAP PCR assays carried out at University of Liverpool using the Johne-PureSpin kit (FASMAC Ltd) and qPCR performed using Reso-Light (RL) IS900 qPCR assay as described by Kawaji et al. (Kawaji et al., 2020), and BioPrep MAP-1 & MAP-2, Bio-Extract with PCR performed using Bio-T kit *Mycobacterium avium paratuberculosis* (Biosellal Ltd). Full details are provided in the supplementary material document 1.

Further, individual animal faecal and blood samples were analysed by MAP PCR and serology for detection of antibodies to *Chlamydia abortus, Toxoplasma gondii,* and *Fasciola hepatica* for a subset of animals in Study 4 (performed by APHA) and for metabolic profiling in Study 2 (performed by Edinburgh University).

Data analysis

Data was stored and processed in Microsoft Excel, statistical analysis was performed in Minitab22 with the exception of within flock prevalence (WFP) estimates which were calculated using six different frequentist methods (A–F) described in the literature using the pooled prevalence calculator available at https://epitools.ausvet.com.au/pooledprevalence (Toribio & Sergeant, 2007; Cowling et al., 1999; Mita et al., 2016) and described in detail in Table S6 of supplementary materials document 1. Results from each prevalence estimation methodology are included to communicate the variation in and between prevalence distributions intrinsic to methodology used as this variation influences the degree to which the number of positive pools can be interpreted as an indicator of within flock prevalence. Calculations are based on the number of individual animals represented in each pool, the total number of pools tested and the proportion of positive pools. Flocks were classified as infected if one or more pools returned a positive MAP PCR result.

Where either none or all the pools for a flock tested are positive, methods A-F to calculate WFP estimates and confidence limits are invalid. In the case of methods A and B, which both assume perfect specificity and sensitivity, WFP estimates are 0 % and 100 % respectively, the latter of which is biologically improbable. For the same scenario using methods C-E, WFP estimates are invalid as the proportion of positive pools must be greater than the false positive rate (1 – specificity) and less than or equal to the true positive rate (sensitivity). Hence WFP estimates cannot be calculated when all of the pools are positive as this high proportion of positive pools is inconsistent with assumed test sensitivity. For Studies 2, 3 and 5, specificity (100 %) and sensitivity (84 %) values were used for methods C-E, based on test characteristics originally reported by (Kawaji et al., 2020). For methods C-E in Studies 1 and 4, specificity (100 %) and sensitivity (86 %) was used based on the results comparing pooled versus individual sampling involving 119 samples in Study 4. Method F was used for estimating overall prevalence (animal level prevalence) across all flocks (both negative and positive) within each study and across all five studies. This method was also used for estimating animal level prevalence for all the infected flocks which

Table 1
Summary of Mycobacterium avium subspecies paratuberculosis (MAP) prevalence in sheep flocks in GB (Studies 1–4) and NZ (Study 5) between 2017 and 2023 as determined by pooled faecal qPCR. Both overall animal level prevalence and animal level prevalence for infected flocks were estimated across all flocks for each study using Method F, a linear frequentist model (Williams & Moffitt, 2001).

	Study 1	Study 2	Study 3	Study 4	Study 5
Number of sheep flocks sampled (GB or NZ)	53 (GB)	40 (GB)	59 (GB)	10 (GB)	83 (NZ)
Sampled animal selection	Cull ewes	Randomly selected breeding ewes	1. Randomly selected ewes; 2. Thin ewes of any age; 3. Old ewes in good body condition	1. Randomly selected pregnant ewes; 2. Non-pregnant ewes	1. Randomly selected pregnant ewes; 2. Non-pregnant ewes
Number of ewes sampled	626	800	3490	245	1690
Number of pools (pools per flock)	157 (1–7)	80 (2)	349 (4–6)	49 (4–8)	338 (4–6)
Number of MAP positive pools (proportion of positive pools)	78 (0.497)	39 (0.487)	140 (0.401)	22 (0.449)	164 (0.485)
Between-flock level MAP prevalence	42 of 53 (79 %)	25 of 40 (63 %)	41 of 59 (69 %)	6 of 10 (60 %)	73 of 83 (88 %)
Overall animal level prevalence [all pools from all flocks] (95 % CI)	15.4 % (12.4 %-18.8 %)	6.5 % (4.7 %-8.7 %)	5.0 % (4.2 %-5.9 %)	11.2 % (7.3 %-16.3 %)	12.4 % (10.7 %-14.3 %)
Animal level prevalence for infected flocks [flocks with one or more MAP positive pools only] (95 % CI)	21.5 % (17.3 %-26.1 %)	14.0 % (10.0 %-19.1 %)	8.1 % 6.9 %-9.5 %)	23.2 % (15.2 %-33.3 %)	14.8 % (12.7 %-17.0 %)

had at least one positive MAP pool, in each study and across all five studies. Further, method F was used for WFP calculations for infected flocks in which fewer than 100 % of the pools analysed were positive. For those flocks where all pools tested were positive, WFP estimates using method F resulted in extremely large confidence intervals inferring a significant degree of unreliability at the distribution extremes and therefore were not included for these flocks.

Analysis of relative pool sample MAP prevalence and associated metadata was conducted by pregnancy group (pregnant versus non-pregnant) within and between farms in Minitab22 including chi-squared and Fisher exact tests. Non-parametric Mood's median statistical analysis of pool PCR assay Ct-value distributions between groups was conducted in Mintab22. A binary logistic regression model was built to analyse the relationship between pregnancy outcome at pregnancy diagnosis (pregnant / non-pregnant) and individual ewe MAP status based on individual PCR results in addition to a range of associated ewe metadata: farm, age, body condition score, seroconversion to *Chlamydophila abortus*, *Toxoplasma gondii* and *Fasciola hepatica*. Model fit was assessed by Pearsons and Hosmer-Lemeshow tests in Minitab22. Extrapolation of relative risk from model outputs was performed using baseline non-pregnant ewe prevalence data from AHDB (Stocktake 2016, 2016).

Longevity in MAP infected versus uninfected flocks was assessed in a mixed effect linear model in Minitab22 using the proxy metrics of the percentage of the flock aged four years old and above, and percentage of one-year olds (replacement rate) as the outcomes relating to flock longevity. The model included data for 59 flocks from (Studies 1, 3 and 4) where good quality age structure data was available for at least 1 year and where flocks did not practice routine culling of ewes based on age. Flock size, year, study and MAP status were included as fixed factors within the model. Model fit was assessed visually by residual distribution. In New Zealand (Study 5) since most flocks routinely culled ewes at a set age, rather than according to individual ewe performance and fitness, insufficient flocks with suitable data remained in the dataset to model the longevity outcomes.

Results

Between-flock prevalence estimates

The estimated between-flock level prevalence of MAP PCR positive farms ranged from 60 % to 79 % across the four GB study populations and was 88 % in the NZ study, Table 1. Across all regions of GB and NZ the majority of flocks tested positive. Overall animal level prevalence (considering all pools from all flocks) ranged from 5 % to 15.4 % across the four GB study populations and was 12.4 % in the NZ study. Animal level prevalence considering only infected flocks ranged from 8.1 % to 23.2 % across the four GB study populations and was 14.8 % in the NZ study.

Comparison of within flock prevalence by pooled and individual sampling

In study 4, pooled MAP PCR and individual MAP PCR data was recorded. WFP estimates in this study could be derived from pooled and individual animal PCR results, allowing comparison between the two estimation approaches in the six flocks where MAP was detected. Individual animal PCR positive prevalence for each flock ranged from 23 % to 73 %, with a mean of 46 % (CI 45–47 %). Estimates of the sensitivity and specificity of pooled PCR were calculated based upon the individual qPCR results. All the pools which had reported a positive result corresponded to at least one of the five individual samples testing positive. The mean number of positive PCR individual samples per positive pool was 2.4 and a mode of 2. However, 38 % of the apparently MAP negative pools contained at least one positive MAP individual sample and in one case a pool of five samples that had tested negative contained two positive samples when tested individually. Extrapolating from this small number of samples, a sensitivity and specificity of 86 % and 100 % respectively was estimated for the Pooled PCR. The positive predictive value (PPV) and negative predictive values (NPV) of single pool test results was 100 % and 63 % respectively. The mean WFP estimates based upon the statistical extrapolation from the pooled PCR results (methods A-F) consistently and substantially underestimated the MAP prevalence in each of the three flocks as determined by the proportion of individual animal PCR positive results (Table 2) (NB. The methods A-F can only be applied when fewer than 100 % of the pools test positive, this was the case in three of the six flocks).

Within-flock prevalence (WFP) estimates by pooled MAP PCR

WFP estimates were derived using pooled PCR data alone from 124 flocks across Studies 1, 2, 3 and 5. Flocks with either no or all pools positive could not be used for WFP calculations due to the limitations of the statistical methods.

In Study 1, using a sampling frame of cull ewes, between-flock prevalence was estimated at 79.2 % (42 of 53 flocks) while pool level prevalence was 49.7 % (78 of 157 pools). Of the 42 flocks which tested positive, 18 tested positive in all pools. WFP cannot be accurately estimated when all pools are either positive or negative. WFP was calculated

Table 2
Summary of individual and pooled Mycobacterium avium subspecies paratuberculosis faecal PCR results from Study 4. Within-flock prevalence (WFP) estimates as determined by frequentist methods A-F described in the pooled prevalence epitools calculator included for amalgamated data and individual farm data where possible. N/A denotes instances where the combination of pool results falls outside the parameters of the estimation method.

Farm	Individual ewe prevalence positive qPCR results	% of positive qPCR pools	Within-flock prevalence stochastic estimators Methods A-F. Mean % (95 % CI)					
			Method A	Method B	Method C	Method D	Method E	Method F
A	73.1 % [19 of 26]	85.7 % [6 of 7]	32.2 % (7.6–56.8)	32.2 % (10.4–67.5)	68.1 % (<1 - >100)	68.1 % (12.6 – N/A)	68.1 % (<1- >100)	32.2 % (13.2 – 61.2)
B	10.5 % [2 of 19]	25.0 % [1 of 4]	5.6 % (<1 – 16.3)	5.6 % (<1 – 28.0)	6.6 % (<1–19.6)	6.6 % (<1–42.5)	6.6 % (<1–19.6)	5.6 % (<1–22.6)
C	23.3 % [7 of 30]	42.8 % [3 of 7]	10.6 % (<1 – 22.1)	10.6 % (2.1 – 28.7)	12.9 % (<1–27.7)	12.9 % (2.4–44.8)	12.9 % (<1–27.7)	10.6 % (2.7–25.6)
D	57.1 % [4 of 7]	100 % [3 of 3]	N/A					
E	60.0 % [9 of 15]	100 % [4 of 4]	N/A					
F	63.6 % [14 of 22]	100 % [5 of 5]	N/A					

from the remaining 24 MAP positive flocks with the proportion of positive pools ranging from 14 % to 75 %. The WFP in these 24 flocks ranged between 3.3 % and 34 % with a mean of 16.6 % (CI 13.7 % – 19.5 %) using method F only, as methods A-E are not applicable to datasets with variable pool size. No correlation was observed between the number of animals per pool or the number of pools per flock and the proportion of flocks defined as MAP qPCR positive.

In Study 2, samples from randomly selected breeding ewes were pooled, giving a between-flock prevalence of 62.5 % (25 of 40 flocks) while pool level prevalence was 48.8 % (39 of 80 pools). The small number of pools analysed per flock in Study 2 only allowed for a single WFP estimate to be calculated for those flocks where one out of the two pools were positive. WFP calculations were not possible for those flocks where both pools were positive using the same pooled prevalence methods. Further, the large sample theory applied by methods A, C and E resulted in negative lower confidence levels. Fourteen flocks were positive on both pools analysed and 11 flocks were positive on a single pool. WFP estimates for flocks with one positive pool using the six methods were as follows: 6.7 % for methods A and B (CI <1 % - 19.6 % and <1 % - 35.4 % respectively), 8.6 % for methods C,D and E (CI <1 % - 27.3 %, <1 % - N/A and <1 % - 27.3 % respectively) and 6.7 % (CI <1 % - 27.9 %) for method F. Of the five farms that kept only sheep, two were positive for MAP and three were negative. For the mixed species farms, 23 were MAP positive and 13 were negative.

In Study 3, pools were formed from three groups of sheep: (1) randomly selected ewes; (2) thin ewes of any age; and (3) old ewes in good body condition, giving a between-flock prevalence of 69.5 % (41 of 59 flocks). Cattle were also sampled, where present, giving a between-herd prevalence of 59.2 % (29 of 49 herds). Forty out of the 41 positive flocks had sufficient numbers of breeding ewes for the full 60 individual samples to be collected resulting in six pooled results (the one remaining positive farm had one positive pool out of five collected). The number of positive pools detected in the MAP positive flocks and the estimated WFP according to the six different pooled prevalence calculator methods is shown in Table 3. WFP estimates ranged from 1.8 % to 38.3 % dependent on the number of positive pools and methodology used excluding those flocks where all the pools were positive. In these flocks, WFP couldn't be calculated by the estimation methods used but it may be reasonably assumed to be higher than for those flocks with a lower proportion of positive pools.

Detection rates of positive flocks varied according to the group of ewes sampled ('random', 'thin of any age', or 'old ewes (full mouth) in

Table 3
Summary of within-flock prevalence (WFP) estimates, as determined by frequentist methods A-F described in the pooled prevalence epitools calculator, for varying proportions of Mycobacterium avium subspecies paratuberculosis (MAP) positive faecal pools from each study involving sheep flocks in Great Britain and New Zealand. NB. WFP algorithms cannot be applied to extremities of the distribution where all or none of the pools from a flock test positive, therefore those farms with all or nil positive pools are excluded from WFP estimate analysis and the higher WFP of MAP in flocks where all pools were positive is not reflected in these results.

Country (Study)	Pools +ive	Number of Farms	Estimated Within-Flock Prevalence. Mean % (95 % CI)					
			Method A	Method B	Method C	Method D	Method E	Method F
Great Britain (Study 1)	**14–75 %**	18	N/A	N/A	N/A	N/A	N/A	16.6 % (13.7– 19.5)
Great Britain (Study 2)	**(50 %) 1 of 2**	11	6.7 % (<1–19.6)	6.7 % (<1–35.4)	8.6 % (<1 %- 27.3)	8.6 % (<1 - N/A)	8.6 % (<1–27.3)	6.7 % (<1–27.9)
Great Britain (Study 3)	**(17 %) 1 of 6**	11	1.8 % (<1–5.3)	1.8 % (<1–9.7)	2.2 % (<1 – 6.5)	2.2 % (<1 – 13.4)	2.2 % (<1 – 6.5)	1.8 % (<1 – 7.7)
	(33 %) 2 of 6	4	3.9 % (<1 – 9.4)	3.9 % (<1 – 13.9)	4.9 % (<1 – 12.0)	4.9 % (<1 – 22.8)	4.9 % (<1 – 12.0)	4.0 % (<1 – 11.9)
	(50 %) 3 of 6	5	6.7 % (<1 – 14.2)	6.7 % (1.2 – 19.2)	8.6 % (<1 – 19.4)	8.6 % (1.5 – N/A)	8.6 % (<1 – 19.5)	6.7 % (1.7 – 16.9)
	(67 %) 4 of 6	6	10.4 % (<1 – 20.5)	10.4 % (2.5 – 26.9)	14.6 % (<1 – 33.2)	14.6 % (3.0 – N/A)	14.6 % (<1 – 33.5)	10.4 % (3.2 – 23.9)
	(83 %) 5 of 6	3	16.4 % (1.4 – 31.4)	16.4 % (4.3 – 42.1)	38.3 % (<1 - >100)	38.3 % (5.4 – N/A)	38.3 % (<1 - >100)	16.4 % (5.7 – 36.7)
New Zealand (Study 5)	**(25 %) 1 of 4**	25	0.6 % (<1 – 16.3)	0.6 % (<1 – 28.0)	6.8 % (<1 – 20.2)	6.8 % (<1 – 47.3)	0.7 % (<1 – 20.2)	5.6 % (0.3– 22.6)
	(50 %) 2 of 4	19	12.9 % (<1 – 30.0)	12.9 % (1.4 – 41.7)	16.5 % (<1 – 40.6)	16.5 % (1.7 – N/A)	16.5 % (<1 – 40.7)	12.9 % (2.2 – 36.0)
	(75 %) 3 of 4	16	24.2 % (<1 – 49.9)	24.2 % (4.2–63.7)	36.0 % (<1 – 96.4)	36.0 % (5.1– N/A)	36.0 % (<1 – 97.0)	24.2 % (6.3 – 56.1)

good body condition'); 63 % (26 out of 41) of the MAP infected flocks were identified as positive by one pool collected from thin ewes of any age and 39 % (16 out of 41) by one pool collected from old ewes in good body condition. Four pools were collected from a random selection of ewes in each flock and 90 % (37 out of 41) of positive flocks were detected by at least one of the four pools. Simulation of results based upon sampling of only one, two or three of the four random pools estimated detection rates of 59 %, 70 % and 77 % respectively.

In Study 5 (NZ) randomly selected pregnant ewes and non-pregnant ewes were sampled as separate groups. Between-flock prevalence was 88 % (73 out of 83 flocks) while pool level prevalence was 48.5 % (164 out of 338 pools). WFP estimates varied from < 1–6.8 % for flocks with one positive pool up to 24.2–36 % for farms with 3 of 4 pools test positive with substantial confidence intervals, Table 3.

Comparative prevalence in cattle and sheep by farm type

Between species prevalence within farms could be estimated in Study 3, where both sheep and cattle were sampled. For the farms that kept only sheep (n = 20), 10 flocks were MAP positive and 10 were MAP negative (prevalence 50 %). In contrast, of the mixed species farms (n = 39), 31 flocks were positive whilst only eight were negative (prevalence 79 %). Positive MAP flock status differed significantly between the two different farm categories (p = 0.022), indicating that flocks on mixed species farms were significantly more likely to be MAP positive compared to flocks from sheep only farms.

A similar trend was observed between the 15 cattle-only farms where eight herds were positive for MAP and seven were negative (prevalence 53 %) whilst on 34 mixed species farms (32 beef-only herds and two herds with dairy stock), 21 were positive and 13 were negative (prevalence 62 %). The sample size of farms was insufficient to establish statistical significance for cattle only farms.

Relationship between MAP infection and reproductive failure

Ewes in Study 4 and 5 were selected from pregnant and non-pregnant groups with ewes in each group tested in pools of 5 ewes per pool. The percentage of MAP PCR positive pools was consistently higher for the non-pregnant group compared to the pregnant group in both the GB and NZ studies with 81 % versus 64 % in GB flocks (n = 30 pools, 6 flocks) and 63 % versus 55 % respectively in NZ flocks (n = 164 pools, 73 flocks). In five out of the six positive GB flocks, individual ewe faecal PCR results demonstrated higher individual animal MAP prevalence among the non-pregnant ewes compared to their pregnant flock mates, with the non-pregnant group prevalence ranging from 22 % to 100 % (mean 58 %) compared to the pregnant group prevalence ranging from 25 % to 88 % (mean 39 %). MAP shedding load, as measured by the Ct control standardised distribution as an inverse proxy, from non-pregnant pools was lower than the pregnant pools in the GB flocks with a median of 16.98 (IQR: 15.18, 18.29) versus 17.31 (IQR: 16.92, 18.40) respectively, indicating higher MAP load in the non-pregnant group. The corresponding Ct analysis in the larger sample of NZ flocks identified the same relationship with a significantly larger difference between non-pregnant ewe pools, median of 11.4 (IGR:10.8, 12.1) compared to 14.1 (IQR:12.9,15.2), (p < 0.001).

At the individual ewe level, the relationship between a range of variables including MAP shedding, and the likelihood of a ewe failing to be diagnosed pregnant were assessed by case-control comparison from the six farms in Study 4. Pairwise analysis of variables identified that non-pregnant ewes tended towards lower mean BCS than pregnant ewes (2.20 vs 2.54). MAP shedding ewes also tended towards lower mean BCS (2.28 vs 2.44). Binary logistic regression model results, after accounting for exposure to other common pathogens associated with poor reproductive performance and physiological factors with an influence on reproductive performance (age and BCS), indicated MAP shedding was associated with a significant and substantial increased risk of being diagnosed non-pregnant with an odds ratio of 3.346 and an extrapolated 3 times relative risk increase of reproductive failure, Table 4.

Longevity

Flock age profile is described by the proportion of the breeding flock in each successive annual age cohort. The flock age profiles of MAP infected flocks showed a steeper decline in the proportion of ewes in each successive age cohort compared to uninfected flocks (Fig. 2). Longevity was compared between MAP infected and uninfected flocks using flock age structure where it was recorded in Studies 1, 3 and 4. A significantly higher replacement rate was identified in MAP infected flocks (6 % higher median replacement rate of 29 % in infected flocks against a base median rate of 23 % in uninfected flocks which equates to a 26 % increase in replacement rate between reproduction groups) along with a significantly smaller proportion of older ewes (≥4 yrs) in infected flocks (23 % versus 32 %), Table 5.

Discussion

GB and NZ are two of the largest and most sophisticated commercially farmed sheep populations, representing nearly 4.5 % of the global sheep population (World Population Review, 2023). In their respective industries controlling endemic diseases presents opportunities for improved productivity, profitability and sustainability. Paratuberculosis is a neglected disease in small ruminants. Despite its global distribution the importance to the national and global sheep industry is poorly understood. To gain a quantitative understanding of this, better estimates of prevalence and production impact are required. Therefore the first aim of this study was to estimate the prevalence of MAP infection at the farm level and describe the distribution of within-farm prevalence from the sampled farms. The second aim was to investigate the relationship between MAP infection, reproductive performance and longevity.

The between-flock MAP prevalence from Studies 1–4 consistently indicates the substantial majority of GB flocks were infected, ranging from 60 % to 79 %. These figures are comparable to the 88 % flock level prevalence for New Zealand flocks, which itself is an increase on the 76 % between-flock prevalence reported in New Zealand flocks a decade earlier by (Verdugo et al., 2014a, 2014b).

This apparent increase in prevalence over time would be plausible, as once a flock is infected it remains infected due to the resilience of the pathogen, and as the flock level prevalence in a country increases the likelihood of naïve flocks becoming infected increases as well. Both countries have similar climatic conditions and farming practices, both have predominantly extensive pasture-based lamb production systems and sheep breeds of similar genetic origin. Considering these factors, it is not surprising that similar prevalence levels are found in both countries. This is in contrast to some European countries who reported flock level prevalence in Austria of 8.9 % (Schrott et al., 2023) and 46.7 % in Portugal (Coelho et al., 2007). The magnitude of these differences between studies may at least partially be due to the use of faecal PCR for screening in favour of the less sensitive serological methods used in these previous European studies (Nielsen & Toft, 2008) but this does not discount the possibility that substantial differences in flock level prevalence do exist between countries due to a range of biological and management factors such as housing and feeding practices which may influence transmissibility and genetic variation between populations which influence resistance to infection.

The relative performance of different sampling frames within a flock could be observed in Studies 3, 4 & 5 which showed that sampling thin ewes or non-pregnant ewes after ultrasound scanning proved to be the most sensitive. Based on these results, the authors would advise preferentially targeting thin, non-pregnant cull ewes where possible when selecting animals in order to identify the MAP status of a flock. However, random sampling of breeding ewes was also sufficiently sensitive to be appropriate in most cases so long as a sufficient number of pools are

Table 4
Binary logistic regression model of ewe reproductive failure, with a reference category of 'pregnant' and response event of 'non-pregnant' (Study 4, n = 115 ewes). The independent variables were: C.abortus, T.gondii, F.hepatica and MAP infection status which were coded as binary categorical variables with uninfected as the reference category for each variable. Age and body condition score (BCS) were coded as continuous variables with farm as a fixed effect. Statistically significant variables denoted by * and in bold.

Term	Odds Ratio	95 % CI	Coef	SE Coef	Z-Value	P-Value	VIF
Constant			-1.628	0.855	-1.90	0.057	
Age	**0.6624**	**(0.4885, 0.8981)**	**0.412**	**0.155**	**2.65**	**0.008***	**1.39**
BCS	0.5899	(0.3290, 1.0577)	0.528	0.298	1.77	0.076	1.58
C.abortus	0.2764	(0.0077, 9.9788)	1.29	1.83	0.70	0.482	1.51
T.gondii	5.1574	(0.4265, 62.3683)	-1.64	1.27	-1.29	0.197	1.31
MAP	**3.3460**	**(1.1939, 9.3772)**	**-1.208**	**0.526**	**-2.30**	**0.022***	**1.66**
F.hepatica	1.4788	(0.2377, 9.2005)	-0.391	0.933	-0.42	0.675	1.38

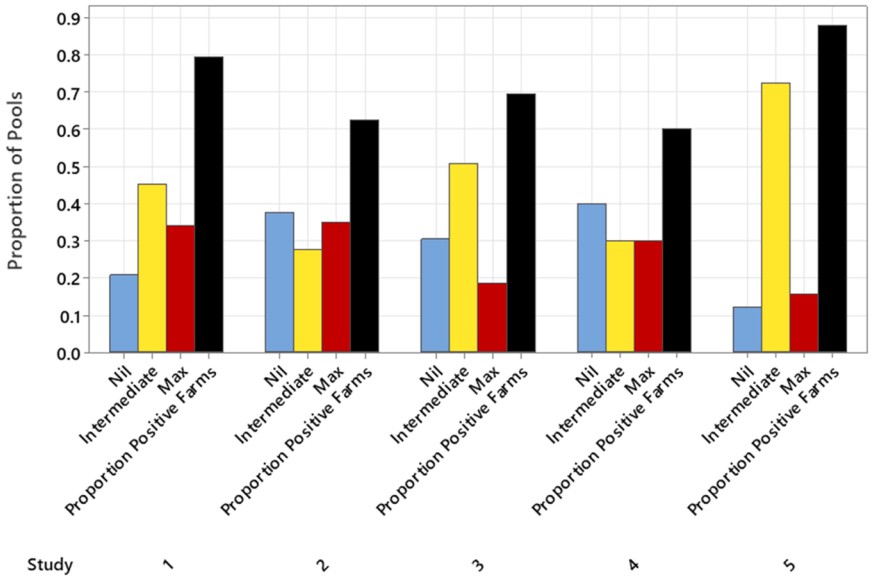

Fig. 1. Proportion of farms by study where Mycobacterium avium spp. paratuberculosis (MAP) was detected by pooled faecal PCR in none of the tested pools (Nil, blue), detected in one or more but fewer than all tested pools (Intermediate, yellow) and where MAP was detected in all tested pools (Max, red). Proportion of farms positive for MAP at either level in black.

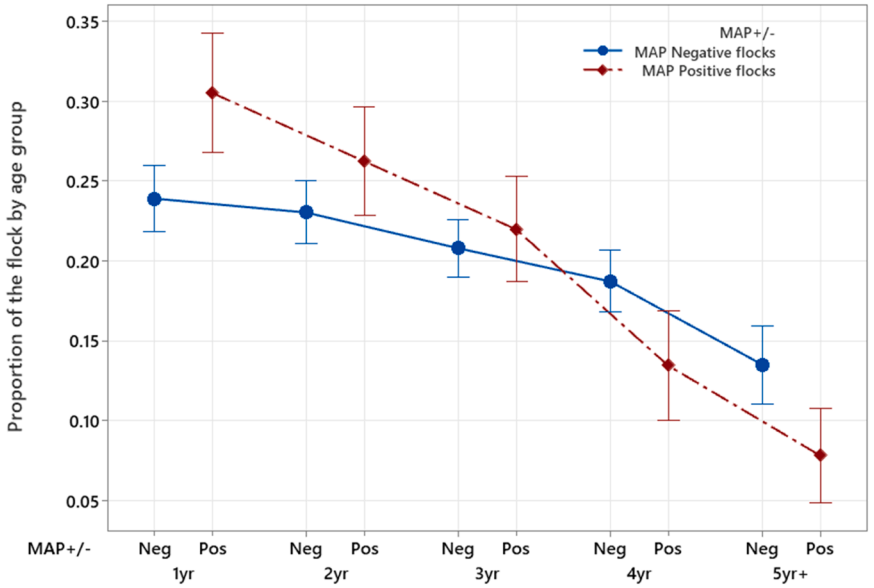

Fig. 2. Mean and 95 % confidence interval plot of age group proportions in Mycobacterium avium spp. paratuberculosis (MAP) infected (positive RED) and MAP uninfected (negative BLUE) flocks as determined by pooled faecal PCR.

Table 5
Mixed effects models of flock mean age, replacement rate and proportion flock ≥ 4 yrs in Mycobacterium avium spp. paratuberculosis (MAP) infected and uninfected flocks by flock size and study. Studies 1, 3 and 4.

Term	Coefficient	SE Coefficient	DF	95 % CI	T-Value	P-Value
Outcome mean flock age (yrs)						
Constant	2.699678	0.074006	52.98	(2.55124, 2.84812)	36.479365	0.000
Flock Size	0.000008	0.000130	44.08	(-0.00025, 0.00027)	0.062094	0.951
MAP						
ref = negative						
Positive	-0.228302	0.045810	57.27	(-0.13658, −0.32003)	-4.983709	0.000
Study						
Ref = 4						
1	0.070865	0.072796	54.67	(-0.07504, 0.21677)	0.973468	0.335
3	0.144479	0.067989	59.55	(0.00846, 0.28050)	2.125032	0.038
Outcome Replacement rate						
Constant	0.248861	0.013917	55.93	(0.220981, 0.276742)	17.881265	0.000
Flock Size	0.030588	0.018070	50.02	(-0.005706, 0.066882)	1.692760	0.097
MAP						
ref = negative						
Positive	0.044650	0.014397	57.73	(0.073473, 0.015828)	3.101280	0.003
Study						
Ref = 4						
1	-0.065764	0.022989	56.01	(-0.111817, −0.019711)	-2.860648	0.006
3	0.009937	0.021297	59.25	(-0.032675, 0.052548)	0.466572	0.643
Outcome proportion of flock 4 yrs old and older (%)						
Constant	0.287799	0.012485	51.40	(0.262738, 0.312860)	23.050913	0.000
Flock Size	0.010042	0.015846	30.70	(-0.022289, 0.042373)	0.633694	0.531
MAP						
ref = negative						
Positive	-0.065111	0.013072	53.77	(-0.038900, −0.091322)	-4.980814	0.000
Study						
Ref = 4						
1	-0.019806	0.020672	49.00	(-0.061348, 0.021736)	-0.958112	0.343
3	0.059771	0.019415	58.70	(0.020917, 0.098625)	3.078590	0.003

tested. Determining the appropriate number of pools to be tested and by which assay is unfortunately not a simple task. In this study we demonstrated on a small sample of farms the pooled PCR technique was substantially less sensitive than individual sampling. However, the high cost of faecal PCR assays mean pooled sampling strategies are the most economically feasible screening option. Further research is urgently required to optimise and standardise the protocols for pooled faecal PCR in sheep due to the biological variability in MAP load in the pooled sample and the substantial between-laboratory variability in MAP PCR test characteristics, as demonstrated by (Worsley & Davies, 2024). Currently accurate validation data is not available to the veterinary practitioner seeking to determine which commercial test to use or the sample size and threshold prevalence to consider in their screening protocol calculation for flocks of varying sizes.

Accurate estimates of WFP are important when attempting to understand production limiting diseases in livestock. The higher the prevalence within a flock the greater the cumulative impact on flock productivity and profitability and the greater the need to identify cost-effective control interventions (Raizman et al., 2011). WFP estimates can also be a measure of success for control programmes over time (Raizman et al., 2011). The findings here suggest not only that a large majority of flocks in both countries are infected with MAP, but also that WFP is high in many infected flocks with the caveat that estimation of prevalence from pooled sampling frames is inherently imprecise. It should also be noted that the sensitivity of pooled sampling is moderate at 86 % compared to individual faecal PCR, presumably due to the dilution effect of uninfected ewes in the pool and detection threshold of the PCR. This means pooled screening approaches may be cheaper and more practical for veterinary practitioners to implement but require cautious interpretation as they may be underestimating the true prevalence within the flock. The range of WFP's between infected flocks may be due to the rate of transmission within a flock which might be influenced by a range of biological and management factors including the proportion of pauci- and multibacillary infected sheep and associated with varying levels of MAP shedding (Whittington et al., 2001), or stocking density (McGregor et al., 2012). Also, the concurrent presence of MAP and overt clinical OJD can not necessarily be assumed as other factors such as host genetics and immunological responses, microbiome dynamics, MAP genotypes and management factors may influence prevalence of clinical OJD (Barkema et al., 2018; Begg et al., 2017; De Silva et al., 2018; Kravitz et al., 2021, p. 2021; Matthews et al., 2021).

The higher MAP prevalence in both flocks and herds on mixed species farms presented here could support the hypothesis that cross-species transmission of MAP between the two species occurs, this relationship may be confounded by other factors such as management or husbandry practices which differ between farm types. Whilst there is evidence in the literature in support of interspecies transmission (Stevenson et al., 2009; Verdugo et al., 2014), reported transmission dynamics varies between countries. Cross-species transmission was reported in Iceland when sheep imported from Germany introduced a Type S strain into the local cattle population which subsequently was passed back to the sheep population following a depopulation and restocking programme (Fridriksdottir, 2000; Whittington et al., 2001). Evidence of interspecies transmission has also been reported in New Zealand where the same Type S strain subtypes were found in beef cattle and sheep co-grazing on the same farms (Verdugo et al., 2014). Conversely a study in Australia reported that the risk of natural transmission of Type S strains from sheep to cattle was likely to be low and only thought to occur when susceptible animals received high infective doses of MAP (Moloney & Whittington, 2008). Given the high proportion of mixed, cattle-sheep enterprises in GB, NZ and many other countries throughout the world, the role of interspecies transmission is important as it has ramifications for the efficacy of control measures in cattle, sheep and goats and certainly justifies further research.

The second aim was to explore the association between MAP, reproductive performance and longevity to test the supposition that production loss, other than on-farm mortality, associated with MAP is detectable and substantial. In both GB and NZ, a clear pattern of higher MAP prevalence and shedding was observed among ewes who failed to become pregnant after the breeding season. Higher MAP shedding was

associated with a lower BCS as well as the expected and well-known association between lower BCS and lower reproductive performance. In NZ and GB reproductive failure is one of the most common reasons for culling breading ewes (Ridler et al., 2025) (McLaren et al., 2020). The age profile data from MAP infected flocks showed a significantly smaller proportion of older ewes compared to uninfected flocks and a correspondingly higher proportion of young, replacement ewes.

Large scale, multi-farm observational cross-sectional and case-control studies such as these are very useful for establishing associations with multiple risk factors and quantitatively estimating the potential importance of a disease such as paratuberculosis. In the case of diseases with long latency and high diagnostic costs like paratuberculosis they are especially useful as an initial epidemiological research tool. These study designs are inherently limited to the description of associations and cannot demonstrate causality or mechanism of action. In these studies the findings show the high prevalence of MAP within and between farms in both countries, and the associations between MAP shedding, ewe body condition, reproductive failure and reduced flock longevity all support the hypothesis that MAP contributing to increased culling of ewes in the majority of flocks. However, causation cannot be conclusively established or claimed at this point without further research to explore the longitudinal relationship between the pathogen and host pathophysiology.

The authors hypothesise that these associations between reproductive failure, longevity and MAP shedding are linked as follows. Previous studies have demonstrated using experimental infections that MAP infected ewes do not all develop the same clinical presentation (De Silva et al., 2018). On the contrary several different clinical presentation phenotypes were observed. These included MAP shedding by apparently asymptomatic sheep, and resilient sheep which shed MAP transiently. As well as those which progressed to rapid clinical wasting phenotype, defined as a body weight loss of ≥ 10 % in a month. Reproductive performance was not included in the experimental infection study and it is unclear from the De Silva study to what extent body weight, body condition or nutritional status fluctuated with MAP shedding over the long observation period, or if the diverse range of clinical phenotypes would occur with naturally acquired as well as experimental infections. However, the authors suggest that the associations reported here, in naturally infected flocks in GB and NZ using an observational study design could be explained by a sub-clinical disease phenotype, intermediate between the resilient and the rapid clinical wasting phenotype. We speculate that a sub-clinical subgroup of infected ewes (Phenotype A, see Fig. 3) develops slowly progressive or transient symptoms characteristic of Johne's disease where granulomatous enteritis leads to malabsorption and protein losing enteropathy (Allen et al., 1974), as observed in the longitudinal clinicopathological study of naturally infected sheep (Dennis et al., 2011). These factors combined with the metabolic cost associated in mounting the chronic inflammatory response may then impose ever greater demands upon the ewe, pushing her into negative energy and protein balance. In this state the ewe partitions the available resources towards essential systems and down-regulates non-essential systems, principally the reproductive systems (Dobson et al., 2012). This will manifest as an increased risk of infertility, detected by routine pregnancy diagnosis ultrasound screening (scanning). It is also possible that implantation, placentation or maternal recognition of pregnancy may be disrupted by the nutritional stress experienced by the ewe, leading to increased first trimester embryonic loss which would be indistinguishable from true infertility by routine mid-gestation ultrasound examination. Given the slow rate of progression of the disease, several weeks to months as observed by (Dennis et al., 2011) and others, the authors suggest it is plausible that the first detectable consequence of the disease may be the increased likelihood of being diagnosed as non-pregnant by ultrasound. At this point in the sheep production cycle, there is a strong economic imperative for the farmer to sell these non-pregnant ewes as quickly as possible at a point in the year when 'cull' ewe values are relatively high and to avoid wasting expensive winter feed on unproductive ewes. This means non-pregnant ewes typically do not undergo veterinary investigation to determine the cause of infertility or reproductive failure. If not addressed, then the proportion of ewes suffering MAP associated

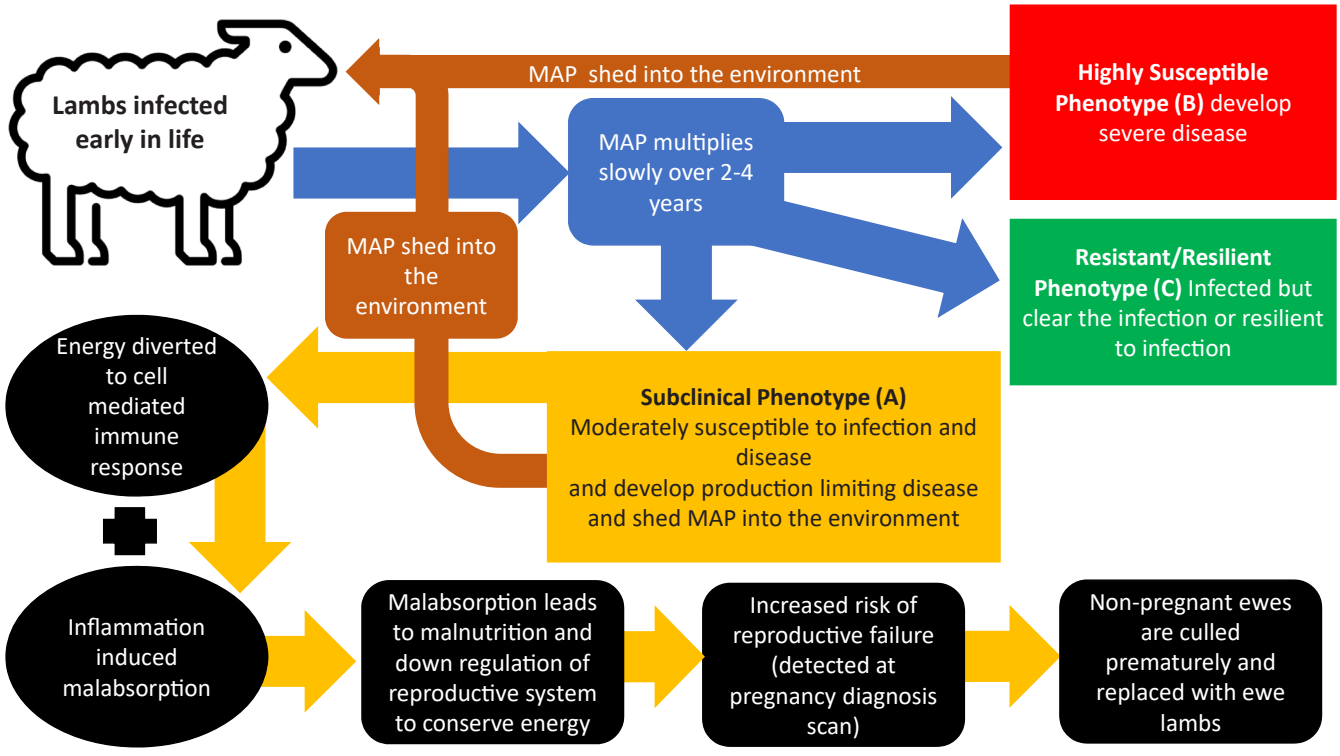

Fig. 3. Conceptualization of MAP epidemiology with three phenotypes A, B and C where A is characterised by insidious reproductive failure and increased forced culling rate.

infertility or reproductive failure, will increase in the flock as the prevalence rises, and this will lead to earlier culling and a shorter average productive life for breeding ewes. Most noticeably the MAP infected flock will be forced to replace a larger number of ewes per year to compensate for the MAP associated reproductive failure culls. The finding that MAP prevalence was higher among non-pregnant ewes and MAP shedding was also higher among non-pregnant ewes supports this hypothesis and the 26 % increase in median replacement rate identified in the MAP positive flocks compared to the MAP negative flocks (29 % versus 23 %), along with the significantly smaller proportion of older ewes in those MAP infected flocks further supports the hypothesis. The negative impact on replacement rates is an important aspect to emphasise as flock replacements represent a large fraction of the total variable costs of a sheep farming enterprises, the third largest expense after feed and labour costs (*Stocktake 2016*, 2016). Higher replacement rates represent unnecessary and preventable waste in the entire production system. Historically, replacement rates receive less emphasis than other key performance indicators such as lambing percentage. Increasing awareness of the opportunities to increase productive longevity and reduce replacement rates could improve not only productivity and profitability but also the sustainability and carbon footprint of the sheep sector.

Whilst these studies relate to GB and NZ the potential implications of their findings have a global significance. With a global sheep population of 1.3billion, a large proportion in low- and middle-income countries where the sheep is the economic foundation of rural communities and a pathogen with known global distribution, the insidious impact of MAP on ewe productivity may be far larger than previously estimated. Robust international epidemiological studies are required to understand both the prevalence of ovine MAP in highly sheep reliant countries and how it is associated with production performance under different host genetic, husbandry and management conditions.

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Conclusion

MAP is widespread throughout GB and NZ, infecting the large majority of sheep flocks. A high prevalence of MAP shedding ewes, associated with an increased risk of reproductive failure is a common feature of infected flocks. Management practices cull out these unproductive ewes at a relatively early, pre-clinical stage of the disease but not before causing sub-clinical productivity and financial losses. The 6 % difference in replacement rate between infected and uninfected flocks equates to 26 % more replacement ewes being required per year in infected flocks compared to their uninfected counterparts. This is a very substantial increase in costs per flock. It is vital for farmers, vets and advisors to appreciate the potentially expensive, wasteful impact of MAP on their flocks. Efforts should be made to encourage farmers to screen for MAP infection in order to establish their disease status. A clearer understanding of flock infection status will enable more informed and appropriate management decisions to be taken including vaccination and biosecurity. Further work is required to better understand the mechanisms of the relationship between the host and pathogen in order to better understand options for control, mitigation and prevention of infection in GB, NZ and the wider global sheep population where the potential benefits may be greatest.

CRediT authorship contribution statement

Peers Davies: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joanna Urbaniec:** Investigation. **Laura Worsley:** Writing – original draft, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Amy Wedley:** Investigation. **Anne Ridler:** Writing – review & editing, Project administration, Investigation, Data curation.

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Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2026.106600.

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