Developments in diagnosis and control of bovine paratuberculosis

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Abstract

Bovine paratuberculosis can be costly to farmers who, as a consequence, may be interested in control of the causative agent, *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Between-herd spread is primarily due to movement of MAP-infected livestock, and within-herd transmission most often occurs when susceptible calves are exposed to the faeces of infectious adult cattle. This review provides an update on options to control MAP infections via the use of diagnostics, with an emphasis on dairy cattle. Control of MAP infections can be achieved through improved hygienic measures and reducing the exposure of susceptible animals to the milk and faeces of infected animals. However, cost-effectiveness may depend on labour costs, and strategic use of diagnostics may have certain appeals through the information provided. Current bulk tank milk tests are not deemed to have a role in MAP control, whereas other herd-level diagnostics can be useful to guide establishment of control schemes by estimating whether MAP infections are prevalent or not. Then, test strategies based on screenings using cow-level tests can be used to identify the majority of infectious animals. The proportion of detected infectious animals depends on the timing of testing. If test results are used for risk-based management, then it is of utmost importance that they are still valid when used. Test results 3–6 months old may have lost a significant part of their validity. However, more research on the validity of test results over time to determine the infectiousness of animals should be carried out to enable the design of better test strategies.

Keywords: Paratuberculosis, Control, test strategy, ELISA, PCR, Culture.

Introduction

Paratuberculosis is a chronic infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in cattle and other ruminants. Infection usually occurs via the faecal–oral route, although *in utero* infections also happen [5]. MAP are phagocytized by macrophages and usually reside intracellularly, resulting in pathological forms that can be characterized as focal, multifocal or diffuse forms depending on the stage and severity of infection [6].

The lesions primarily result from the immune responses. Initially, the macrophages containing MAP interact with T-cells in an attempt to control MAP. Pro-inflammatory responses delimit the infection, primarily in the focal forms, through cytokines such as interferon-γ (IFN-γ) [7–9]. The immune-responses in this Th1-dominated phase are generally considered to control MAP [8], while a Th2-dominated phase or a T-cell anergy may result in a lack of control [9] and development of multifocal or diffuse forms [7]. Although it does not appear that there is a

strict split between the pro- and anti-inflammatory responses, Th2-cells may gradually occur with the production of immunoglobulin G1 (IgG1) [9]. The IgG1 increase is primarily directed at crude antigens such as purified protein derivatives, and not necessarily other antigens such as heat shock proteins Hsp65 and Hsp70 and lipoarabinomannan (LAM) [10]. IFN-γ is frequently detected when tissue damage is most severe (diffuse forms), but can be found in all lesion types [7]. In comparison, IgG1 was not detectable in most of the cows with focal lesions, but was detected in 92% of the cattle with diffuse lesions. Immune responses are thus associated with the pathological lesions, although the pro-inflammatory reactions may be less specific to the different lesion stages.

MAP-specific IgG reactions have also been associated with reduced milk yield [11], reduced slaughter weight and value [12] and excretion of MAP [13]. The reduced milk yield and reduced body condition may not be apparent initially, but they eventually become obvious. The intestinal lesions result in malabsorption and protein-losing diarrhoea, resulting in death if the cow is not culled [5]. Cattle with end-stage MAP are also very likely to transmit MAP to their foetus *in utero,* or just after calving [14]. Fertility may also be affected, but the data are sparse on this aspect, although an increased number of days open has been associated with the occurrence of MAP-specific IgG [15] and increased calving intervals have been associated with high levels of MAP shedding [16].

The direct effects of MAP infections on the individual cow are thus multiple: reduced animal welfare of the clinically affected cow, reduced production traits and increased mortality. However, the indirect effects are also significant due to premature culling, loss of genetic potential and spread of MAP to herd mates, resulting in significant economic losses [17–19]. Furthermore, MAP is suspected of being implicated in Crohn's disease of humans and is thus, a potential zoonosis [20]. Consequently, major dairy producing countries have a vested interest in the control of MAP infections [21], but successful control programmes are sparse [22]. Few countries have established programmes, and the aims of the programmes are often quite diverse if specified at all [3].

This review summarizes options for control of MAP with a review of recent advances in diagnostic test strategies, which may aid in the control schemes. The emphasis is on dairy cattle, although beef cattle are also addressed to a small extent.

Control Options

Paratuberculosis control should minimize the risk of introduction of MAP to non-infected herds and minimize the exposure of susceptible cattle to MAP in infected herds [23]. Introduction of MAP to non-infected herds may occur via the purchase of infected cattle and exposure to MAP infected wildlife. MAP introduction via the movement of infected livestock is a commonly acknowledged risk factor [24, 25], whereas the impact of wildlife is much more uncertain [26]. Therefore, external control should primarily focus on closed herd management and if purchase of replacement stock is required, purchases should be made from low-risk farms [27]. Identification of 'low-risk' farms needs to be done through repeated testing of the herds, because history and a once negative herd screening are no guarantee for a herd being free of MAP [28], particularly for smaller herds, where few adult animals are available for testing [29].

Internal control is basically based on identification of infected or infectious animals to avoid transmission of MAP to susceptible animals. Because MAP is excreted in faeces and milk, and uptake is oral, then susceptible animals should be protected from the faeces and milk of infectious animals. Calves are usually considered the most susceptible, and adults have the highest risk of being infectious. Therefore, identified risk factors also centre on areas where calves are exposed to the milk and faeces of adults. This is primarily the calving environment [2]. The role of milk and colostrum is considered of less importance than faeces [2]. It is recommended that risk assessments should be performed on individual farms [30], because milk and colostrum management is farm specific.

The above-mentioned factors usually lead to the development of guidelines for control. As an example, the specific Danish recommendations in 2006 were defined as: calves should not be fed milk or colostrum from infectious cows, but the use of milk and colostrum from non-infectious cows is allowed; calves should be removed within 2 hours from infectious cows, while this was not required from non-infectious cows; calving environments should be cleaned when an infectious cow had been present in the calving area, and calving environments should not be shared by infectious and non-infectious cows. Furthermore, infectious cows should be culled as soon as possible [31]. The challenge in such a setup is to identify the infectious animal, for which purpose diagnostics are required. The alternative is to not use diagnostics and simply consider all animals as potentially infectious, a strategy that may be financially the most attractive [32–34]. The reason for this is the cost of the information provided by the diagnostics. Vaccination is also an option, but is not considered to provide the necessary reduction in transmission [32, 35]. Furthermore, vaccination may interfere with the diagnosis of *M. bovis*, and is therefore prohibited in many countries. As a result, vaccination will not be discussed any further here. The principles for control of MAP are similar in dairy and beef cattle, but different management practices in rearing dairy and beef cattle can lead to modifications in the guidelines. Notably, dams and calves are often reared together in beef production, which challenges the

Table 1 Examples of purposes that can be used to define test strategies in relation to paratuberculosis in a dairy cattle herd.

A	Make certain that the level of MAP in milk used for human consumption is below a level, where MAP are killed during pasteurization
B	Screening to estimate prevalence of MAP infections in a herd prior to establishment of a control scheme
C	Testing to identify animals where MAP infections – affect animal welfare – affect farming profitability – expose humans to MAP through milk – transmit to susceptible animals

recommendation that calves should not be exposed to faeces of infectious adults.

If diagnostics are used, it should be clear how the test results are converted into management actions. Test-and-cull strategies are not considered cost-effective based on simulation models [4, 32, 36], whereas strategies based on combinations of test-and-management and test-and-cull might be depending on the costs of labour [37]. These conclusions are based on simulation studies, because field studies are immensely expensive and would require too many years to carry out. Therefore, appropriate field validations have not been done to assess the validity of the simulation models [38].

Although the best strategy may be management without testing, test results may provide a certain appeal because they offer information, which can be used not only to separate the tested cattle into infectious and non-infectious, but also to monitor the prevalence, to estimate losses, to identify low-risk herds and so forth [39]. However, it should be noted that different test strategies may be more applicable under specific circumstances [40–42].

Still, simulation studies suggest that test-and-cull must be supplied with management and the ideal test strategy differs between different settings. The Danish control programme is risk-based, combining test-and-management and test-and-cull. Nonetheless, only the test-cull part has proven efficient [24], despite these test-and-cull strategies alone are not considered to be effective [4, 32, 36]. Therefore, either the simulation models have yet to capture some essential elements of MAP control, or the above-mentioned study [24] did not capture the management-part sufficiently. While both options are likely contributors to an explanation, diagnostic testing deserves a bit more scrutiny.

Diagnostic Testing

Ante mortem animal-level MAP diagnostic tests are usually divide into three groups based on detection of (a) MAP or parts of it, e.g. polymerase chain reaction (PCR) or culture-based diagnostics for faecal samples or milk; (b) cell-mediated immune-diagnostics, e.g. tests detecting IFN-γ in blood; and (c) IgG detection using indirect ELISA on serum or milk [1]. Other paraclinical tests are also available [43], but will not be discussed here. The laboratory-based tests can be supplemented with clinical information such as occurrence of reduced milk yield, diarrhoea or more unspecific features such as 'general poor performance'. Whereas the latter may be difficult to define in practice, they are not necessarily uncommonly used by the trained herdsman.

The use of diagnostic tests should be featured in a test strategy; that strategy should be associated with a purpose of the intervention and a purpose of testing, to adhere to the OIE fitness-for-purpose criterion [44, 45]. In the control programme, the purpose of the intervention may be implied simply by the word 'control', which could mean 'any effort directed towards reducing the frequency of existing disease to levels biologically and/or economically justifiable or otherwise of little consequence' [46]. Here lies a major challenge with MAP infections: there are multiple effects, which may not all be of priority to all engaging in control, and may vary between farms. Therefore, it is usually important to specify the elements that are of importance. The consequences may be split into major categories, namely those where: animal health and welfare are affected (see above); farming profitability is affected [19] or humans are exposed to MAP via food products such as milk and beef [47–51]. Each of these may require different test strategies, initially or long term. Examples of purposes of testing are listed in Table 1.

A screening may initially be performed to establish if MAP infections are actually present in a population, and if so, be used to estimate the prevalence. Bulk tank milk (BTM) antibody testing has been used for several infectious agents such as bovine virus diarrhoea virus [52], but is not really useful for MAP infections, because of low within-herd prevalence and potentially protracted infections in the individual [53]. Bacteriological culture of composite faecal samples offers a better tool to determine herd infection status [54–56]. Herd-level sensitivity of this method depends on the number of samples per herd, e.g. one study estimated that one sample per herd would provide a sensitivity of approximately 33%, and five samples a sensitivity of about 56% in low-prevalence herds [56]. A clear advantage of composite samples is the lower cost, while disadvantages not only include the low

Table 2 Summary of the sensitivity and specificity of selected diagnostic tests used for various purposes related to control of MAP in dairy cattle, including cautions related to the individual test. More detailed explanations are given in the text.

Test	Purposes	Characteristics	Notes and cautions
Indirect ELISA on BTM	None.	Non-informative.	Should not be used.
Culture of BTM	Surveillance of MAP level in BTM.	Analytical sensitivity may be lower than PCR, but specificity potentially higher.	MAP quantification dubious.
PCR on BTM	Surveillance of MAP level in BTM.	Analytical sensitivity may be higher than culture, but may also detect dead cells.	MAP quantification dubious.
Indirect ELISA for serum or milk	Detection of infection with MAP.	Low sensitivity and early stages not detected, but increasing with age. Specificity of current commercial ELISAs >98%.	Many dubious test evaluations published.
	Detection of MAP infectious animals or animals with progressed infection.	High sensitivity of several current commercial ELISAs. No real difference in accuracy between serum or milk.	Requires frequent testing to have valid test results which can be used for risk-based management. Milk and serum results should not be used together. 'Non-specific' results can occur while IgG may occur before an animal is infectious.
Culture of faecal samples	Detection of infection with MAP.	Low sensitivity and early stages rarely detected.	Sensitivity comparable to indirect ELISA. Non-specific results possible in high-prevalence herds.
	Detection of MAP infectious animals or animals with progressed infection.	High sensitivity, but potentially low specificity.	Many animals shedding low numbers of MAP may be non-infectious and infection may never deteriorate.
PCR on faecal samples	Detection of infection with MAP	Low sensitivity and early stages rarely detected.	Sensitivity comparable to indirect ELISA.
	Detection of MAP infectious animals or animals with progressed infection.		
IFN-γ ELISA for blood	Detection of animals exposed to MAP.	Antigen-dependent.	May detect cured animals as well. Assays based on appropriate assays still to be developed and validated.

sensitivity, but also the lack of results on an individual level. Such results can be obtained using cow-level tests such as indirect ELISAs detecting IgGs in milk or serum, or bacterial culture or PCR used on faecal samples. There are also different advantages and disadvantages associated with all of these methods, some of which are summarized in Table 2. I will elaborate on some of these issues for some of the tests.

BTM culture and BTM PCR

BTM culture and BTM PCR can be used to determine, if MAP is present in the BTM on the specific day of testing, and the level of MAP can be quantified. This could be important if it is deemed that milk delivered for human consumption should be free of MAP, or if the MAP concentration should be below a certain threshold to ensure that pasteurization is effective [57]. However, the level of MAP in BTM may be greatly affected by indirect contamination via faeces from MAP excreting cows [58].

Because faeces from the same high-excreting cow is relatively unlikely to contaminate the BTM every day, then the days exceeding 'acceptable' levels are likely to be few and will not be detected [58]. Therefore, it seems pointless to use BTM tests for surveillance. A positive BTM will, however, be relatively specific and may be a good indicator of herd infection. The sensitivity for detection of herd infection has, however, not been determined.

Culture and PCR of individual cow's milk samples

MAP can be excreted through milk [59, 60], but the most common source of MAP in milk samples obtained ante mortem is likely faecal contamination [58]. Although cattle that tests positive in IgG ELISA or bacteriological culture of faeces are more likely to test positive in both bacteriological culture and PCR of the milk samples [61], the pathogenesis related to the occurrence of MAP in milk is still poorly described. MAP detection in milk has

been associated with the level of MAP excreted in faeces [59]. The high apparent prevalence of MAP excretion in milk reported in some studies [61], where the prevalence appeared to be higher than the overall MAP within-herd prevalence [62], may suggest that faecal contamination of the tested milk samples is not an uncommon phenomenon in many studies. A consequence would be difficulties in interpretation of many studies.

The largest study with paired samples conducted so far suggested that IS900-based PCR was more sensitive than bacteriological culture, with PCR classifying 13% and culture 3% of 1493 cows of unknown infection statuses as positive [63]. However, other studies come to the opposite conclusion [61]. Culture may take 3 months to perform, whereas PCR can be done within a day. Because of the risk of faecal contamination, where the test result may not relate to the MAP infection status of the cow from which a sample was obtained, the interpretation of test results can easily be dubious. If strictly sterile samples can be obtained, PCR may offer a faster and more sensitive testing option, but local laboratory expertise may be the primary determinant in choice of method. So far, there seems to be limited motivation to detect MAP from individual cow's milk as part of control schemes.

Culture and PCR of faecal samples

Detection of MAP in faecal specimens by culture is a technique that has been around for a century, while PCR has been available for decades, and the latter has made gradual improvements, e.g. to enable high throughput [64]. However, detection of MAP or parts thereof in faeces requires that shedding occurs. Shedding of MAP from infected cows is not a continuous event, especially in the early stages of infection [13, 65–67]. Still, few longitudinal studies have been carried out, and shedding patterns are therefore poorly characterized. Detection of shedding can occur more or less throughout the entire infection period [65], but only a few animals test positive in the early phases of infection, and at early ages following natural infection [68, 69].

The motivation and relevance of detection of these early cases is critical to establish, prior to assessing the diagnostic accuracy. Again, the purpose of testing needs identification. If infected animals are identified and culled, then identification of animals excreting even minimal amounts of MAP may be ideal. However, few will be detected, because most cattle are relatively young and with early stage infections, and early stage infections are rarely detectable. For example, among adult cattle with focal MAP lesions at slaughter, only 13% have been described to be detected by bacteriological culture and 30% by a real-time IS900-based PCR [7]. The cattle in that study were on average 4.5 years of age, and age is an important factor when interpreting such sensitivity estimates [70]. Another study demonstrated infection in only 80% of cows with a previous positive culture of faecal samples in high prevalence herds [71], proposing that up to 20% of cattle with a positive faecal culture may be non-infected in high-prevalence herds. Cows ingesting MAP without being infected have been labelled 'passive shedders' [72]. On average, they may reduce the specificity of a faecal culture to about 98% [73], but in herds with animals shedding high numbers of MAP, the use of PCR may result in a very high apparent prevalence, which may be a result of passive shedding [74].

Detection of MAP infected cattle is not always ideal, because these cattle may never become diseased, experience production losses or even be a liability to their herd mates in terms of infection risk. Consequences of detecting MAP infected cattle may be culling or uncertainty about what to do with the animal. Culling of many infected animals that will not be diseased may negatively affect farming profitability, whereas keeping one infected animal that will eventually spread MAP to the rest of the herd can also negatively affect the profitability. So the risk that an animal may become infectious is likely the most important feature, because of the potential impact to the herd.

The risk of infection for susceptible animals is lower following low or single doses of MAP [5, 66], but the actual requirements for an infectious dose is not known, although doses of 10^6 colony-forming units (CFU) may be sufficient, whereas 5×10^8 CFU is considered more reliable in experimental infection models [75]. A positive test result from a culture or PCR does not reflect a bacterial concentration. Therefore, the relevance of a positive culture or PCR result based on a faecal sample relative to detection of infectious animals remains to be characterized. Discrimination between low and high MAP shedders, or even high shedders and supershedders may offer a potential solution, but requires that quantification is done. Excretion of high levels of MAP has been associated with milk production losses and reduced fertility, without simultaneous detection of these production effects among cows with low MAP shedding [16, 76].

The ideal test might detect animals just before they become infectious, so they can be culled before they become a threat to their herd-mates. This test should also preferably be able to predict production losses (reduced body condition, milk yield and fertility) attributable to the MAP infection. Culture and PCR of faecal samples based on a dichotomous positive/negative test interpretation are not able to discriminate between the animal that may merely be a passive shedder or have a past infection, and those with a current infection that will deteriorate in the life-time of the cow.

Serum and milk MAP-specific IgG ELISA

Indirect ELISAs for detection of MAP-specific IgG are widely used for serum or milk samples. IgG1 generally

occur in higher concentrations than IgG2 in milk [77, 78], and MAP-specific IgG1 is generally associated with the anti-inflammatory immune responses dominated by Th2 cells occurring when the cow appears to lose control over the MAP infection [9]. Therefore, existing commercial IgG1-detecting ELISAs can be expected to be relatively specific to the later stages of MAP infection, whereas they will generally not be useful in the early stages. This is also exemplified by the inability to detect animals with focal lesions, whereas animals with diffuse lesions are more easily detected [7]. ELISAs using LAM as an antigen are available, but have gained limited popularity due to low specificity [1].

Specificity may be a concern, but the origin of non-specific reactions should be understood to properly interpret test results. Current commercial ELISAs are not 100% specific, partly due to exposure of some cattle to environmental mycobacteria [79]. Exposure to environmental bacteria is likely more common for cattle kept on pasture compared with cattle kept indoor all year round. Consequently, beef cattle kept on pasture may be more likely to be exposed to environmental bacteria, particularly in areas where dairy cattle are mainly kept in barns. Under controlled circumstances e.g. in research projects, the specificity may still be estimated to almost 100% for some tests [80]. In practice, the situation may be different, for example if a low cut-off has been chosen to increase sensitivity, or due to misidentification of samples during sampling, cross-contamination, etc. The magnitude of the latter has not been characterized, but is also likely to differ from farm to farm, laboratory to laboratory, etc. Another important reason might be that the animal is indeed infected, but has been classified as non-infected because MAP cannot be identified in faeces or tissues, despite being present [81]. In a recent Danish study comprising longitudinal population-based data, we took an alternative approach to evaluate an ELISA. Rather than dividing the cattle into those with or without pathological lesions, or those excreting detectable amounts of MAP, and so forth as used in traditional test evaluations [1], we divided the population into those that would develop an anti-inflammatory immune response in 'their expected lifetime', and those that would not. The expected lifetime was based on the distribution of the actual lifetimes of the Danish cattle population when this study was performed. The rationale behind this approach was in principle that those that did not develop an anti-inflammatory immune response would not be diseased, have production loss or be infectious in their life-time, whereas among those that did, it was important to determine when this happened. Those that were infected, but did not develop an anti-inflammatory immune response in the expected life time were not important from a population perspective and consequently not for estimation of 'sensitivity' and 'specificity'. Therefore, a farm-management relevant intermediary between 'infection' and 'diseased with excretion of infectious MAP doses or affected production features' could be established to estimate the sensitivity and specificity. The specificity was estimated to be 98.66, while the sensitivity was age-specific ranging from approximately 20% at 2 years of age to around 70% at 5 years of age, depending on the definitions used [82]. This was higher than the sensitivity estimates for detection of MAP infection summarized to around 7–39% in a previous review [1], but the interpretation is also fairly different.

There seems to be limited differences between the accuracy of milk or serum ELISA [73, 83, 84]. Differences might be detectable if comparing paired samples; however, test results from the same test-dates should not be compared with deem one test better than the other, because the odds of testing positive in milk ELISA and serum ELISA do not peak at the same time in lactation [85]. The choice of sample specimens used for testing may, therefore, rely more on logistics than on anything else. Some herds participate in milk recording schemes, where milk samples are readily available, while milk recording is rarely done in other herds, e.g. beef herds. Milk samples cannot be obtained from dry cows, and samples from the first 3–5 days of lactation are unreliable [86]. Some herds may already collect serum for other diagnostic purposes, and then these samples might be ideal to use. The cost of indirect ELISAs may be only 10–20% of the costs of agent detection [29], which also has a certain appeal to some decision-makers.

Timely detection of infectious cows is dependent on the frequency of testing. MAP-specific IgGs usually occur before significant shedding of MAP [13], but is also dependent on the specific ELISA and its characteristics. Characterization of the sensitivity of a test should be done relative to the day the animal becomes infectious. This day cannot be defined, because we do not know the infectious dose. Instead, the day can be defined relative to, for example, 'intermittent' or 'persistent' shedding as illustrated in Figure 1. Here, it can be seen that if the cow is tested on the date she starts persistent shedding, then approximately 93% of the persistent shedders will be detected. Had she been tested 1 year prior to the occurrence of persistent shedding, then the sensitivity would have only been approximately 28%. The ELISA is obviously less sensitive for detection of intermittent shedders, which is expected according to the pathogenesis.

To summarize, serum and milk ELISA results are almost ineffective for detection of early infection, whereas agent-detecting methods such as culture and PCR are more sensitive in the earlier infection stages. However, because occurrence of MAP-specific IgG is usually related to progression of the infection to the stage where production losses and infectivity become issues, then ELISA tests may have a useful diagnostic potential as a predictor of disease progression. The lower cost may also warrant more frequent testing.
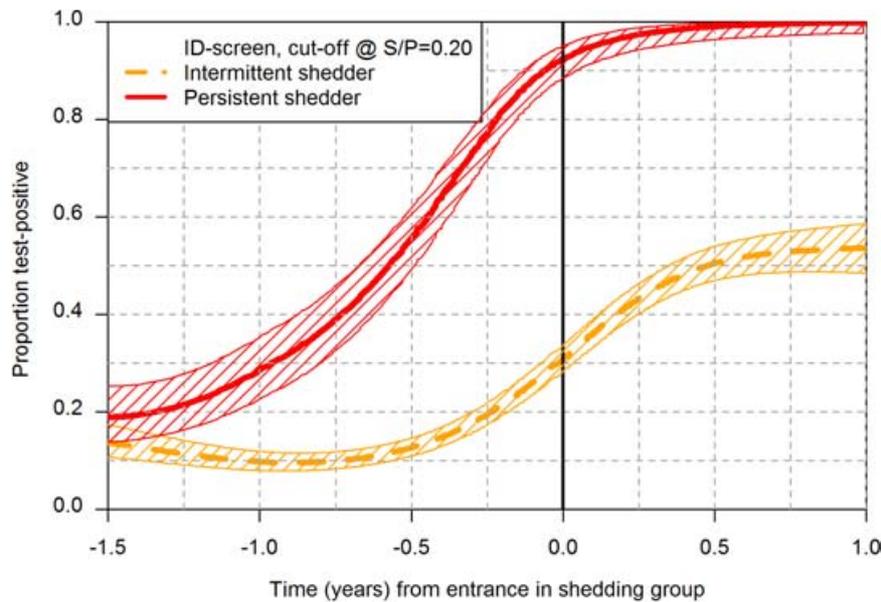
Figure 1 Time from entering a specific shedding group to testing positive in the ID-Screen milk ELISA at cut-off 0.20 sample-to-positive (S/P) ratio. Data were collected as described by Nielsen [13], but only a random subset of the data was tested with the ID-Screen milk ELISA. This graph illustrates when a cow which became an intermittent shedder at Time=0, would be 30% more likely to test positive at Time=0 and only 10% likely to test positive one year earlier. A cow persistently shedding MAP at Time=0 would be >90% likely to test positive on the day she became a persistent shedder, but one year earlier, she would only be 30% likely to test positive using the ELISA test. The definitions of 'persistent' and 'intermittent' were based on bacterial culture of repeated faecal samples.

Use of Diagnostics for Control

The diagnostic tests have different sensitivities and specificities, and consequently different pros and cons in different stages and for different purposes, as summarized in Table 3. Indirect ELISAs and culture have been used in practice for decades, and therefore, significant experiences and numerous evaluations have included these tests. Newer tests like PCR and IFN-γ have provided less and still insufficient data to provide consistent evidence of their potential use, although PCR is up-and-coming and may be used more and more, and soon provide sufficient data to allow better interpretation in practice.

Low-cost tests may be more useful to support management schemes for MAP control than higher cost tests, even if the latter are more sensitive [42]. Management practices that include generally high levels of hygiene at low within-herd prevalence, or very large herd testing, may not be advisable at all [42]. However, few studies have actually been carried out to assess the effects. Two very similar studies in the USA and Denmark focused on the use of ELISA for risk-based management and demonstrated that it is feasible to reduce the test-prevalence [24, 87]. However, only culling of test-positive and the purchase of a higher number of cattle could be associated with a reduction in test-prevalence, whereas significant prevalence reductions could not be associated with the management of high-risk animals [24], although trends could be seen for some management practices.

Therefore, culling seems to have a major effect, and it may be supported by changes in management practices.

The value of the diagnostic test results decrease over time (Figure 1), and the results should be up-to-date when they are used, as illustrated in Figure 2. There is no point in using out-dated results for management, and there is no point in testing if results are not used. Then, the money spent for testing could be saved.

There are some challenges when using indirect tests such as antibody detection to predict when an animal is infectious. There is a need to better understand and characterize the relation between test-positivity and infectiousness. However, first, the MAP shedding patterns with inclusion of quantities of MAP sheddings need much better characterization. Once these have been established, the dynamics of IgG responses relative to specific shedding patterns, as well as the occurrence of milk yield reduction and other clinical signs can be characterized, and better test strategies designed. However, the establishment of the infectious dose or infectious doses required to infect a susceptible animal would also be neat information to have.

Antibody ELISAs are currently the least costly test to perform. It is not always 100% specific, but because of the low cost, increases in specificity can be achieved through follow-up testing of positive cows [82]. Follow-up testing using culture has often been used, but because ELISA and culture appears to be conditionally independent given infection [73], the serial testing and interpretation used

Table 3 Summary of sensitivity (Se) and specificity (Sp) of the most common diagnostics used for different purposes associated with MAP control.

	Purpose of testing – detection of:							
	MAP infected		MAP infectious[1]		Affected by MAP[2]		MAP excretion in milk	
	Se	Sp	Se	Sp	Se	Sp	Se	Sp
IFN-γ	+	+	+	+	?	?	?	?
IgG ELISA	(+)	+ + +	+ + +	+ +	+ + +	+ + +	+	−
Culture	+	+ +	+ + +	+	+ + +	+ + +	+	−
PCR	+	+	+ + +	+	+ + +	+ + +	+ +	−

[1]Presumes that testing is done just prior to the animal becoming infectious.
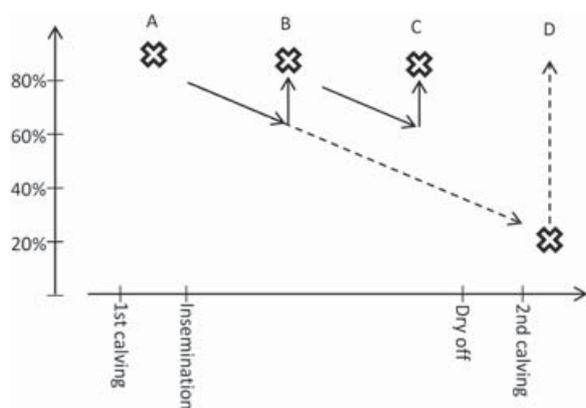[2]Combined with clinical sign e.g. low/reduced milk yield; loss of body condition; diarrhoea.

Figure 2 Schematic description of decreases in sensitivity and consequently, the diagnostic value over a lactation lasting approximately 1 year. From time points A to B, the sensitivity decreases about 20–30%; however, from A to D, it is a decrease of about 60–70%, corresponding to the decreases seen in Figure 1. However, the results only need to be up-to-date when they are used. For example, if testing is done at time point A to determine if a cow should be inseminated or not, then there is no need to test at time-point B. If test results should be used at calving, however, it would be required to do testing at time-point C, or at least before calving. If the results are used continuously throughout lactation, then testing at time-points A, B, C and D is required, if a sensitivity above 60% should be retained.

through this approach significantly reduces the overall diagnostic sensitivity. This does not appear to be the case for repeated ELISA tests, but they should primarily be used with parallel interpretation. A likely explanation is the cause of false-positive results: if they are caused by laboratory errors or misidentification of samples, then these errors would normally be independent of infection status. Therefore, the gains in sensitivity from repeated testing allow parallel interpretation without the loss in specificity that normally results from parallel interpretation. Another aspect that is rarely discussed in scientific literature is the role of the herdsmen. A trained herdsman can often sense if an animal is not performing as it should, without being able to specifically diagnose the specific condition. However, if diagnostic test results are readily available from recurrent screenings, then it is possible to

quickly rule-in MAP infection if test-positive results occur. This will mean that the animal can be culled immediately without spreading MAP for too long. Should the farmer wait 1–2 weeks for test results, then a lot of MAP can be spread in the meantime. The value of this information is also hard to define, because it will vary significantly from manager to manager.

Detection of infected cattle cannot be deemed a viable approach to controlling within-herd transmission, because we do not know if MAP infected animals are able to cure infection or endure a latent infection throughout their lifetime. Culling these cattle can prove to be an extremely expensive strategy, and therefore, the development of more sensitive tests for this purpose seems pointless. If only prediction of progression was feasible, then the more sensitive test would have a role to play in practice. However, more sensitive tests would be preferable to control the between-herd spread of MAP. Tests to rule out MAP infection in an animal that should be moved are highly desirable. Currently, we have to rely on probability diagnoses, but historical and current test-information should enable decent estimates of low risk for a specific herd if sufficient animals are tested [29].

Alternative approaches to test-evaluations may provide new insights into the performance of diagnostics in the future, but irrespective of whether new tests are developed or old tests are used in new strategies, they need to be fit-for-purpose and evaluated in view of that purpose [45]. Once control of MAP infections is on track, we need to be able to establish if a herd is free or still infected. Test strategies for this purpose have been developed [29], but historical information also needs to be included to account for the possible latency of MAP infections. Another challenge that still requires management is the potential survival of MAP in the farm environment after the removal of infected cattle [88].

Conclusion/Summary

Control of MAP infections can likely be achieved by improving farm hygiene. However, the use of diagnostic tests can target the efforts, e.g. through risk-based

approaches. The use of herd-level tests or herd-screenings might be useful to decide if a control scheme should be established, but has a limited value in the actual risk-based management. Here, milk- or serum-based ELISAs are the most cost-effective tests, although the specific test strategy can differ significantly between different production systems and herd sizes. A key to a successful test strategy can be one, where timely detection of infectious animals with their subsequent specific management is pivotal. Current commercial ELISAs are ineffective for early detection, where agent-detecting tests are better although still relatively insensitive. Future research should focus on characterization of the infectious animal and when an infected animal becomes a liability to the herd, rather than focusing on the infected animal itself.

Acknowledgements

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