Animal Health Research Reviews

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Alternatives to antibiotics in veterinary medicine: considerations for the management of Johne's disease

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Review Article

Cite this article: O'Connell LM, Coffey A, O'Mahony JM (2023). Alternatives to antibiotics in veterinary medicine: considerations for the management of Johne's disease. *Animal Health Research Reviews* **24**, 12–27. https://doi.org/10.1017/S146625232300004X

Received: 15 September 2021
Revised: 5 August 2022
Accepted: 9 June 2023
First published online: 16 June 2023

Keywords:
antibiotic resistance; Johne's disease; mycobacteriophage; veterinary medicine

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Abstract

Antibiotic resistance has become a major health concern globally, with current predictions expecting deaths related to resistant infections to surpass those of cancer by 2050. Major efforts are being undertaken to develop derivative and novel alternatives to current antibiotic therapies in human medicine. What appears to be lacking however, are similar efforts into researching the application of those alternatives, such as (bacterio)phage therapy, in veterinary contexts. Agriculture is still undoubtedly the most prominent consumer of antibiotics, with up to 70% of annual antibiotic usage attributed to this sector, despite policies to reduce their use in food animals. This not only increases the risk of resistant infections spreading from farm to community but also the risk that animals may acquire species-specific infections that subvert treatment. While these diseases may not directly affect human welfare, they greatly affect the profit margin of industries reliant on livestock due to the cost of treatments and (more frequently) the losses associated with animal death. This means actively combatting animal infection not only benefits animal welfare but also global economies. In particular, targeting recurring or chronic conditions associated with certain livestock has the potential to greatly reduce financial losses. This can be achieved by developing novel diagnostics to quickly identify ill animals alongside the design of novel therapies. To explore this concept further, this review employs Johne's disease, a chronic gastroenteritis condition that affects ruminants, as a case study to exemplify the benefits of rapid diagnostics and effective treatment of chronic disease, with particular regard to the diagnostic and therapeutic potential of phage.

Introduction

Infectious disease has been a major cause of mortality, with early outbreaks referred to as plagues and blamed on unrelated factors, such as climate and religious beliefs (Bazin, 2003). By the late 19th century, these early explanations of contagious illness were replaced with germ theory (Valent *et al*., 2016). In 1910, Paul Ehrlich synthesized the first antibiotic agent, arsphenamine (Salvarsan®; Valent *et al*., 2016; Vernon, 2019). Salvarsan® was an organoarsenic compound that was a popular treatment for syphilis (Vernon, 2019). It would take Alexander Fleming a further 18 years to identify penicillin and more than 20 years after that for the Golden Age of Antibiotic Discovery to peak (Table 1; Hutchings *et al*., 2019). Since Fleming's game-changing discovery, antibiotics have become a cornerstone of human medical treatment and have extended the average life expectancy by an average of 23 years (Hutchings *et al*., 2019). Veterinary medicine and livestock farming have also greatly benefited from the advent of antibiotic treatment, with routine antibiotic therapy preventing the dissemination of zoonotic diseases amongst large herds of livestock (Landers *et al*., 2012).

However, we are now facing the concerning rise in antibiotic resistance (AR), which is the result of prolonged exposure of bacteria to antibiotic agents, usually as a consequence of failed medical treatment or, in the case of livestock farming, routine administration of antibiotics to pre-empt potential infections (Palma *et al*., 2020). AR is now one of the most concerning threats against human and veterinary health, and it is predicted that by 2050, human deaths resulting from AR infections will outnumber those by cancer and result in major economic losses globally (Dadgostar, 2019). These losses include the farming sector, due to a reduction in breeding and trading of livestock, as well as animal death and culling due to resistant infection (Bengtsson and Greko, 2014; Dadgostar, 2019). For this reason, it is not only important to adapt human medicine in accordance with increased incidence of AR infections but also veterinary medicine.

A brief history of veterinary medicine

Veterinary medicine likely dates back to 9000 BC when the Neolithic man first began domesticating animals (Hunter, 2018). Archaeological evidence supports this, as instances of cranial

Table 1. Excerpt of the parallel timelines of antibiotic discovery and the development of antibiotic resistance

Decade	Antibiotic(s) discovered	Antibiotic resistances identified
1928	Penicillin	Salvarsan
1930–1939	Sulphonamides and gramicidin	Sulphonamides
1940–1949	Streptomycin, bacitracin, cephalosporins, chloramphenicol, chlortetracycline, and neomycin	Penicillin
1950–1959[a]	Oxytetracycline, erythromycin, vancomycin, and kanamycin	
1960–1969[a]	Gentamicin, spectinomycin, and clindamycin reported	Methicillin-resistant *Staphylococcus aureus*, plasmid-borne resistance to sulphonamides
1970–1979	Tobramycin and cephamycin	

[a]Golden age of antibiotic discovery.
Source: Adapted from Fair and Tor (2014) and Hutchings *et al*. (2019).

surgery have been identified in animal skulls dating from that period (Ramirez Rozzi and Froment, 2018). As agriculture developed, a king of ancient Babylon incorporated laws pertaining to the payment and responsibilities of veterinary surgeons into what is now known as 'The Code of Hammurabi' (Samad, 2016).

The advent of what can be considered as 'modern' veterinary medicine, which is scientifically informed and consistent, occurred much more recently. In 1761, the first veterinary school was established in Lyon, France, by Cladude Bourgelat (Cáceres, 2011; Samad, 2016). During the 18th century, rinderpest (cattle plague) was a major concern for cattle health, and a physician named Giovanni Maria Lancisi had proposed very effective control measures (e.g. separating sick and healthy animals; Mourant *et al*., 2018). It was Bourgelat's goal to train veterinarians in Lancisi's methods of maintaining animal health (Cáceres, 2011).

Today, there are more than 650 veterinary colleges across the globe (Gyles, 2015; Samad, 2016). There is also a new approach to medicine known as 'One Health', which was developed after the emergence of severe acute respiratory disease and recognizes the link between human and animal health, as well as the threat to food security and agricultural economies posed by zoonoses and animal illness (Samad, 2016; Mackenzie and Jeggo, 2019). One aspect of the One Health concept of medicine is understanding the connection between antibiotic use (and misuse) in human and animal medicine and the rise in AR (Collignon and McEwen, 2019; Mackenzie and Jeggo, 2019; More, 2020; Palma *et al*., 2020).

Antibiotic use in veterinary medicine

As new antibiotic classes were discovered, and new products were rolled out to human medicine, they were also introduced to veterinary therapies (Economou and Gousia, 2015). Unlike human medicine, antibiotics are employed more extensively within veterinary health (e.g. in 2014, 8927 tons of antibiotics were used in veterinary medicine in the EU versus 3821 tons in human medicine), as they also serve nontherapeutic functions in agriculture, such as prophylactic supplements and growth promoters (Cuong *et al*., 2018; Collignon and McEwen, 2019).

These additional functions of antibiotic treatment in livestock animals serve to prevent the transmission of potential infections (such as mastitis in dairy cattle), as well as marginally promote weight gain (Economou and Gousia, 2015; Collignon and McEwen, 2019). While the use of antibiotic agents as growth promoters has been banned in the European Union since 2006, ionophores (nutrient-utilization-promoting antibiotics) are still heavily employed in European feedlots (Economou and Gousia, 2015; More, 2020).

Similarly, the US frequently uses tylosin, a macrolide used exclusively in veterinary medicine, in 88% of pigs and 42% of beef calves to promote growth (Landers *et al*., 2012). In 2014, the agriculture sector was responsible for approximately 70% of total antibiotic consumption in the US (interestingly, the 8927 tons of antibiotic used in animal medicine in the EU, also equates to approximately 70% of total antibiotic consumption in 2014; Cuong *et al*., 2018).

Unfortunately, reliable quantitative data regarding antibiotic usage in agriculture are not readily available, in part as there is a general lack of infrastructure to support the documentation of antibiotic use. This is to change, as the EU Veterinary Medicines Regulations shall require EU members to report antibiotic use in food animals in national databases from 2027 (Martin *et al*., 2020). Currently, however, the data surrounding antibiotic use in livestock are relatively limited, and there is no clear distinction between therapeutic and nontherapeutic use (Collignon and McEwen, 2019; More, 2020). What is far more evident is the risk posed by excessive and/or long-term antibiotic exposure to zoonotic and environmental bacteria (Manyi-Loh *et al*., 2018).

Antibiotic resistance associated with veterinary disease

AR infections, such as methicillin-resistant *Staphylococcus aureus*, were once largely associated with hospitals, where organisms were likely to be subjected to selective pressure caused by exposure to residual antibiotics (Duerden *et al*., 2015). Increasingly, community-acquired infections and environmental isolates are displaying AR (Whittaker *et al*., 2019; Hua *et al*., 2020; Donner *et al*., 2022). There is considerable evidence that supports the over-use of antibiotics in agriculture contributing to the increase in non-hospital associated AR, with specific respect to food animals shedding subclinical levels of antibiotics into the environment and introducing AR-organisms into the food chain (Martin *et al*., 2015; Manyi-Loh *et al*., 2018; Hua *et al*., 2020). Of particular concern is the apparently high prevalence of AR (33–67%) to commonly used veterinary antibiotics, such as tetracycline, chloramphenicol, and beta-lactams, associated with food isolates (Manyi-Loh *et al*., 2018).

As AR associated with community infections and foodborne illness is a major concern for human health, it stands to reason

https://doi.org/10.1017/S146625232300004X Published online by Cambridge University Press

that it is also a major consideration in treating animal illness (Bengtsson and Greko, 2014; Palma *et al.*, 2020). By treating livestock prophylactically, it may lead to treatment failure should an animal acquire an infection that is typically treated with the same or similar antibiotic (Bengtsson and Greko, 2014). For example, penicillin was previously a first-line antibiotic treating mastitis in dairy animals, but it is now not an advised therapy due to widespread resistance (Bengtsson and Greko, 2014; Käppeli *et al.*, 2019).

Unfortunately, literature relating to AR originating from agriculture is biased toward discussing the impact on human health, as opposed to the risk that animals may contract AR infections that exclusively affect their species (Bengtsson and Greko, 2014). This bias needs to be addressed, not only to ensure animal welfare in general, but to avoid an unfair and unrealistic distribution of therapeutic resources within the One Health concept. Similarly, alternatives to antibiotics should be extensively examined for potential applications in veterinary medicine. This will not only increase the arsenal for treating animal illness but will in turn reduce the burden of AR in human infection by limiting the evolution of AR in food isolates.

Alternatives to antibiotics in veterinary medicine

Within the EU, only ionophores are used as feed additives and several countries, such as France, Sweden, and the Netherlands, have implemented further controls to reduce the prescription of antibiotics by veterinarians (Economou and Gousia, 2015; Wong, 2019; Nowakiewicz *et al.*, 2020). While limiting the prescribing of antibiotics may help reduce the incidence of AR in animal illness, it begs the question – how else will animal health be managed? A possible answer is developing novel prophylactics which could offer some protection against disease to healthy animals. Novel prophylactics would not only improve and maintain animal health but would remove the perceived necessity of dosing animals with subclinical levels of antibiotics.

Pre- and probiotics in animal health

Prebiotics are nondigestible dietary fiber compounds which support the growth of beneficial gut bacteria, which have been shown to aid digestion, improve weight gain, and reduce levels of potentially pathogenic bacteria in several species (Arowolo and He, 2018; Markowiak and Śliżewska, 2018; Asha and Khalil, 2020). Probiotics are live microbes that confer health benefits when consumed in adequate quantities (Asha and Khalil, 2020). Several studies have found that the inclusion of probiotic yeast strains in dairy cattle feed aids rumen digestion and reduces oxidative stress (respectively improving gut health and reducing seasonal variation in milk yield; Pinloche *et al.*, 2013; Mirzad *et al.*, 2019). Similarly, bacterial probiotics have been shown to positively affect the rumen and improve weight gain in livestock, with several feed types including lactic acid bacteria, such as *Lactobacillus* (Arowolo and He, 2018; Alayande *et al.*, 2020; Direkvandi *et al.*, 2020; Bhogoju and Nahashon, 2022).

Despite the benefits, several limitations exist which prevent the widespread application of pre- and probiotics in animal feed, including inconsistent effects, difficulties registering novel feed additives, and a lack of regulation surrounding probiotic usage (Cheng *et al.*, 2014; Markowiak and Śliżewska, 2018; Direkvandi *et al.*, 2020). For example, China has formally approved 12 probiotics, but up to 50 are in use (Cheng *et al.*, 2014). Until these issues are addressed, particularly the inconsistent outcomes, it's unlikely that pre- and probiotics will be ushered into a new generation feed additives.

Vaccination

Preventative vaccination could be a cost-effective alternative to prophylactic antibiotics. The goal of vaccination is to stimulate an antibody-mediated immune response to a pathogen without exposure to a virulent organism, usually by injection of dead/attenuated pathogen or immunogenic elements of the pathogen (Meeusen *et al.*, 2007; McVey and Shi, 2010). Vaccines are highly regarded for their proven effectiveness and are widely employed in veterinary medicine (e.g. vaccines against rabies and *S. aureus*-mediated bovine mastitis; McVey and Shi, 2010; Cheng *et al.*, 2014). Vaccination has also demonstrated positive results beyond disease prevention, for instance, a vaccine against the etiological agent of ileitis in pigs improved mortality rates and weight gain of the animals (Bak and Rathkjen, 2009). Encouragingly, the efficacy of this vaccine-reduced antibiotic treatment for ileitis by 80% in pigs and in a larger study, most farms noted reduced antibiotic usage and associated costs (Bak and Rathkjen, 2009; Hoelzer *et al.*, 2018). Unfortunately, vaccination remains a neglected means of disease control in animals, as many smallholder farmers and poor rural populations do not have access to appropriate vaccines, and it is difficult to demonstrate the value of vaccination, as many vaccines are against zoonoses which have little to no clinical effect on the animals (Donadeu *et al.*, 2019). Alongside the issues surrounding accessibility and understanding of vaccines, the regrettable state of the art is that there are many diseases, which currently lack an approved vaccine.

Other alternatives

Prophylactic alternatives aside, what remains to be reviewed are alternatives to antibiotic therapy. One-third of antibiotics used in agriculture are employed for therapeutic purposes, i.e. actively treating illness (Martin *et al.*, 2015; Nowakiewicz *et al.*, 2020). This highlights their importance in maintaining animal health, and consequently their use cannot be entirely erased without the provision of a therapeutic alternative. One such alternative to therapeutic antibiotic use, which has garnered renewed interest in medicine, is bacteriophage (phage) therapy (PT).

A brief history of phage therapy

Remarkably, while PT is under much investigation in the current century as an alternative to antibiotics, it actually predates the discovery of penicillin (McCallin *et al.*, 2019). By 1917, the phage phenomenon had been described twice in independent reports, including one by Félix d'Herelle, who is considered the founder of PT (Wittebole *et al.*, 2014; McCallin *et al.*, 2019).

d'Herelle proposed the name 'bacteriophage,' by combining 'bacteria' and 'phagein', from the Greek for 'to devour', as the phage appeared to devour cells (Sulakvelidze *et al.*, 2001; Dublanchet and Bourne, 2007; Wittebole *et al.*, 2014). It is now understood that it is the lytic cycle of phage (i.e. phage infection resulting in bacterial lysis) that causes the collapse of the culture (Fig. 1; Lin *et al.*, 2017; Furfaro *et al.*, 2018). d'Herelle determined that phage-targeted specific bacterial hosts, and he commonly isolated disease-specific phage from the filtered stool of convalescents (Dublanchet and Bourne, 2007; Wittebole *et al.*, 2014). PT involving strictly lytic phages became a popular treatment in

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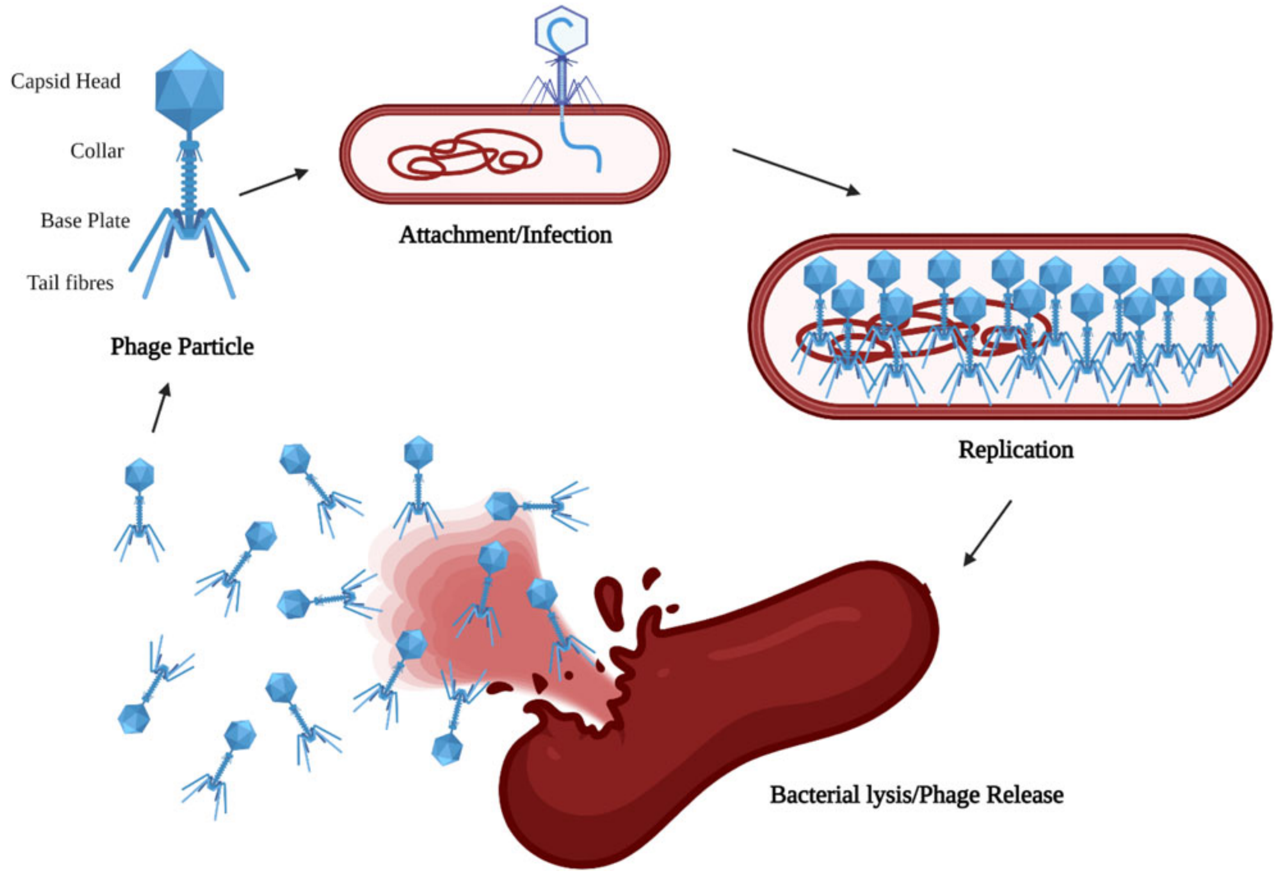

Figure 1. Lytic cycle of phage. Attachment/infection. The phage particle (blue) recognizes specific receptors on the surface of the bacterium (red) via binding proteins in its tail fibers and injects its genetic material into the cell. (Replication) A combination of host and phage factors allows the phage particles to replicate to high numbers within the host cell. (Bacterial lysis/Phage release) Newly synthesized phage particles are released during bacterial lysis caused by phage endolysins rupturing the cell wall and are free to restart the attachment/infection phase with neighboring bacteria. Created with BioRender.com.

the pre-antibiotic decades, particularly in the former USSR (Summers, 2012; Wittebole *et al.*, 2014; Furfaro *et al.*, 2018; Allué-Guardia *et al.*, 2021).

In the post-antibiotic era, PT fell out of favor, partly because the highly specific nature of phage was perceived as a limitation when compared to broad-spectrum antibiotics (Sulakvelidze *et al.*, 2001; Allué-Guardia *et al.*, 2021). There was also controversy surrounding the variable success rates/regulation of treatment and limited understanding of phage biology (Lin *et al.*, 2017). Nowadays, PT, or 'compassionate PT,' is a last-resort option for patients whose infection has evaded all attempts at antibiotic treatment (e.g. multi-drug resistant *Acinetobacter baumanii* and *Pseudomonas aeruginosa*; Furfaro *et al.*, 2018; McCallin *et al.*, 2019; Anomaly, 2020; Allué-Guardia *et al.*, 2021). Using that same logic, it's reasonable to presume PT could be employed in compassionate circumstances in veterinary medicine, particularly diarrheal diseases (which might display AR). There could also be an opportunity for PT to entirely replace certain therapeutic antibiotics in agriculture, as the global call to reduce their usage in this sector may support the routine use of PT in livestock.

Bacteriophage in veterinary medicine

Currently, there is no United States Food and Drug Administration (USFDA) approved PT for use in veterinary medicine, though several have approval for use in food processing, such as the use of phage against *Listeria monocytogenes* to decontaminate surfaces (Kahn *et al.*, 2019; Allué-Guardia *et al.*, 2021). This lack of approved PT for use in animals is liable to change as more evidence is presented regarding their safety and efficacy.

Many studies investigating PT have involved animal models, e.g. murine models of *Escherichia coli* (*E. coli*) infections, which have generally returned positive results and suggest the potential for PT to be used against similar animal illnesses (Atterbury, 2009). This has been practically demonstrated during investigations of PT in food animals. Huff *et al.* (2006) found that treating *E. coli*-challenged chickens with either phage DAF6 or SPR02 reduced mortality associated with colibacillosis by 41%. Similar poultry studies have shown that PT generates significant reductions in *Salmonella* and *Campylobacter* colonization (Atterbury, 2009). Larger livestock, such as calves, piglets, and lambs, have also undergone PT trials that demonstrated phage were capable of reducing the burden of verotoxigenic and enterotoxigenic *E. coli*, with one study demonstrating both effective prophylactic use and remission after the onset of clinical signs (Smith and Huggins, 1983; Atterbury, 2009). Interestingly, during the PT trials conducted by Smith and Huggins (1983), 11 out of 13 calves treated with phage after the onset of diarrhea recovered, while the entire control group died. This drastic contrast between the control and test groups very clearly illustrates how animal death-related profit losses can be reduced by employing PT.

https://doi.org/10.1017/S146625232300004X Published online by Cambridge University Press

While the therapeutic application of phage is not yet standard practice in veterinary health, Smith and Huggins (1983) have also indirectly revealed that the compassionate application of PT may be ideal for use in the terminal stage of a (potentially AR) diarrheal disease, such as Johne's disease (JD), which shall become the focus of this review.

Johne's disease

JD is a chronic gastrointestinal illness of ruminants that features granulomatous enteritis, diarrhea, and nutrient malabsorption, with subsequent weight loss and muscle wasting (Fig. 2; Rathnaiah *et al*., 2017; Stinson *et al*., 2018; Field *et al*., 2022). The signs of JD in cattle were first described in the early 19th century and were likened to 'consumption', i.e. tuberculosis (TB; Skellett, 1807; Dziedzinska and Slana, 2017; Moonan, 2018). It was presumed that *Mycobacterium tuberculosis* (the causative agent of TB) was the causative agent of JD until 1895, when veterinary pathologists Dr Heinrich Johne and Dr Langdon Frothingham identified *Mycobacterium pseudotuberculosis*, later termed *Mycobacterium avium* subsp. *paratuberculosis* (MAP; Harris and Barletta, 2001; Sechi and Dow, 2015; Davis *et al*., 2017; Dziedzinska and Slana, 2017).

Mycobacterium avium *subsp.* paratuberculosis

In veterinary medicine, non-tuberculosis mycobacteria (NTM) infections present more frequently than TB (non-primates rarely develop active TB; Hlokwe *et al*., 2017). One such NTM infection is *paratuberculosis*, or JD, caused by MAP. MAP is an obligate intracellular pathogen that belongs to the *Mycobacterium avium complex* (Garvey, 2020; Matthews *et al*., 2021). It differs from other species in this complex by its ability to infect non-immunocompromised ruminants, its exceptionally slow cultivation (up to 16 weeks or more), its inability to produce the iron-chelator mycobactin and the presence of multiple copies of insertion element IS*900* in its genome (Harris and Barletta, 2001; Tiwari *et al*., 2006; Robertson *et al*., 2017; Cunha *et al*., 2020; Okuni *et al*., 2020). It appears that MAP is more heat stable than other mycobacteria, as previous research has demonstrated its ability to survive pasteurization (Rathnaiah *et al*., 2017; Gerrard *et al*., 2018). Therefore, a test for its rapid detection would therefore not only be extremely useful in veterinary medicine but also in dairy processing.

There is an apparently cyclical relationship between the environmental distribution of MAP and the incidence of JD, as the infected animals shed MAP into the soil, and slurry/run-off introduces MAP to water sources (Salgado *et al*., 2015; Garvey, 2018). Broadly speaking, the extra-intestinal lifecycle of MAP is not well understood, though it has been suggested that free-living amoebae may act as a non-mammalian host that contributes to their environmental persistence, with one study practically demonstrating the survival of MAP in *Acanthamoeba castellani* in vitro (Salgado *et al*., 2015; Samba-Louaka *et al*., 2018; Okuni *et al*., 2020). The thick, waxy cell wall is also considered to be a major factor in the persistence of MAP in the environment, where it appears to contribute to its stability under various heat, UV, and low pH conditions (Okuni *et al*., 2020). Previous studies have found that MAP can remain viable for several months to a year in manure, soil, and/or water, with shaded pastures and troughs proving the most permissible to MAP survival (Whittington *et al*., 2004; Whittington *et al*., 2019). Considering its environmental distribution, an important consideration for designing novel detection assays would be whether the assay can be applied to a wide range of sample types (e.g. blood, soil, or water) to maximize usefulness.

The mycolic acid-rich cell wall and the intracellular lifecycle of MAP also explain the worryingly intrinsic AR of this species to certain antibiotics (e.g. isoniazid; Brown-Elliott *et al*., 2012; Franco-Paredes *et al*., 2018). This emphasizes the need for alternative therapies and control strategies to be found to combat JD, as the intrinsic AR of MAP already limits treatment options. What would be a great benefit, is a clinical treatment and/or detection method that is not unhindered by the mycolic acid-rich cell wall but exploits it. By closely monitoring the farm environment in tandem with animal testing, it should be possible to actively sever routes of transmission, which are discussed below in the context of dairy herds.

Transmission of Mycobacterium avium *subsp.* paratuberculosis

Vertical transmission of MAP involves the prenatal exposure of unborn calves by MAP-positive cows/heifers through transplacental infection (Park *et al*., 2017; Garvey, 2020). The possibility that susceptibility to MAP infection is inheritable has been analyzed to be low to moderate, so it is likely that the prevalence of vertical transmission is in some way related to the prevalence of MAP within the herd (Park *et al*., 2017). An issue inherent to analyses of vertical transmission is difficulty in differentiating vertical from horizontal transmission (Judge *et al*., 2006; Park *et al*., 2017; Mitchell *et al*., 2019).

Horizontal transmission occurs postnatally (pseudo-vertically), often within the first month of life when calves consume contaminated colostrum/milk or are exposed to the fecal matter from the infected mother or other calves (Wolf *et al*., 2015; Al-Mamun *et al*., 2017; Field *et al*., 2022). Horizontal transmission has been occasionally observed in older cattle, primarily through the fecal–oral route, whereby MAP-infected animals shed the bacteria and thereby contaminate the environment, as described in a previous section (Garvey, 2020). This draws attention to the necessity for a highly effective diagnostic test. An ideal test would also have a rapid turnaround time in order to quickly detect and isolate animals who may be shedding MAP and actively infecting other livestock in the herd.

Interestingly, MAP infection does not guarantee progression to JD, as additional factors such as herd size, animal age, and milk production contribute to disease prognosis (Wolf *et al*., 2015; Garvey, 2018; Garvey, 2020). Overall, it is understood that calves are the most at risk of infection due to their underdeveloped immune system (Windsor and Whittington, 2010; Wolf *et al*., 2015; Facciuolo *et al*., 2016; Garvey, 2020).

Pathogenesis of Johne's disease

Progression to JD involves MAP cells breaching the mucosal defences of the small intestine and establishing a niche resulting in granulomatous lesions along the wall of the ileum (Weiss *et al*., 2006; Martcheva *et al*., 2015; DeKuiper and Coussens, 2019). MAP transverse the epithelium by exploiting fibronectin receptors on the surface of microfold (M) cells. M cells are specialized epithelial cells responsible for 'sampling' the lumen contents and transporting potential antigens, including live bacteria, to the underlying lymphoid follicles (Dillon and Lo, 2019; Kobayashi

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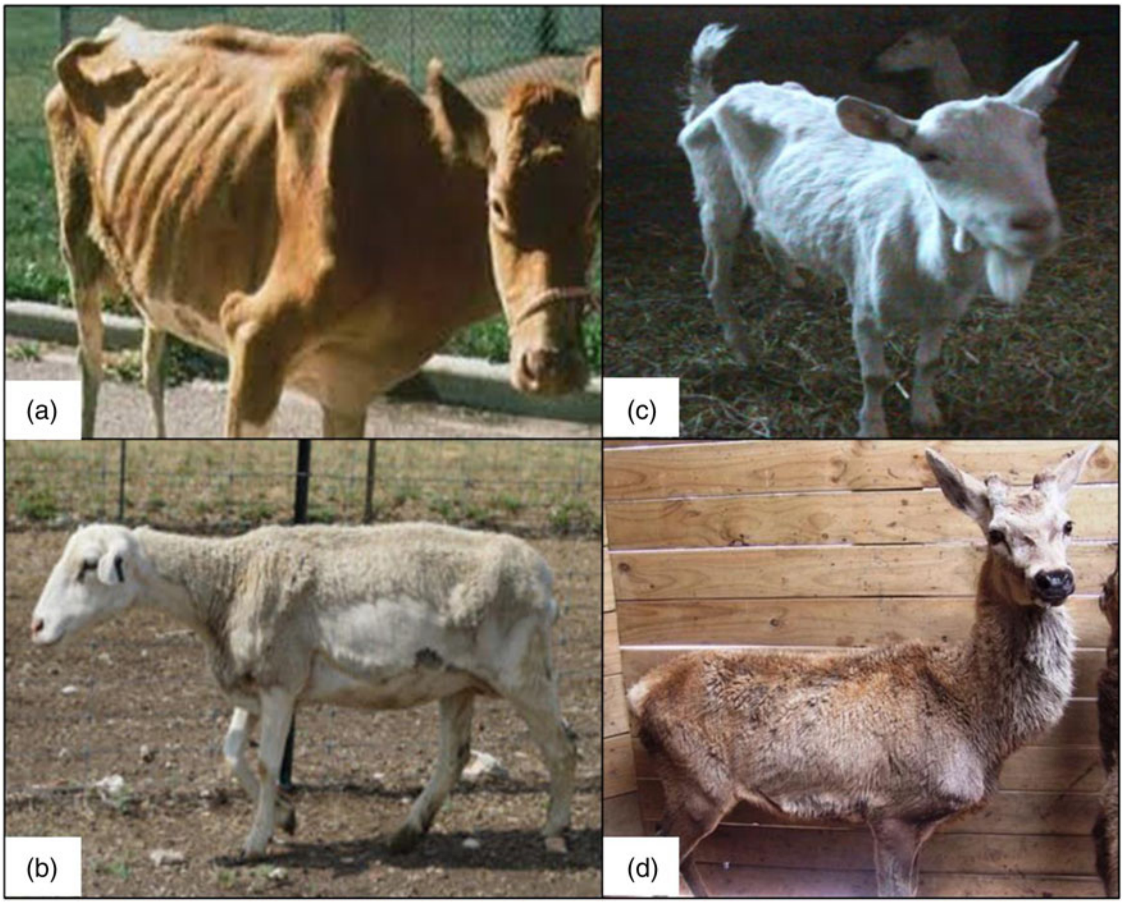

Figure 2. Ruminant animals suffering from clinical Johne's disease. (a) Cow. (b) Goat. (c) Sheep. (d) Deer. Images adapted from https://johnes.org/.

et al., 2019). The lymphoid follicles, termed Peyer's patches, are home to macrophages, which phagocytose the MAP at the basal side of the M cells (Fig. 3; Patel *et al.*, 2006; Wagner *et al.*, 2018; Garvey, 2020). Infected macrophages enter the lymphatic system, which further disseminates the MAP infection (Patel *et al.*, 2006; Tiwari *et al.*, 2006).

During early infection, some MAP survives within the phagosome by preventing lysosome fusion (Broxmeyer *et al.*, 2002). This poses an additional problem in terms of developing novel treatments and rapid diagnostic tests, as the pathogens effectively have a mammalian cell shield preventing the drug or the probe accessing its target. This becomes a greater issue as the infection progresses, as infected macrophages eventually differentiate into epithelioid cells, which aggregate to form granulomas to suppress bacterial growth (Martcheva *et al.*, 2015; Rice *et al.*, 2019). Within the granuloma, MAP enters a dormant state and can gradually reactivate in later stages of infection, resulting in intermittent shedding of MAP in mid-stage disease (Martcheva *et al.*, 2015; Rice *et al.*, 2019; Garvey, 2020). The granulomas attract T lymphocytes to the site of infection, which release inflammatory cytokines that contribute to the pathology of JD (DeKuiper and Coussens, 2019; Rice *et al.*, 2019). For clarity, JD is typically described in four stages based on the severity of signs and likelihood of a positive result from a serological diagnostic test (Table 2; Whitlock and Buergelt, 1996).

Stage I is the preclinical phase and does not display any noticeable pathologies and only post-mortem tissue culture to recover MAP returns a positive diagnosis (Whittington *et al.*, 2017). Similarly, Stage II is the subclinical phase and features no clinical signs, but animals may intermittently shed MAP (Wright *et al.*, 2019; Garvey, 2020). Dairy cows may yield less milk during Stage II which can lead to culling prior to diagnosis (Tiwari *et al.*, 2006). Diagnostic testing at this stage can be difficult, due to the intermittent nature of shedding causing fecal culture to be unreliable and anti-MAP antibodies are usually only detectable shortly before progression to Stage III (Tiwari *et al.*, 2006; Berry *et al.*, 2018). Due to the slow growth rate of MAP and potential for the bacteria to enter a dormant phase within granulomas, infected animals remain in Stages I–II for prolonged incubation periods of 2–5 years (Garvey, 2020; Elsohaby *et al.*, 2021). Clinical signs develop at Stage III, at which point illness is apparent, with the hallmarks of JD (major diarrhea and weight loss) evident while vital signs remain normal (e.g. heart rate; Tiwari *et al.*, 2006; Berry *et al.*, 2018). At this stage, both fecal culture and serological testing will return a positive result for MAP infection (Tiwari *et al.*, 2006). Stage IV is considered advanced clinical disease and is fatal (Künzler *et al.*, 2014; Whittington *et al.*, 2017). The clinical of Stage III worsen, and animals become too weak to stand, anemic, and very skeletal in appearance (Fig. 1a; Tiwari *et al.*, 2006; Garvey, 2020). Diagnostic tests will return positive results but relatively few animals reach this stage due to culling in Stage II or III, typically as an outcome of test-and-cull policies (Forde *et al.*, 2015). While test-and-cull may appear to be a quick and logical process of disease control, the sad truth is that these

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Table 2. Stages of Johne's disease

Stage	Symptoms	Granulomatous lesions	Serology results
I	None	None (potentially not detected)	Negative
II	None	Occasionally	Results vary
III	Nutrient malabsorption, diarrhea, weight loss/muscle-wasting, coat-roughening, and reduced productivity	Observed in the small intestine, particularly the terminal ileum	Positive
IV	Nutrient malabsorption, anemia, dehydration, weakness, fatigue, diarrhea, emaciation, jaw swelling, and high mortality	Observed in the small intestine as well as secondary infection sites, such as mammary glands, lymph nodes, and lymph nodes	Positive

Source: Adapted from the National Research Council Committee on and Control of Johne's Disease (2003) and Tiwari *et al.* (2006).

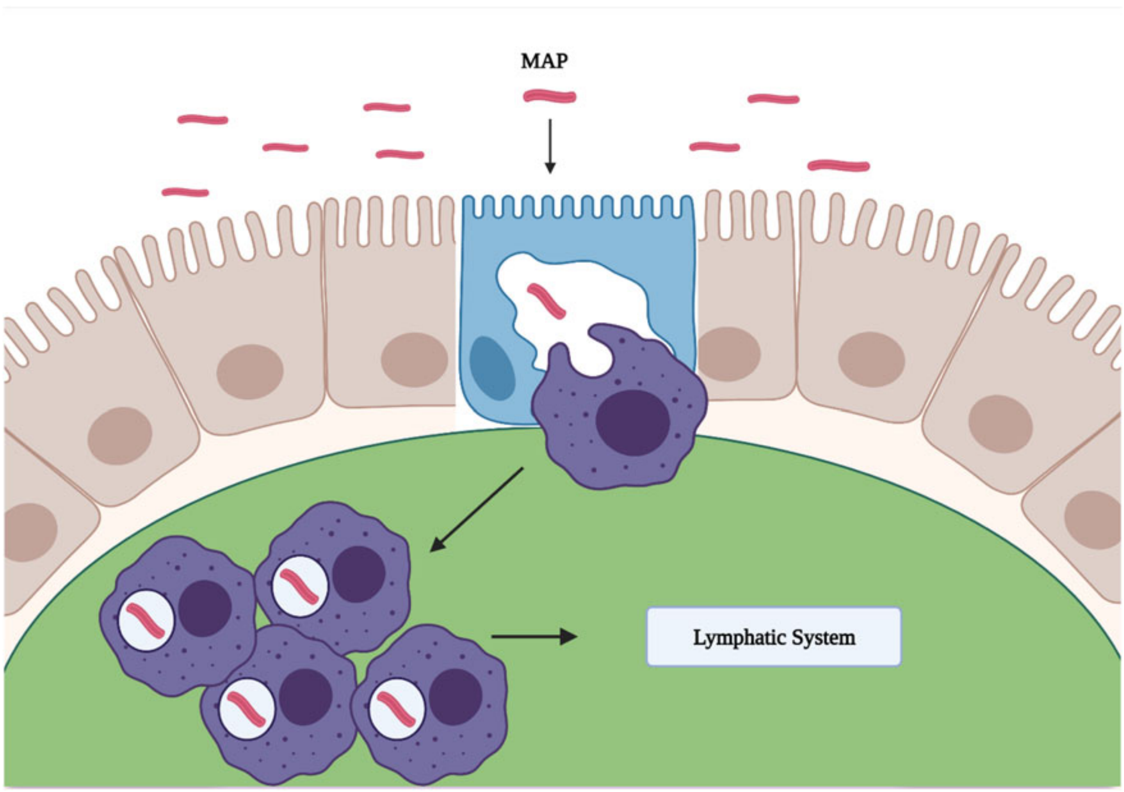

Figure 3. Schematic of MAP uptake by M cells. The MAP (red) interacts with the fibronectin receptors on the surface of the M cell (blue), triggering endocytosis. MAP is released from the endosome at the basal side of the M cell and is presented to macrophages (purple) resident in Peyer's patches (green). The macrophages phagocytose the MAP and will eventually aggregate into granulomas. MAP-infected macrophages may also enter the lymphatic system through the Peyer's patch, which creates a systemic infection and secondary sites of infection, such as in the mammary glands. Created with BioRender.com.

policies are fundamentally flawed, as a result of the unreliability of existing testing methods.

Test-and-cull to limit the transmission of Johne's disease

Culling is believed to be an important control measure for JD (Al-Mamun *et al.*, 2017; Garvey, 2020). Adult cows are typically culled once their milk yield reduces enough to affect overall productivity of the farm, potentially prior to any testing to diagnose subclinical JD (Tiwari *et al.*, 2006; Lavers *et al.*, 2013). As a result of more targeted efforts at reducing the impact of JD, test-based culling has become the standard, with newly bought calves and heifers undergoing testing for MAP infection. A negative result allows the new purchase to be integrated into the wider herd, while a positive result leads to culling (Jordan *et al.*, 2020). An inherent issue with test-and-cull methods is that no testing method is both 100% specific and 100% sensitive and existing programs are expensive and limited by the ability of farmers and veterinarians to recognize JD (Windsor and Whittington, 2010). The three main methods of MAP detection are fecal culture, polymerase chain reaction (PCR)-based assays, and serological tests (Fig. 4).

Fecal culture

Fecal culture is a diagnostic test that determines the cause of diarrhea resulting from a presumed bacterial infection (Hewison *et al.*, 2012). While fecal culture to recover MAP offers a 100% specific result, it is usually only 30% sensitive and, in the context of

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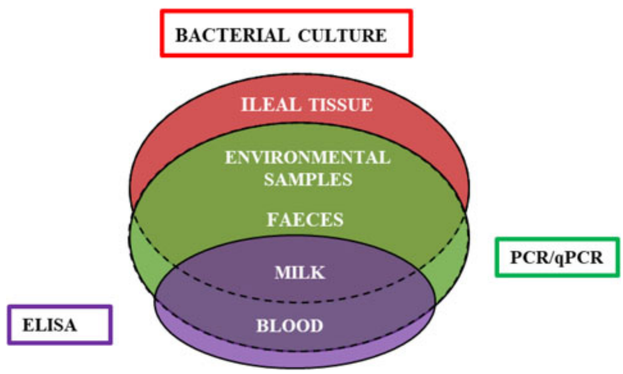

Figure 4. Venn diagram illustrating the types of tests performed on various samples to diagnose JD.

large-scale testing on dairy farms, its cost outweighs the benefits (Lavers *et al*., 2013; Al-Mamun *et al*., 2017). This method of testing is also inefficient for diagnosing JD in a timely manner, due to the long cultivation periods associated with MAP, even when using optimized conditions (Wolf *et al*., 2015; Al-Mamun *et al*., 2017; Okuni *et al*., 2020; Dane *et al*., 2022). Likewise, fecal culture is an unreliable test for JD, due to the intermittent nature of MAP shedding in the subclinical stage and the potential for ingested MAP to be passively shed (i.e. no true infection), leading to incorrect diagnoses (Corbett *et al*., 2019; Whittington *et al*., 2019). Fecal culture may also not be widely available in certain parts of the world, such as Saudi Arabia (Elsohaby *et al*., 2021).

Polymerase chain reaction

Feces-based PCR tests are rapid and comparably sensitive to fecal culture, and the MAP-specific genetic elements, such as the insertion element IS*900* (gold standard), f57, and open reading frame MAP0865, provide prime targets for analysis (Semret *et al*., 2006; Imirzalioglu *et al*., 2011; Cunha *et al*., 2020; Ramovic *et al*., 2020; Elsohaby *et al*., 2021). Quantitative real-time PCR (qPCR) methods for IS*900* detection have improved MAP sensitivity compared to conventional PCR (Sonawane and Tripathi, 2013; Albuquerque *et al*., 2017; Beinhauerova *et al*., 2021) and Acharya *et al*. (2017) have provided solutions to PCR inhibition that improved qPCR sensitivity to 80% compared to fecal culture. qPCR has also been shown to be highly effective at detecting infective MAP in environmental samples (Albuquerque *et al*., 2017; Ramovic *et al*., 2020). However, fecal-qPCR tests can be liable to return false positives due to passive MAP shedding, which can lead to premature culling (Forde *et al*., 2015; Corbett *et al*., 2019; Whittington *et al*., 2019; Beinhauerova *et al*., 2021).

Enzyme-linked absorbance assays

Enzyme-linked absorbance assays (ELISA) have been developed to detect MAP-related antigens, anti-MAP antibodies and elevated interferon-γ (an inflammatory cytokine associated with JD; Harris and Barletta, 2001; Whittington *et al*., 2019). ELISA is a quick and cost-effective method and several user-friendly kits have been created to aid veterinary risk assessments and management plans (Kennedy *et al*., 2016; Whittington *et al*., 2019; Jordan *et al*., 2020). Unlike PCR, ELISA is highly effective at detecting MAP in bulk milk samples (Beaver *et al*., 2017). Unfortunately, though the most economically favorable, ELISA is the least sensitive, with testing complicated by common antigens between MAP and other mycobacteria, such as *Mycobacterium bovis*, and the fact that the immune response in the early stages of JD are cellular (i.e. phagocyte mediated) as opposed to humoral (i.e. antibody mediated; Harris and Barletta, 2001; Beaver *et al*., 2017; Elsohaby *et al*., 2021). Consequently, current strategies rely on using ELISA to determine the overall JD status of herd and ELISA-positive cattle undergo further testing, usually fecal culture, to confirm the diagnosis. However, a positive ELISA does not necessarily predict a positive fecal culture (due to intermittent shedding of MAP; Beaver *et al*., 2017; Whittington *et al*., 2019; Ramovic *et al*., 2020). Research also suggests that veterinarians are not appropriately advised on the handling of serum samples prior to testing, which can affect the outcome of the ELISA (Alinovi *et al*., 2009).

The future of test-and-cull methods

Evidently, vast improvements must be made upon the existing testing methods before test-and-cull becomes a truly efficient method of JD control. Similarly, veterinarians will likely require further education and training regarding JD and associated testing to ensure tests are carried out correctly and in a timely manner. Consequently, an ideal novel diagnostic assay should not only be highly sensitive and specific, but easy to use with clear sample handling guidelines and have a rapid turnaround time to benefit animals whose clinical signs were recognized late. Ideally, a novel test will also be capable of detecting the antibiotic susceptibilities of MAP.

However, until such novel diagnostics are developed and marketed, the unfortunate reality is that the combination of inefficient diagnostics tests, intermittent MAP shedding, and the slow prognosis of JD will inevitably lead to high global prevalence of the disease and subsequent economic losses associated with culling and replacing cattle.

Prevalence and economic impact of Johne's disease

JD is a global issue, though disease prevalence varies between countries and appears to be correlated to whether an informed control program is in available/complied with, and the accessibility/quality of diagnostics (Whittington *et al*., 2019; Jordan *et al*., 2020; Klopfstein *et al*., 2021). The unreliability of the diagnostic tests described in the previous section makes accurately determining prevalence difficult (Windsor and Whittington, 2010; Beaver *et al*., 2017; Mitchell *et al*., 2019; Garvey, 2020). This is reflected in a retrospective analysis undertaken by Lombard *et al*. (2013) that determined the apparent prevalence of JD to be 70.4% in 2007, while the apparent prevalence for that year was 91.1%. The discrepancy between the apparent and true prevalence is important to address in terms of disease control but also in relation to economic losses.

According to Lombard *et al*. (2013), in 1996 the US prevalence of JD was 21.6%, and for the same year Losinger (2005) reports losses of \$200 million ± \$160 million to the US economy. By 2010, the global economic losses tied to JD were more than US \$ 1.5 billion, correlating to the increase in MAP prevalence in the US and Europe in the intervening years (Johnston *et al*., 2010; Lombard *et al*., 2013; Garvey, 2020). The economic impact associated with JD is largely related to the reduction in milk production and the culling of infected animals further reducing the productivity of dairy farms (Harris and Barletta, 2001; Johnston *et al*., 2010; Wolf *et al*., 2015; Rasmussen *et al*., 2021). For instance, MAP-positive dairy farms in Germany lose approximately 1.41% of gross milk revenue and 34–41% of total losses

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incurred by the Irish dairy industry are attributed to premature culling and decreased slaughter value (Rasmussen *et al.*, 2021). There are also additional veterinary costs related to treating MAP-associated mastitis, diagnostic testing, and implementing other control measures (such as routine testing; Garvey, 2020; Jordan *et al.*, 2020). Fewer costs are associated with treatment of JD, as most guidelines promote test-and-cull, but actively treating MAP infection could provide a solution to culling-related losses and expenses (i.e. diagnostic testing and purchasing replacement cattle). It is therefore important to consider how to effectively treat JD, while abiding by the legislation surrounding antibiotic use in food animals and limiting the risk of AR development.

Treatment of Johne's disease and the risk of antibiotic resistance

Presently, clinical JD is considered untreatable, and there is no recommended drug therapy or preventive vaccine (Garvey, 2020), although Australian researchers have recently published optimistic data from a large-scale vaccination trial, in which herd immunity (~70% immunity) was achieved amongst sheep against ovine JD (Links *et al.*, 2021). Some available treatments include anti-inflammatory drugs, steroids, and monoclonal antibodies, but these are only partially effective, and relapse is common (Click, 2011b). Proposed antibiotic treatments for MAP infection involve a multi-drug approach over several months, as is the case for human TB infection (Slocombe, 1982; St-Jean and Jernigan, 1991; Davis *et al.*, 2017).

AR, and the potential development of AR, is an important consideration when designing antibiotic regimens to treat JD. Ramovic *et al.* (2020) observed during a pilot study that MAP and AR Gram-negative bacteria can co-exist within the same herd, thus posing a threat that a reservoir of AR genes is accessible to MAP. Similar to other mycobacteria, MAP has intrinsic resistance to certain antibiotics, such as isoniazid (Harris and Barletta, 2001; Garvey, 2020). Notably, macrolide resistance in MAP may be aided or even mediated by a 'reluctant' dimethyl-transferase, Erm (38), which has been identified in the *M. tuberculosis* complex (Madsen *et al.*, 2005; Brown-Elliott *et al.*, 2012). As traditional culture-based antibiotic susceptibility testing is inefficient in the context of slow-growing MAP (i.e. it can several months to confidently determine susceptibility), the ability for a rapid diagnostic test to not only determine infection status, but the AR status of a positive result, would be a major advantage.

Considering the potential for MAP to become resistant to typical anti-mycobacterial drugs, other approaches have been considered. Forbes *et al.* (2015) have developed a high-throughput screening method to identify small molecules with anti-mycobacterial potential. Probiotics have also shown promise, with the bacterium Dietzia demonstrating the ability to prevent JD development following in utero or neonatal MAP infection, as well as the ability to improve the signs of Stage IV JD to a point of clinical remission (Click, 2011*a*, 2011*b*).

At the time of writing this review, PT has yet to be practically employed as a treatment for JD. However, interest in phage that infect mycobacteria or mycobacteriophage (MP), has been renewed in recent years as incidence of AR associated with human mycobacterial infections (including NTM infections) has increased (Azimi *et al.*, 2019). Similarly, as the global prevalence of JD has increased over recent decades, MP has been explored in lab settings as a potential control measure in the management of JD.

Mycobacteriophage

The first isolated MP targeted *Mycobacterium smegmatis* (*M. smegmatis*) and was identified in 1947. To date, approximately 12,000 MP have been isolated according to the Actinobacteriophage Database (https://phagesdb.org/hosts/genera/1/; accessed 25 July 2022) (Gardner and Weiser, 1947; Allué-Guardia *et al.*, 2021). Most MP was discovered during large screening efforts involving programs aimed at second- and third-level students. For instance, the Phage Hunters Integrating Research and Education (PHIRE) program was developed in the early 2000s and has identified more than 300 novel MP (Hatfull, 2018). Later, the University of Pittsburgh collaborated with the Science Education Alliance to create the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, which has discovered thousands of MP, over 2000 of which have been sequenced (Jacob *et al.*, 2020; Hatfull, 2022).

The sequenced MP revealed extensive genetic diversity. It became apparent that MP genomes are architectural mosaics (meaning specific regions have evident evolutionary lineages, as opposed to the complete genome) with largely conserved gene organizations, featuring structural operons to one end of the genome and the genes required for infection and the lytic/lysogenic lifecycles to the other (Lima-Junior *et al.*, 2016; Allué-Guardia *et al.*, 2021). As a consequence of their mosaic architectures, it is exceedingly difficult to describe the evolution of MP in terms of biological relatedness. Therefore, the phage are grouped into 'clusters' and 'subclusters' according to overall nucleotide sequence similarity (Hatfull, 2018; Sinha *et al.*, 2020). Each cluster is composed of phage which shares a minimum of 35% of their genes and members of subclusters share 90% (Pope *et al.*, 2017; Hatfull, 2018). MP which does not meet the minimum sequence homology required for inclusion in a cluster is termed 'singletons' (Suarez *et al.*, 2020). For a comprehensive review of the MP clusters and genomics, please see Hatfull *et al.* (Hatfull, 2022).

All MP described to date is double-stranded DNA viruses with icosahedral capsids and tails (Fig. 5; Allué-Guardia *et al.*, 2021). The tail proteins are important factors which determine the host specificity and infectability of MP (Hatfull, 2018). Generally, phage recognize host-specific receptors present on the surface of bacteria, but unfortunately identifying mycobacterial receptors of importance for MP has proven to be a challenge and the basis of MP–host interactions remains elusive (McNerney and Traoré, 2005; Allué-Guardia *et al.*, 2021).

Despite the insufficient understanding of how MP interact with their hosts, it has been noted that MP mutate in order to broaden their host specificity, with a relatively high frequency of 1 in every 100,000 acquiring such a mutation (which results in a single amino acid change in the tail proteins; Jacobs-Sera *et al.*, 2012). This adaptability suggests the potential for MP which infects non-pathogenic strains of *M. smegmatis* to readily adapt to infect clinical mycobacteria, such as MAP. MAP-sensitive MP could then be used to aid the biocontrol of JD, by detecting and potentially treating MAP infections.

Applications of mycobacteriophage in the biocontrol of Johne's disease

Mycobacteriophage-based detection of Mycobacterium avium *subsp.* paratuberculosis

MAP-sensitive MP have been applied in novel assays used to detect MAP in herds, bulk milk tanks and the farm environment

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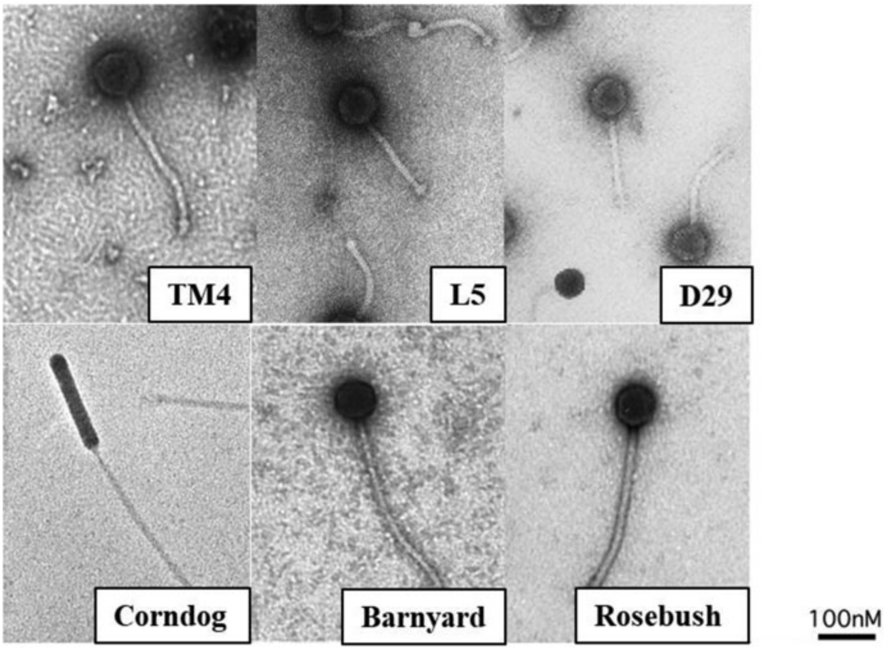

Figure 5. Electron micrographs of several MP. As the lack of genetic relatedness between MP does not easily lend itself to a systematic naming system, in combination with the fact that students are often responsible for their discovery, MP is not named according to any logical nomenclature. As a result, the monikers of MP range from the seemingly typical, such as TM4, L5, and D29, to the delightfully random, such as Corndog, Barnyard, and Rosebush. Adapted from Pedulla *et al.* (2003).

(Fig. 6; Foddai and Grant, 2020). The *FASTPlaque*TB assay was the first MP-based test to be used to detect viable MAP in milk, when researchers in the UK adapted the TB diagnostic tool. The basis of the test is phage-amplification. Viable mycobacteria present in the sample will allow for amplification or propagation of the MP, resulting in an increase in MP-plaque numbers by the end point of the test (Fig. 6a; Stanley *et al.*, 2007). This modified assay was then combined with IS*900*-PCR to determine if the plaques contained MAP DNA to increase the specificity of the assay (Stanley *et al.*, 2007).

More recently, the same UK research group developed a rapid diagnostic test capable of detecting low levels of MAP in blood samples, called Actiphage®. This assay does not require plaques to form prior to PCR analysis, instead the lytic effect of phage D29 is exploited to release DNA from mycobacterial cells present in a sample prior to IS*900*-PCR (Fig. 6b; Swift *et al.*, 2020). During validation, Actiphage® was shown to be more sensitive and as specific as earlier phage-amplification-PCR methods, with Actiphage® detecting MAP in 87% of experimentally infected calves (0% in the control group) while the phage-amplification-PCR detected 66% (Swift *et al.*, 2020).

A similar MAP-detection assay was developed by Foddai and Grant (2020). This assay resembles Actiphage®, in that it is based on the PCR detection of MAP DNA following phage-related lysis, but its procedure builds on a previous assay developed by this group that involves magnetic beads (Foddai *et al.*, 2011). The beads are coated with D29, which retain the ability to interact with MAP. An additional magnet is used to remove the bead-phage-MAP complex, which is then resuspended in fresh media to allow for infection and subsequent cell lysis. Once the cells have been lysed, the released DNA undergoes qPCR analysis to identify samples positive for MAP (Fig. 6c; Foddai and Grant, 2020). A similar phage coated magnetic bead assay also been optimized for detection MAP in milk (Hosseiniporgham *et al.*, 2022)

Something that these assays do not address is AR in MAP. As described in a previous section, MAP possesses intrinsic resistance to certain antibiotics and may have access to a pool of resistance genes present in AR-Gram-negative commensals. There is a TM4-based assay which employs the phage-amplification technique to determine antibiotic sensitivities of *M. smegmatis* (Fig. 6d; Crowley *et al.*, 2019). *Mycobacterium smegmatis* is incubated with the minimum inhibitory concentration of the drug before TM4 is added to the culture (Crowley *et al.*, 2019). Plaque assays are performed similarly to the *FASTPlaque*TB test (Stanley *et al.*, 2007; Crowley *et al.*, 2019). An increase in plaque numbers at the end point of the assay suggests the *M. smegmatis* cells were still viable and therefore resistant to the antibiotic (Crowley *et al.*, 2019). It is very plausible that this assay can be optimized to determine AR-profiles of other mycobacteria, including MAP. This would likely reduce the turnaround considerably compared to traditional culture-based AR-susceptibility testing, because MP-based assays generally take 48 h to obtain results, while MAP culture can exceed 4 months (Broxmeyer *et al.*, 2002; Crowley *et al.*, 2019). The optimized assay could be modified to resemble the *FASTPlaque*TB-IS*900*-PCR assay, so it could simultaneously determine the AR-profile and have high specificity for MAP (Stanley *et al.*, 2007). This could aid the design of more effective antibiotic therapies and lead to a reduction in treatment failure, which would thereby reduce culling and economic losses. Similar loss-reductions could also be achieved, if safe and effective MP therapy (MPT) is developed as an alternative to antibiotic treatment.

Mycobacteriophage-based treatment of Johne's disease

At the time of writing, there have been no published attempts of clinically administering MP in an effort to treat JD. It appears the majority of studies involving MP and MAP only investigated the potential for MP to be used as diagnostic/detection tools, but the notion that they can be developed into a viable treatment option has been entertained before (Emery and Whittington, 2004; Allué-Guardia *et al.*, 2021). While exploration into the MPT in veterinary health is lacking, the rising incidence of extensively drug-resistant TB, and the concurrent rise in NTM and AR-NTM infections, has heightened the interest in MPT within human medicine (Azimi *et al.*, 2019; Allué-Guardia *et al.*, 2021).

Several MP has been investigated for efficacy in reducing the bacterial burden in TB infections, including the model MP D29

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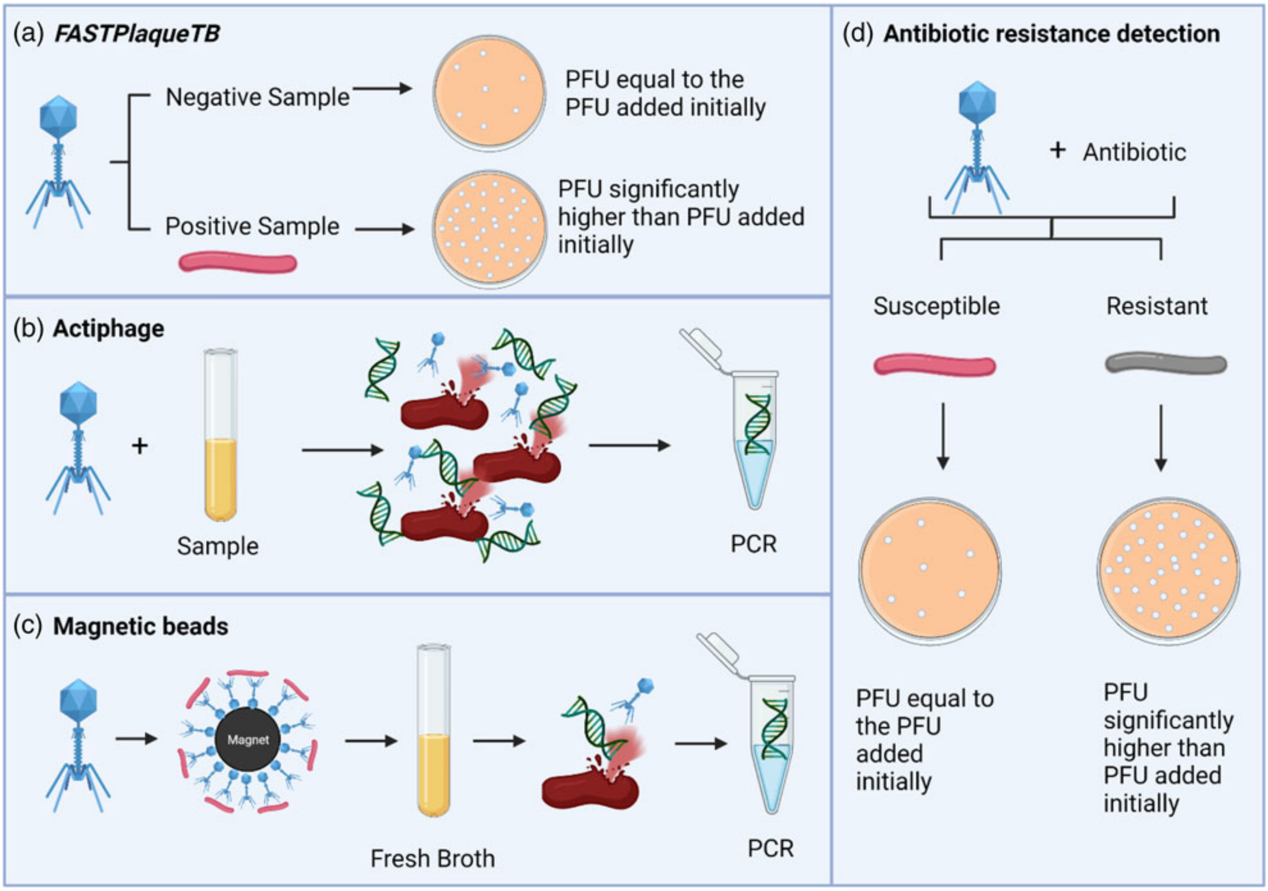

Figure 6. Mycobacterial detection assays. (a) *FASTPlaqueTB*. A known concentration of phage-forming units (PFU) ml^{-1} is added to the samples. If there are no mycobacteria present in the sample, the endpoint PFU ml^{-1} will remain the same as the initial PFU ml^{-1} the phage is unable to propagate. If there are mycobacteria in the sample, the phage will infect the cells and propagate, resulting in an increase in PFU ml^{-1} at the endpoint of the assay relative to the initial concentration. (b) Actiphage. Phage is added to a sample to induce the lysis of any mycobacteria that may be present. Released DNA is then isolated and used in PCR-based detection methods to confirm the presence of mycobacteria. (c) Magnetic beads. Phage is attached to magnetic beads and used to bind to mycobacteria on the sample, which is then removed using a second magnet. The beads are subsequently placed in fresh broth to allow for infection and lysis prior to DNA isolation and PCR-based detection methods. (d). Antibiotic resistance detection. A known concentration of PFU ml^{-1} are added to mycobacterial cultures following the addition of an antibiotic. If the culture is susceptible to that antibiotic, the cells will have been killed prior to phage addition and thus the endpoint PFU ml^{-1} will remain the same as the initial PFU ml^{-1}. If the culture is resistant to that antibiotic, the cells will still be viable at the point of phage addition and the endpoint PFU ml^{-1} will be increased relative to the initial PFU ml^{-1}. Created with BioRender.com.

and TM4 (Azimi *et al*., 2019). These studies have largely been conducted in vitro, and to date only two in vivo studies concerning MPT have been performed (Allué-Guardia *et al*., 2021). While the first in vivo study conducted by Sula *et al*. (1981) demonstrated the therapeutic effect of MP DS-6A against disseminated TB infection in guinea pigs (resulting in fewer lesions in the spleen, liver, and lungs), the second study found that MPT was less effective than isoniazid in treating disseminated TB in the same model (however fewer granulomas were observed in the phage treated guinea pigs; Zemskova and Dorozhkova, 1991). Although the curative properties of MP remain hypothetical, the reduction in granuloma formation associated with disseminated TB could mean that combining MPT and traditional antibiotic regimens could improve prognoses and aid recovery. Likewise, there appears to be hope in prophylactic MPT, as a recent investigation found that mice treated with nebulized D29 prior to TB inhalation had a significantly reduced bacterial burden compared to the control group (Carrigy *et al*., 2019). These results suggest that it may be possible to prophylactically treat dairy cattle (and other susceptible ruminants) against MAP infection, and therefore JD.

Compassionate MPT could also be a viable treatment option, should a MAP infection prove resistant to the recommended antibiotic therapies. For example, a recent case study of an AR-NTM infection in a cystic fibrosis patient has demonstrated the efficacy of genetically engineered-MP treatment in resolving AR mycobacterial infections (Dedrick *et al*., 2019). While evidence that this success story will translate effectively into bovine JD is lacking, the amazing accomplishments of PT associated with compassionate use in human medicine offers hope that its introduction to veterinary medicine will be similarly effective in treating late-stage JD with MP.

Potential limitations of mycobacteriophage therapy

Aside from the lack of legislation surrounding PT and Western society, PT (and therefore MPT) is still largely regarded as an 'experimental' treatment that has little public understanding. For a comprehensive review on the limitations of MPT, please refer to Allué-Guardia *et al*. (2021).

Briefly, host specificity can limit the applicability of any one phage (which is why PT often involves phage cocktails) and there are several concerns regarding the risks posed by phage to mammals (Allué-Guardia *et al*., 2021). These include the risk of toxic shock as a consequence of cytotoxic components being released from lysed bacterial cells following phage infection (Henein, 2013; Allué-Guardia *et al*., 2021). Similarly, the bacterial debris and the phage themselves could possibly trigger an allergic reaction (although no trials in any mammalian model have observed such a reaction; Cisek *et al*., 2017; Allué-Guardia *et al*., 2021). Another possible hindrance is anti-phage antibodies, which may clear the phage before the infection is treated (Cisek *et al*., 2017). Additionally, intracellular pathogens, such as MAP, are presumed to be well concealed by the mammalian cell, thus preventing recognition by phage (Azimi *et al*., 2019; Allué-Guardia *et al*., 2021).

Despite this presumption, it has been observed that phage can exploit transcytosis to cross the epithelial barrier and enter the bloodstream and other organs. It is estimated that 31 billion phage transcytose the epithelial barrier in the gut daily (Nguyen *et al*., 2017; Otero *et al*., 2019). Pathogen-infected phagocytes are also capable of internalizing phage via endocytosis, at which point the endosome and phagosome can merge (as typically only lysosome fusion is inhibited by bacteria), thereby granting phage access to the pathogen (Broxmeyer *et al*., 2002; Jończyk-Matysiak *et al*., 2017). Other investigations have demonstrated the ability of genetically engineered phage to induce endocytosis and kill intracellular bacteria (Bárdy *et al*., 2016; Møller-Olsen *et al*., 2018).

Regarding MAP/mycobacterial infections specifically, there is much interest in the use of *M. smegmatis* or liposomes to facilitate a 'Trojan horse' approach (Broxmeyer *et al*., 2002; Nieth *et al*., 2015; Azimi *et al*., 2019; Otero *et al*., 2019). Non-virulent *M. smegmatis* harboring MP can present antigens to MAP-infected phagocytes and following internalization, can protect the MP from degradation and deliver the phage to the target bacteria (Broxmeyer *et al*., 2002). Similarly, encapsulating MP in liposomes can shield them from undesirable conditions and enable transcytosis of MP by interacting with the lipid membranes of epithelial and phagocytic cells (Otero *et al*., 2019).

While yet to be explored in a clinical setting, it appears that the limitations that may arise during the design of MPT have a good chance of being overcome, particularly as understanding of phage-host and phage-mammalian cell interactions advances. However, it is vital that every potential barrier to effective MPT (and PT in general) is addressed in extreme detail, both to quell any concerns held by the agricultural and veterinary sectors and (especially in the case of compassionate MPT) to confirm the safety and efficacy of treatment.

Concluding remarks

Employing JD as a case study, this review has highlighted the negative impact of this disease on both the physical wellbeing of infected ruminants and the profit margin of the dairy industry. The current limitations associated with diagnosing JD and the risk that its etiological agent, MAP, could develop AR have also been addressed. Ergo, developing novel diagnostic approaches and highly targeted treatments would benefit both the agricultural and health sector, by reducing the economic impact of JD and the potentially negative impact of antibiotic use in livestock. The notion that MP may constitute a highly effective basis for rapid MAP-detection assays and possibly phage-based medications, which can greatly aid JD control programs, has been emphasized in this review. While the introduction of MP-based control measures for JD is a relatively novel approach, and currently there is little evidence supporting the in vivo efficacy of MPT, the potential for these simple organisms to transform how JD is managed is considerable.

Acknowledgments. The authors would like to acknowledge the financial contribution of the Risam PhD Scholarship Fund.

References

Acharya KR, Dhand NK, Whittington RJ and Plain KM (2017) PCR inhibition of a quantitative PCR for detection of *Mycobacterium avium* subspecies *paratuberculosis* DNA in feces: diagnostic implications and potential solutions. *Frontiers in Microbiology* **8**: 115.

Al-mamun MA, Smith RL, Schukken YH and Gröhn YT (2017) Use of an individual-based model to control transmission pathways of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle herds. *Scientific Reports* **7**: 11845.

Alayande KA, Aiyegoro OA and Ateba CN (2020) Probiotics in animal husbandry: applicability and associated risk factors. *Sustainability* **12**: 1087.

Albuquerque PPFD, Santos ADS, Souza Neto OLD, Kim PDCP, Cavalcanti EFTSF, Oliveira JMBD, Mota RA and Júnior JWP (2017) Detection of *Mycobacterium avium* subsp. *paratuberculosis* in bovine milk from the state of Pernambuco, Brazil. *Brazilian Journal of Microbiology* **48**; 113–117.

Alinovi CA, Ward MP, Lin TL and Wu CC (2009) Sample handling substantially affects Johne's ELISA. *Preventive Veterinary Medicine* **90**: 278–283.

Allué-Guardia A, Saranathan R, Chan J and Torrelles JB (2021) Mycobacteriophages as potential therapeutic agents against drug-resistant tuberculosis. *International Journal of Molecular Sciences* **22**: 735.

Anomaly J (2020) The future of phage: ethical challenges of using phage therapy to treat bacterial infections. *Public Health Ethics* **13**: 82–88.

Arowolo MA and He J (2018) Use of probiotics and botanical extracts to improve ruminant production in the tropics: a review. *Animal Nutrition* **4**: 241–249.

Asha MZ and Khalil SFH (2020) Efficacy and safety of probiotics, prebiotics and synbiotics in the treatment of irritable bowel syndrome: a systematic review and meta-analysis. *Sultan Qaboos University Medical Journal* **20**: e13–e24.

Atterbury RJ (2009) Bacteriophage biocontrol in animals and meat products. *Microbial Biotechnology* **2**: 601–612.

Azimi T, Mosadegh M, Nasiri MJ, Sabour S, Karimaei S and Nasser A (2019) Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review. *Infection and Drug Resistance* **12**: 2943–2959.

Bak H and Rathkjen PH (2009) Reduced use of antimicrobials after vaccination of pigs against porcine proliferative enteropathy in a Danish SPF herd. *Acta Veterinaria Scandinavica* **51**: 1.

Bárdy P, Pantůček R, Benešík M and Doškař J (2016) Genetically modified bacteriophages in applied microbiology. *Journal of Applied Microbiology* **121**: 618–633.

Bazin H (2003) A brief history of the prevention of infectious diseases by immunisations. *Comparative Immunology, Microbiology & Infectious Diseases* **26**: 293–308.

Beaver A, Sweeney RW, Hovingh E, Wolfgang DR, Gröhn YT and Schukken YH (2017) Longitudinal relationship between fecal culture, fecal quantitative PCR, and milk ELISA in *Mycobacterium avium* ssp. *paratuberculosis*-infected cows from low-prevalence dairy herds. *Journal of Dairy Science* **100**: 7507–7521.

Beinhauerova M, Beinhauerova M, Mccallum S, Sellal E, Ricchi M, O'brien R, Blanchard B, Slana I, Babak V and Kralik P (2021) Development of a reference standard for the detection and quantification of *Mycobacterium avium* subsp. *paratuberculosis* by quantitative PCR. *Scientific Reports* **11**: 11622.

Bengtsson B and Greko C (2014) Antibiotic resistance – consequences for animal health, welfare, and food production. *Upsala Journal of Medical Sciences* **119**: 96–102.

Berry A, Wu C-W, Venturino AJ and Talaat AM (2018) Biomarkers for early stages of Johne's disease infection and immunization in goats. *Frontiers in Microbiology* **9**: 2284.

Bhogoju S and Nahashon S (2022) Recent advances in probiotic application in animal health and nutrition: a review. *Agriculture* **12**: 304.

Brown-Elliott BA, Nash KA and Wallace Jr RJ (2012) Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clinical Microbiology Reviews* **25**: 545–582.

Broxmeyer L, Sosnowska D, Miltner E, Chacón O, Wagner D, Mcgarvey J, Barletta RG and Bermudez LE (2002) Killing of *Mycobacterium avium* and *Mycobacterium tuberculosis* by a mycobacteriophage delivered by a nonvirulent mycobacterium: a model for phage therapy of intracellular bacterial pathogens. *The Journal of Infectious Diseases* **186**: 1155–1160.

Cáceres SB (2011) The long journey of cattle plague. *The Canadian Veterinary Journal* **52**: 1140.

Carrigy NB, Larsen SE, Reese V, Pecor T, Harrison M, Kuehl PJ, Hatfull GF, Sauvageau D, Baldwin SL, Finlay WH, Coler RN and Vehring R (2019) Prophylaxis of *Mycobacterium tuberculosis* H37Rv infection in a preclinical mouse model via inhalation of nebulized bacteriophage D29. *Antimicrobial Agents and Chemotherapy* **63**: e00871-19.

Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L and Yuan Z (2014) Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in Microbiology* **5**: 217–217.

Cisek AA, Dąbrowska I, Gregorczyk KP and Wyżewski Z (2017) Phage therapy in bacterial infections treatment: one hundred years after the discovery of bacteriophages. *Current Microbiology* **74**: 277–283.

Click RE (2011a) A 60-day probiotic protocol with Dietzia subsp. C79793–74 prevents development of Johne's disease parameters after in utero and/or neonatal MAP infection. *Virulence* **2**: 337–347.

Click RE (2011b) Successful treatment of asymptomatic or clinically terminal bovine *Mycobacterium avium* subspecies *paratuberculosis* infection (Johne's disease) with the bacterium Dietzia used as a probiotic alone or in combination with dexamethasone: adaption to chronic human diarrheal diseases. *Virulence* **2**: 131–143.

Collignon PJ and Mcewen SA (2019) One Health - its importance in helping to better control antimicrobial resistance. *Tropical Medicine and Infectious Disease* **4**: 22.

Corbett CS, De Jong MCM, Orsel K, De Buck J and Barkema HW (2019) Quantifying transmission of *Mycobacterium avium* subsp. *paratuberculosis* among group-housed dairy calves. *Veterinary Research* **50**: 60.

Crowley G, O'Mahony J, Coffey A, Sayers R and Cotter P (2019) A rapid viability and drug-susceptibility assay utilizing mycobacteriophage as an indicator of drug susceptibilities of anti-TB drugs against *Mycobacterium smegmatis* mc^2 155. *International Journal of Mycobacteriology* **8**: 124–131.

Cunha MV, Rosalino LM, Leão C, Bandeira V, Fonseca C, Botelho A and Reis AC (2020) Ecological drivers of *Mycobacterium avium* subsp. *paratuberculosis* detection in mongoose (*Herpestes ichneumon*) using IS900 as proxy. *Scientific Reports* **10**: 860.

Cuong NV, Padungtod P, Thwaites G and Carrique-Mas JJ (2018) Antimicrobial usage in animal production: a review of the literature with a focus on low- and middle-income countries. *Antibiotics* **7**: 75.

Dadgostar P (2019) Antimicrobial resistance: implications and costs. *Infection and Drug Resistance* **12**: 3903–3910.

Dane H, Koidis A, Stewart LD and Grant IR (2022) Optimization of the composition of a solid culture medium for *Mycobacterium avium* subsp. *paratuberculosis* using factorial design and response surface methodology. *Journal of Applied Microbiology* **132**: 4252–4265.

Davis WC, Kuenstner JT and Singh SV (2017) Resolution of Crohn's (Johne's) disease with antibiotics: what are the next steps? *Expert Review of Gastroenterology & Hepatology* **11**: 393–336.

Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, Gilmour KC, Soothill J, Jacobs-Sera D, Schooley RT, Hatfull GF and Spencer H (2019) Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. *Nature Medicine* **25**: 730–733.

Dekuiper JL and Coussens PM (2019) Inflammatory Th17 responses to infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cattle and their potential role in development of Johne's disease. *Veterinary Immunology and Immunopathology* **218**: 109954.

Dillon A and Lo DD (2019) M cells: intelligent engineering of mucosal immune surveillance. *Frontiers in Immunology* **10**: 1499.

Direkvandi E, Mohammadabadi T and Salem AZM (2020) Effect of microbial feed additives on growth performance, microbial protein synthesis, and rumen microbial population in growing lambs. *Translational Animal Science* **4**: 203.

Donadeu M, Nwankpa N, Abela-Ridder B and Dungu B (2019) Strategies to increase adoption of animal vaccines by smallholder farmers with focus on neglected diseases and marginalized populations. *PLoS Neglected Tropical Diseases* **13**: e0006989.

Donner L, Staley Zachery R, Petali J, Sangster J, Li X, Mathews W, Snow D, Howe A, Soupir M, Bartelt-Hunt S and Auchtung Jennifer M (2022) The human health implications of antibiotic resistance in environmental isolates from two Nebraska watersheds. *Microbiology Spectrum* **10**: e02082-21.

Dublanchet A and Bourne S (2007) The epic of phage therapy. *The Canadian Journal of Infectious Diseases & Medical Microbiology* **18**: 15–18.

Duerden B, Fry C, Johnson AP and Wilcox MH (2015) The control of methicillin-resistant *Staphylococcus aureus* blood stream infections in England. *Open Forum Infectious Diseases* **2**: ofv035.

Dziedzinska R and Slana I (2017) *Mycobacterium avium* subsp. *paratuberculosis* – An overview of the publications from 2011 to 2016. *Current Clinical Microbiology Reports* **4**: 19–28.

Economou V and Gousia P (2015) Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance* **8**: 49–61.

Elsohaby I, Fayez M, Alkafafy M, Refaat M, Al-Marri T, Alaql FA, Al Amer AS, Abdallah A and Elmoslemany A (2021) Serological and molecular characterization of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from sheep, goats, cattle and camels in the Eastern Province, Saudi Arabia. *Animals* **11**: 323.

Emery DL and Whittington RJ (2004) An evaluation of mycophage therapy, chemotherapy and vaccination for control of *Mycobacterium avium* subsp. *paratuberculosis* infection. *Veterinary Microbiology* **104**: 143–155.

Facciuolo A, Gonzalez-Cano P, Napper S, Griebel PJ and Mutharia LM (2016) Marked differences in mucosal immune responses induced in Ileal versus Jejunal Peyer's patches to *Mycobacterium avium* subsp. *paratuberculosis* secreted proteins following targeted enteric infection in young calves. *PLoS One* **11**: e0158747.

Fair RJ and Tor Y (2014) Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry* **6**: 25–64.

Field NL, Mcaloon CG, Gavey L and Mee JF (2022) *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle – a review in the context of seasonal pasture-based dairy herds. *Irish Veterinary Journal* **75**: 12.

Foddai ACG and Grant IR (2020) A novel one-day phage-based test for rapid detection and enumeration of viable *Mycobacterium avium* subsp. *paratuberculosis* in cows' milk. *Applied Microbiology and Biotechnology* **104**: 9399–9412.

Foddai A, Strain S, Whitlock RH, Elliott CT and Grant IR (2011) Application of a peptide-mediated magnetic separation-phage assay for detection of viable *Mycobacterium avium* subsp. *paratuberculosis* to bovine bulk tank milk and feces samples. *Journal of Clinical Microbiology* **49**: 2017–2019.

Forbes L, Ebsworth-Mojica K, Didone L, Li SG, Freundlich JS, Connell N, Dunman PM and Krysan DJ (2015) A high throughput screening assay for anti-mycobacterial small molecules based on adenylate kinase release as a reporter of cell lysis. *PLoS One* **10**: e0129234.

Forde T, Pruvot M, De Buck J and Orsel K (2015) A high-morbidity outbreak of Johne's disease in game-ranched elk. *The Canadian Veterinary Journal* **56**: 479–483.

Franco-Paredes C, Marcos LA, Henao-Martínez AF, Rodríguez-Morales AJ, Villamil-Gómez WE, Gotuzzo E and Bonifaz A (2018) Cutaneous mycobacterial infections. *Clinical Microbiology Reviews* **32**: e00069-18.

Furfaro LL, Payne MS and Chang BJ (2018) Bacteriophage therapy: clinical trials and regulatory hurdles. *Frontiers in Cellular and Infection Microbiology* **8**: 376.

Gardner GM and Weiser RS (1947) Discovery of a bacteriophage for *Mycobacterium smegmatis*. *Journal of Bacteriology* **54**: 272.

Garvey M (2018) *Mycobacterium avium* subspecies *paratuberculosis*: a possible causative agent in human morbidity and risk to public health safety. *Open Veterinary Journal* **8**: 172–181.

Garvey M (2020) *Mycobacterium avium paratuberculosis*: a disease burden on the dairy industry. *Animals* **10**: 1773.

Gerrard ZE, Swift BMC, Botsaris G, Davidson RS, Hutchings MR, Huxley JN and Rees CED (2018) Survival of *Mycobacterium avium* subspecies *paratuberculosis* in retail pasteurised milk. *Food Microbiology* **74**: 57–63.

Gyles C (2015) Veterinary schools – which ones are the best? *The Canadian Veterinary Journal* **56**: 787–790.

Harris NB and Barletta RG (2001) *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. *Clinical Microbiology Reviews* **14**: 489–512.

Hatfull GF (2018) Mycobacteriophages. *Microbiology Spectrum* **6**: 10.1128/microbiolspec.GPP3-0026-2018

Hatfull GF (2022) Mycobacteriophages: from Petri dish to patient. *PLoS Pathogens* **18**: e1010602.

Henein A (2013) What are the limitations on the wider therapeutic use of phage? *Bacteriophage* **3**: e24872.

Hewison CJ, Heath CH and Ingram PR (2012) Stool culture. *Australian Family Physician* **41**: 775–779.

Hlokwe TM, Said H and Gcebe N (2017) *Mycobacterium tuberculosis* infection in cattle from the Eastern Cape Province of South Africa. *BMC Veterinary Research* **13**: 299.

Hoelzer K, Bielke L, Blake DP, Cox E, Cutting SM, Devriendt B, Erlacher-Vindel E, Goossens E, Karaca K, Lemiere S, Metzner M, Raicek M, Collell Suriñach M, Wong NM, Gay C and Van Immerseel F (2018) Vaccines as alternatives to antibiotics for food producing animals. Part 1: Challenges and needs. *Veterinary Research* **49**: 64.

Hosseiniporgham S, Rebechesu L, Pintore P, Lollai S, Dattena M, Russo S, Ruiu A and Sechi LA (2022) A rapid phage assay for detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Scientific Reports* **12**: 475.

Hua M, Huang W, Chen A, Rehmet M, Jin C and Huang Z (2020) Comparison of antimicrobial resistance detected in environmental and clinical isolates from historical data for the US. *BioMed Research International* **2020**: 4254530.

Huff WE, Huff GR, Rath NC and Donoghue AM (2006) Evaluation of the influence of bacteriophage titer on the treatment of colibacillosis in broiler chickens. *Poultry Science* **85**: 1373–1377.

Hunter P (2018) The genetics of domestication: research into the domestication of livestock and companion animals sheds light both on their "evolution" and human history. *EMBO Reports* **19**: 201–205.

Hutchings MI, Truman AW and Wilkinson B (2019) Antibiotics: past, present and future. *Current Opinion in Microbiology* **51**: 72–80.

Imirzalioglu C, Dahmen H, Hain T, Billion A, Kuenne C, Chakraborty T and Domann E (2011) Highly specific and quick detection of *Mycobacterium avium* subsp. *paratuberculosis* in feces and gut tissue of cattle and humans by multiple real-time PCR assays. *Journal of Clinical Microbiology* **49**: 1843.

Jacob C, Alvarez Y, Carney T, Castro C, Alvarez R and Connors BJ (2020) Genome sequences of mycobacteriophages Joselito, Patt, and Tydolla. *Microbiology Resource Announcements* **9**: e00519–20.

Jacobs-Sera D, Marinelli LJ, Bowman C, Broussard GW, Guerrero Bustamante C, Boyle MM, Petrova ZO, Dedrick RM, Pope WH, Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) Program, Modlin RL, Hendrix RW and Hatfull GF (2012) On the nature of mycobacteriophage diversity and host preference. Virology 434: 187–201.

Johnston C, Coffey A, O'Mahony J and Sleator RD (2010) Development of a novel oral vaccine against *Mycobacterium avium paratuberculosis* and Johne disease: a patho-biotechnological approach. *Bioengineered Bugs* **1**: 155–163.

Jończyk-Matysiak E, Weber-Dąbrowska B, Owczarek B, Międzybrodzki R, Łusiak-Szelachowska M, Łodej N and Górski A (2017) Phage-phagocyte interactions and their implications for phage application as therapeutics. *Viruses* **9**: 150.

Jordan AG, Citer LR, Mcaloon CG, Graham DA, Sergeant ESG and More SJ (2020) Johne's disease in Irish dairy herds: considerations for an effective national control programme. *Irish Veterinary Journal* **73**: 18.

Judge J, Kyriazakis I, Greig A, Davidson RS and Hutchings MR (2006) Routes of intraspecies transmission of *Mycobacterium avium* subsp. *paratuberculosis* in rabbits (*Oryctolagus cuniculus*): a field study. *Applied and Environmental Microbiology* **72**: 398–403.

Kahn LH, Bergeron G, Bourassa MW, De Vegt B, Gill J, Gomes F, Malouin F, Opengart K, Ritter GD, Singer RS, Storrs C and Topp E (2019) From farm management to bacteriophage therapy: strategies to reduce antibiotic use in animal agriculture. *Annals of the New York Academy of Sciences* **1441**: 31–39.

Käppeli N, Morach M, Zurfluh K, Corti S, Nüesch-Inderbinen M and Stephan R (2019) Sequence types and antimicrobial resistance profiles of *Streptococcus uberis* isolated from bovine mastitis. *Frontiers in Veterinary Science* **6**: 234.

Kennedy AE, Byrne N, Garcia AB, O'Mahony J and Sayers RG (2016) Analysis of Johne's disease ELISA status and associated performance parameters in Irish dairy cows. *BMC Veterinary Research* **12**: 43.

Klopfstein M, Leyer A, Berchtold B, Torgerson PR and Meylan M (2021) Limitations in the implementation of control measures for bovine *paratuberculosis* in infected Swiss dairy and beef herds. *PLoS One* **16**: e0245836.

Kobayashi N, Takahashi D, Takano S, Kimura S and Hase K (2019) The roles of Peyer's patches and microfold cells in the gut immune system: relevance to autoimmune diseases. *Frontiers in Immunology* **10**: 2345.

Künzler R, Torgerson P, Keller S, Wittenbrink M, Stephan R, Knubben-Schweizer G, Berchtold B and Meylan M (2014) Observed management practices in relation to the risk of infection with *paratuberculosis* and to the spread of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss dairy and beef herds. *BMC Veterinary Research* **10**: 132.

Landers TF, Cohen B, Wittum TE and Larson EL (2012) A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Reports* **127**: 4–22.

Lavers CJ, Mckenna SLB, Dohoo IR, Barkema HW and Keefe GP (2013) Evaluation of environmental fecal culture for *Mycobacterium avium* subspecies *paratuberculosis* detection in dairy herds and association with apparent within-herd prevalence. *The Canadian Veterinary Journal* **54**: 1053–1060.

Lima-Junior JD, Viana-Niero C, Conde Oliveira DV, Machado GE, Rabello MCDS, Martins-Junior J, Martins LF, Digiampietri LA, Da Silva AM, Setubal JC, Russell DA, Jacobs-Sera D, Pope WH, Hatfull GF and Leão SC (2016) Characterization of mycobacteria and mycobacteriophages isolated from compost at the São Paulo Zoo Park Foundation in Brazil and creation of the new mycobacteriophage Cluster U. *BMC Microbiology* **16**: 111.

Lin DM, Koskella B and Lin HC (2017) Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics* **8**: 162–173.

Links IJ, Denholm LJ, Evers M, Kingham LJ and Greenstein RJ (2021) Is vaccination a viable method to control Johne's disease caused by *Mycobacterium avium* subsp. *paratuberculosis*? Data from 12 million ovine vaccinations and 7.6 million carcass examinations in New South Wales, Australia from 1999-2009. *PLoS One* **16**: e0246411.

Lombard JE, Gardner IA, Jafarzadeh SR, Fossler CP, Harris B, Capsel RT, Wagner BA and Johnson WO (2013) Herd-level prevalence of *Mycobacterium avium* subsp. *paratuberculosis* infection in United States dairy herds in 2007. *Preventive Veterinary Medicine* **108**: 234–238.

Losinger WC (2005) Economic impact of reduced milk production associated with Johne's disease on dairy operations in the USA. *Journal of Dairy Research* **72**: 425–432.

Mackenzie JS and Jeggo M (2019) The One Health approach – why is it so important? *Tropical Medicine and Infectious Disease* **4**: 88.

Madsen CT, Jakobsen L and Douthwaite S (2005) *Mycobacterium smegmatis* Erm(38) is a reluctant dimethyltransferase. *Antimicrobial Agents and Chemotherapy* **49**: 3803–3809.

Manyi-Loh C, Mamphweli S, Meyer E and Okoh A (2018) Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules* **23**: 795.

Markowiak P and Śliżewska K (2018) The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens* **10**: 21.

Martcheva M, Lenhart S, Eda S, Klinkenberg D, Momotani E and Stabel J (2015) An immuno-epidemiological model for Johne's disease in cattle. *Veterinary Research* **46**: 69.

https://doi.org/10.1017/S146625232300004X Published online by Cambridge University Press

Martin MJ, Thottathil SE and Newman TB (2015) Antibiotics overuse in animal agriculture: a call to action for health care providers. *American Journal of Public Health* **105**: 2409–2410.

Martin H, Manzanilla EG, More SJ, O'neill L, Bradford L, Carty CI, Collins ÁB and Mcaloon CG (2020) Current antimicrobial use in farm animals in the Republic of Ireland. *Irish Veterinary Journal* **73**: 11.

Matthews C, Cotter PD and O'Mahony J (2021) MAP, Johne's disease and the microbiome; current knowledge and future considerations. *Animal Microbiome* **3**: 34.

Mccallin S, Sacher JC, Zheng J and Chan BK (2019) Current state of compassionate phage therapy. *Viruses* **11**: 343.

Mcnerney R and Traoré H (2005) Mycobacteriophage and their application to disease control. *Journal of Applied Microbiology* **99**: 223–233.

Mcvey S and Shi J (2010) Vaccines in veterinary medicine: a brief review of history and technology. *The Veterinary Clinics of North America. Small Animal Practice* **40**: 381–392.

Meeusen ENT, Walker J, Peters A, Pastoret P-P and Jungersen G (2007) Current status of veterinary vaccines. *Clinical Microbiology Reviews* **20**: 489–510.

Mirzad AN, Goto A, Endo T, Ano H, Kobayashi I, Yamauchi T and Katamoto H (2019) Effects of live yeast supplementation on serum oxidative stress biomarkers and lactation performance in dairy cows during summer. *The Journal of Veterinary Medical Science* **81**: 1705–1712.

Mitchell RM, Beaver A, Knupfer E, Pradhan AK, Fyock T, Whitlock RH and Schukken YH (2019) Elucidating transmission patterns of endemic *Mycobacterium avium* subsp. *paratuberculosis* using molecular epidemiology. *Veterinary Sciences* **6**: 32.

Møller-Olsen C, Ho SFS, Shukla RD, Feher T and Sagona AP (2018) Engineered K1F bacteriophages kill intracellular *Escherichia coli* K1 in human epithelial cells. *Scientific Reports* **8**: 17559.

Moonan PK (2018) Tuberculosis – the face of struggles, the struggles we face, and the dreams that lie within. *Emerging Infectious Diseases* **24**: 592–593.

More SJ (2020) European perspectives on efforts to reduce antimicrobial usage in food animal production. *Irish Veterinary Journal* **73**: 2.

Mourant JR, Fenimore PW, Manore CA and Mcmahon BH (2018) Decision support for mitigation of livestock disease: rinderpest as a case study. *Frontiers in Veterinary Science* **5**: 182.

Nguyen S, Baker K, Padman BS, Patwa R, Dunstan RA, Weston TA, Schlosser K, Bailey B, Lithgow T, Lazarou M, Luque A, Rohwer F, Blumberg RS and Barr JJ (2017) Bacteriophage transcytosis provides a mechanism to cross epithelial cell layers. *mBio* **8**: e01874-17.

Nieth A, Verseux C, Barnert S, Süss R and Römer W (2015) A first step toward liposome-mediated intracellular bacteriophage therapy. *Expert Opinion on Drug Delivery* **12**: 1411–1424.

Nowakiewicz A, Zięba P, Gnat S and Matuszewski Ł (2020) Last call for replacement of antimicrobials in animal production: modern challenges, opportunities, and potential solutions. *Antibiotics* **9**: 883.

Okuni JB, Hansen S, Eltom KH, Eltayeb E, Amanzada A, Omega JA, Czerny CP, Abd El Wahed A and Ojok L (2020) Paratuberculosis: a potential zoonosis and a neglected disease in Africa. *Microorganisms* **8**: 1007.

Otero J, García-Rodríguez A, Cano-Sarabia M, Maspoch D, Marcos R, Cortés P and Llagostera M (2019) Biodistribution of liposome-encapsulated bacteriophages and their transcytosis during oral phage therapy. *Frontiers in Microbiology* **10**: 689.

Palma E, Tilocca B and Roncada P (2020) Antimicrobial resistance in veterinary medicine: an overview. *International Journal of Molecular Sciences* **21**: 1914.

Park H-T, Park H-E, Cho Y-I, Kim E-H, Jung M, Shin SW, Lee S-H, Kim D-Y and Yoo HS (2017) Potential biomarkers as an indicator of vertical transmission of Johne's disease in a Korean native cattle farm. *Journal of Veterinary Science* **18**: 343–349.

Patel D, Danelishvili L, Yamazaki Y, Alonso M, Paustian ML, Bannantine JP, Meunier-Goddik L and Bermudez LE (2006) The ability of *Mycobacterium avium* subsp. *paratuberculosis* to enter bovine epithelial cells is influenced by preexposure to a hyperosmolar environment and intracellular passage in bovine mammary epithelial cells. *Infection and Immunity* **74**: 2849–2855.

Pedulla ML, Ford ME, Houtz JM, Karthikeyan T, Wadsworth C, Lewis JA, Jacobs-Sera D, Falbo J, Gross J, Pannunzio NR, Brucker W, Kumar V, Kandasamy J, Keenan L, Bardarov S, Kriakov J, Lawrence JG, Jacobs WR Jr, Hendrix RW and Hatfull GF (2003) Origins of highly mosaic mycobacteriophage genomes. *Cell* **113**, 171–182.

Pinloche E, Mcewan N, Marden J-P, Bayourthe C, Auclair E and Newbold CJ (2013) The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. *PLoS One* **8**: e67824.

Pope WH, Mavrich TN, Garlena RA, Guerrero-Bustamante CA, Jacobs-Sera D, Montgomery MT, Russell DA, Warner MH and Hatfull GF (2017) Bacteriophages of *Gordonia* spp. display a spectrum of diversity and genetic relationships. *mBio* **8**: e01069-17.

Ramirez Rozzi F and Froment A (2018) Earliest animal cranial surgery: from cow to man in the neolithic. *Scientific Reports* **8**: 5536.

Ramovic E, Madigan G, Mcdonnell S, Griffin D, Bracken E, Nighallchoir E, Quinless E, Galligan A, Egan J and Prendergast DM (2020) A pilot study using environmental screening to determine the prevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and antimicrobial resistance (AMR) in Irish cattle herds. *Irish Veterinary Journal* **73**: 3.

Rasmussen P, Barkema HW, Mason S, Beaulieu E and Hall DC (2021) Economic losses due to Johne's disease (*paratuberculosis*) in dairy cattle. *Journal of Dairy Science* **104**, 3123–3143.

Rathnaiah G, Zinniel DK, Bannantine JP, Stabel JR, Gröhn YT, Collins MT and Barletta RG (2017) Pathogenesis, molecular genetics, and genomics of *Mycobacterium avium* subsp. *paratuberculosis*, the etiologic agent of Johne's disease. *Frontiers in Veterinary Science* **4**: 187.

Rice JH, Mcdaniel MM, Holland A and Eda S (2019) Modelling bovine granuloma formation in vitro upon infection with *Mycobacterium Avium* subspecies *Paratuberculosis*. *Veterinary Sciences* **6**: 80.

Robertson RE, Cerf O, Condron RJ, Donaghy JA, Heggum C and Jordan K (2017) Review of the controversy over whether or not *Mycobacterium avium* subsp. *paratuberculosis* poses a food safety risk with pasteurised dairy products. *International Dairy Journal* **73**: 10–18.

Salgado M, Alfaro M, Salazar F, Badilla X, Troncoso E, Zambrano A, González M, Mitchell RM and Collins MT (2015) Application of cattle slurry containing *Mycobacterium avium* subsp. *paratuberculosis* (MAP) to grassland soil and its effect on the relationship between MAP and free-living amoeba. *Veterinary Microbiology* **175**: 26–34.

Samad A (2016) Veterinary medical education and profession: past, present and future with especial emphasis to biomedical sciences and One Health concept in Bangladesh. *Bangladesh Veterinary Medical Record* **2**: 1–28.

Samba-Louaka A, Robino E, Cochard T, Branger M, Delafont V, Aucher W, Wambeke W, Bannantine JP, Biet F and Héchard Y (2018) Environmental *Mycobacterium avium* subsp. *paratuberculosis* hosted by free-living amoebae. *Frontiers in Cellular and Infection Microbiology* **8**: 28.

Sechi LA and Dow CT (2015) *Mycobacterium avium* ss. *paratuberculosis* zoonosis – the Hundred Year War – beyond Crohn's disease. *Frontiers in Immunology* **6**: 96.

Semret M, Turenne CY and Behr MA (2006) Insertion sequence IS900 revisited. *Journal of Clinical Microbiology* **44**: 1081–1083.

Sinha A, Eniyan K, Manohar P, Ramesh N and Bajpai U (2020) Characterization and genome analysis of B1 sub-cluster mycobacteriophage PDRPxv. *Virus Research* **279**: 197884.

Skellett E (1807) *A Practical Treatise on the Breeding Cow, and Extraction of the Calf: Before and at the Time of Calving; in which the Question of Difficult Parturition is Considered in All its Bearings, with Reference to Facts and Experience; Including Observations on the Diseases of Neat Cattle Generally; Containing Profitable Instructions to the Breeding Farmer, Cowkeeper, and Grazier, for Attending to their Own Cattle during Illness*, Cornish.

Slocombe RF (1982) Combined streptomycin-isoniazid-rifampin therapy in the treatment of Johne's disease in a goat. *The Canadian Veterinary Journal* **23**: 160–163.

Smith HW and Huggins MB (1983) Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *The Journal of General Microbiology* **129**: 2659–2675.

Sonawane GG and Tripathi BN (2013) Comparison of a quantitative real-time polymerase chain reaction (qPCR) with conventional PCR, bacterial culture and ELISA for detection of *Mycobacterium avium* subsp.

paratuberculosis infection in sheep showing pathology of Johne's disease. *SpringerPlus* **2**: 45.
St-Jean G and Jernigan AD (1991) Treatment of *Mycobacterium paratuberculosis* infection in ruminants. *Veterinary Clinics of North America: Food Animal Practice* **7**: 793–804.
Stanley EC, Mole RJ, Smith RJ, Glenn SM, Barer MR, Mcgowan M and Rees CED (2007) Development of a new, combined rapid method using phage and PCR for detection and identification of viable *Mycobacterium paratuberculosis* bacteria within 48 h. *Applied and Environmental Microbiology* **73**: 1851–1857.
Stinson KJ, Baquero MM and Plattner BL (2018) Resilience to infection by *Mycobacterium avium* subspecies *paratuberculosis* following direct intestinal inoculation in calves. *Veterinary Research* **49**: 58.
Suarez CA, Franceschelli JJ, Tasselli SE and Morbidoni HR (2020) Weirdo19ES is a novel singleton mycobacteriophage that selects for glycolipid deficient phage-resistant *M. smegmatis* mutants. *PLoS One* **15**: e0231881.
Sula L, Sulová J and Stolcpartová M (1981) Therapy of experimental tuberculosis in guinea pigs with mycobacterial phages DS-6A, GR-21T, My-327. *Czechoslovak Medicine* **4**: 209–214.
Sulakvelidze A, Alavidze Z and Morris JG Jr (2001) Bacteriophage therapy. *Antimicrobial Agents and Chemotherapy* **45**: 649–659.
Summers WC (2012) The strange history of phage therapy. *Bacteriophage* **2**: 130–133.
Swift BMC, Meade N, Barron ES, Bennett M, Perehenic T, Hughes V, Stevenson K and Rees CED (2020) The development and use of Actiphage® to detect viable mycobacteria from bovine tuberculosis and Johne's disease-infected animals. *Microbial Biotechnology* **13**: 738–746.
Tiwari A, Vanleeuwen JA, Mckenna SLB, Keefe GP and Barkema HW (2006) Johne's disease in Canada part I: clinical symptoms, pathophysiology, diagnosis, and prevalence in dairy herds. *The Canadian Veterinary Journal* **47**: 874–882.
Valent P, Groner B, Schumacher U, Superti-Furga G, Busslinger M, Kralovics R, Zielinski C, Penninger JM, Kerjaschki D, Stingl G, Smolen JS, Valenta R, Lassmann H, Kovar H, Jäger U, Kornek G, Müller M and Sörgel F (2016) Paul Ehrlich (1854-1915) and his contributions to the foundation and birth of translational medicine. *Journal of Innate Immunity* **8**: 111–120.
Vernon G (2019) Syphilis and Salvarsan. *The British Journal of General Practice: The Journal of the Royal College of General Practitioners* **69**: 246.
Wagner C, Bonnardel J, Da Silva C, Martens L, Gorvel J-P and Lelouard H (2018) Some news from the unknown soldier, the Peyer's patch macrophage. *Cellular Immunology* **330**: 159–167.
Weiss DJ, Evanson OA and Souza CD (2006) Mucosal immune response in cattle with subclinical Johne's disease. *Veterinary Pathology* **43**: 127–135.
Whitlock RH and Buergelt C (1996) Preclinical and clinical manifestations of *paratuberculosis* (including pathology). *Veterinary Clinics of North America: Food Animal Practice* **12**: 345–356.
Whittaker A, Lohm D, Lemoh C, Cheng AC and Davis M (2019) Investigating understandings of antibiotics and antimicrobial resistance in diverse ethnic communities in Australia: findings from a qualitative study. *Antibiotics* **8**: 135.
Whittington RJ, Marshall DJ, Nicholls PJ, Marsh IB and Reddacliff LA (2004) Survival and dormancy of *Mycobacterium avium* subsp. *paratuberculosis* in the environment. *Applied and Environmental Microbiology* **70**: 2989–3004.
Whittington RJ, Begg DJ, De Silva K, Purdie AC, Dhand NK and Plain KM (2017) Case definition terminology for *paratuberculosis* (Johne's disease). *BMC Veterinary Research* **13**: 328.
Whittington R, Donat K, Weber MF, Kelton D, Nielsen SS, Eisenberg S, Arrigoni N, Juste R, Sáez JL, Dhand N, Santi A, Michel A, Barkema H, Kralik P, Kostoulas P, Citer L, Griffin F, Barwell R, Moreira MAS, Slana I, Koehler H, Singh SV, Yoo HS, Chávez-Gris G, Goodridge A, Ocepek M, Garrido J, Stevenson K, Collins M, Alonso B, Cirone K, Paolicchi F, Gavey L, Rahman MT, De Marchin E, Van Praet W, Bauman C, Fecteau G, Mckenna S, Salgado M, Fernández-Silva J, Dziedzinska R, Echeverría G, Seppänen J, Thibault V, Fridriksdottir V, Derakhshandeh A, Haghkhah M, Ruocco L, Kawaji S, Momotani E, Heuer C, Norton S, Cadmus S, Agdestein A, Kampen A, Szteyn J, Frössling J, Schwan E, Caldow G, Strain S, Carter M, Wells S, Munyeme M, Wolf R, Gurung R, Verdugo C, Fourichon C, Yamamoto T, Thapaliya S, Di Labio E, Ekgatat M, Gil A, Alesandre AN, Piaggio J, Suanes A and De Waard JH (2019) Control of *paratuberculosis*: who, why and how. A review of 48 countries. *BMC Veterinary Research* **15**: 198.
Windsor PA and Whittington RJ (2010) Evidence for age susceptibility of cattle to Johne's disease. *The Veterinary Journal* **184**: 37–44.
Wittebole X, De Roock S and Opal SM (2014) A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* **5**: 226–235.
Wolf R, Orsel K, De Buck J and Barkema HW (2015) Calves shedding *Mycobacterium avium* subspecies *paratuberculosis* are common on infected dairy farms. *Veterinary Research* **46**: 71.
Wong A (2019) Unknown risk on the farm: does agricultural use of ionophores contribute to the burden of antimicrobial resistance? *mSphere* **4**: e00433-19.
Wright K, Plain K, Purdie A, Saunders BM and De Silva K (2019) Biomarkers for detecting resilience against mycobacterial disease in animals. *Infection and Immunity* **88**: e00401–e00419.
Zemskova ZS and Dorozhkova IR (1991) Pathomorphological assessment of the therapeutic effect of mycobacteriophages in tuberculosis. *Problemy Tuberkuleza i Boleznei Legkikh* **11**: 63–66.