The use of targeted sampling and risk factor analysis to investigate presence of Johnes disease in dairy herds

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ABSTRACT

The assessment of Johne's disease infection within a herd is fraught with difficulties, given the prolonged asymptomatic stage of infection and the lack of sensitive diagnostic tests to detect sub-clinical infection. The aim of this study was to determine whether analysis of policy for purchasing replacement stock and the risk factors associated with disease spread on individual farms correlated with the clinical history of Johne's disease. Milk and serum samples were also taken from targeted animals within the participating herds and analysed for antibodies to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by ELISA to determine whether this could be an effective screening method for the presence of infection. Herds were defined as being of high, medium and low risk of having Johne's disease according to their re-stocking policy. A greater proportion of high risk herds had clinical Johne's disease implicating the importance of replacement stock in introducing infection, although this was not statistically significant. Herd husbandry practices related to within-herd spread of Johne's disease were quantified using a scoring system. Herds with no clinical history of disease had a lower mean combined score than herds that had experienced Johne's cases. However, although all herds operated husbandry practices that supported within herd spread of disease, farmers were reluctant to implement

appropriate management changes as these could be costly. Targeted sampling identified all herds that had multiple clinical cases of Johne's and could be a cost-effective way of convincing farmers that such changes are necessary.

KEYWORDS

Johnes, ELISA, targeted, risk, prevalence, MAP, *Mycobacterium paratuberculosis,* paratuberculosis

INTRODUCTION

The prevalence of Johne's disease in the UK.

Johne's disease has become an increasing problem in recent years both nationally (Caldow *et al* 2004, Daniels *et al* 2002) and locally within the practice area. There is no recent data regarding the prevalence of Johne's disease in the UK. In an abbatoir survey in the south west of England, Cetinkaya *et al* (1996) used the polymerase chain reaction to identify the presence of *Mycobacterium avium paratuberculosis* (MAP) in mesenteric lymph nodes of cull cows. This resulted in an individual animal prevalence of 3.5 %. This prevalence figure is similar to that achieved by Merkal *et al* (1987) in their national survey of cull cows (2.9 % dairy cows, 0.8 % beef cows) carried out in 32 states in the USA. This suggests that the prevalence of MAP is similar in dairy cows in the south west of England and the USA. Studies from countries with dairy industries not dissimilar to the UK (Collins *et al* 1994, Muskens *et al* 1999) would suggest that herd prevalence in these countries is between 30% - 70% and it is reasonable to suppose that a similar situation exists in the UK.

Merkal *et al* (1975) found in an affected Guernsey herd in the USA that less than 30% of infected cattle were culled for clinical paratuberculosis with most being culled for mastitis (15.9 %), infertility (37.3 %) and other health reasons (16.9 %). Infected cows were more likely to be culled for mastitis or infertility than were non infected herd mates. In the dairy herd it is highly likely that cows are often culled prior to the development of full clinical signs ie during the late sub-clinical phase of the disease.

The fact that paratuberculosis is predominantly a sub-clinical disease is well illustrated by the study of Kallis *et al* (1999) who, using pooled faecal culture, found 46 % of herds that were believed to be uninfected by their veterinary surgeons were in fact infected. This suggests that absence of clinical disease is a poor indicator as to freedom from infection. In dairy herds a significant proportion of infected animals may be culled prior to development of the disease thus masking the presence of infection. Wilson *et al* (1993) found the cull rate to be six times greater among paratuberculosis infected dairy cows than in their non-infected herd mates in a New York herd despite a management decision not to cull on grounds of paratuberculosis.

Reducing farm income has led to an expansion in average herd size and the resultant increase in stock purchases increases the risk of introducing Johne's infected cattle. The risk of within herd spread has also increased as husbandry issues fail to be addressed and the facilities for calving cows are less keenly managed.

Diagnosis and control of Johne's disease

Control of the disease is complicated by its predominantly sub-clinical nature, long incubation period, tests of limited sensitivity (Wells *et al* 2002) and management measures which are problematic to implement. In certain circumstances herds can develop a high incidence of clinical Johne's disease posing major challenges for both the veterinary surgeon and the farmer. The development of effective herd health planning requires systems to prevent both the introduction of disease (biosecurity) and a clear understanding of the likely risk of spread of a disease within a herd. This is achieved with many other infectious agents through bulk milk testing of the respective herds, effective individual animal tests and vaccination of at risk animals. The same approach cannot be applied to Johne's disease.

The test most commonly used for the diagnosis of Johne's disease is the ELISA test for MAP antibodies. However, antibody is only produced relatively late in the disease process thus test sensitivity varies depending on the stage of the disease. Sockett *et al* (1992) investigated the performance of four serological tests for MAP including two ELISA tests in 177 sub-clinically infected cattle. Infection status was confirmed using faecal culture or culture from internal organs if faecal culture was negative ("gold standard"). Test sensitivities (% ± 95% confidence limits) for the two ELISA tests were as follows:

	Shedders n= 108	Non-shedders n = 69	Overall n = 177
IDEXX ELISA	56.5 ± 9.3	24.6 ± 10.2	43.4 ± 7.3
Allied ELISA	65.7 ± 9.0	47.8 ± 11.9	58.8 ± 7.3

Reichel *et al* (1999) found the ELISA test to have an overall sensitivity of 47% when serological results were compared to faecal culture results from 106 confirmed infected cattle. Similar results were obtained in a study using serum samples from 590 MAP infected and 723 uninfected cattle (Dargatz *et al* 2001). Infection status was confirmed using either faecal or tissue culture. Test sensitivity varied from 88% in clinical cases to 15% in cattle classified as "light shedders"

These studies suggest that if the ELISA test is used in the late sub-clinical or early clinical stage that the sensitivity of the test will be higher than if the animals are tested at the early sub-clinical phase. This forms the basis of the “targeted” sampling approach where the farmer and vet identify animals based on physical parameters for testing (scour, milk yield depression, weight loss) as this approach is likely to increase both the sensitivity of the test and the expected prevalence of the disease within the sub-group tested thus increasing the probability of detecting evidence of infection

The value of risk factor analysis

In addition to determining clinical history there is a need to establish the risk of a herd being infected and to evaluate the risk factors associated with spread within a herd. Control of Johne’s infection based on detecting animals once they become clinically affected is costly, will not reduce the herd prevalence of disease and has serious welfare implications. The key to control is to identify infected animals prior to becoming “shedders” and to remove them from the herd or to prevent these animals from entering and contaminating calving areas or infecting calves via their milk or colostrum. Susceptibility to infection by MAP is considered to be greatest in calves. Thus control programmes designed to limit within herd spread focus on reducing exposure of young stock, including ensuring clean calving areas, not feeding pooled colostrum or discarded milk and maintaining young stock separate from adult animals with uncontaminated food and water supplies.

If the true prevalence of Johne's disease is 3.5% in adult dairy cattle as suggested by Cetinkaya *et al* (1996) then the average risk of acquiring an infected cow is 0.035 per purchase i.e. 100% per 30 purchases. This simple calculation illustrates well the risk of introducing MAP with purchased stock. The process of risk factor analysis structures the approach to the investigation of the herd and clearly highlights to both the vet and farmer the risks of purchasing infected stock and the potential for within herd spread.

This allows targeted control measures to be instigated. Groenendaal *et al* (2003), using simulation modelling, suggest that management measures aimed at reducing transmission from adults to calves is essential for control; those based on "test and cull" alone are ineffective.

The purpose of this work was to evaluate if a combination of risk factor analysis and targeted sampling of high risk animals could be used to identify infected herds. In addition, risk factor analysis will pinpoint critical control points allowing effective biosecurity advice to be given both from the point of view of preventing incursion of the disease into "clean herds" and prevention of within herd spread in infected herds.

MATERIALS AND METHODS

23 herds were used for this study. These were all clients of The Park Veterinary Practice, and 14 were on a fortnightly or monthly pre-planned veterinary visit. Two farms had occasional veterinary visits and seven farms were new clients of the practice and testing was performed to help establish the health status of the farm.

Risk of introduction

The risk of spread of disease onto a farm was evaluated using a standard questionnaire modified from a control programme developed in the US (Rossiter *et al* 1998). Herds were classified according to their history of number and frequency of purchases of livestock in the last 13 years (purchases since 1992). The risk of having Johne's infection on the farm was defined as 'high' in herds that had made multiple purchases from multiple suppliers, ‘medium’ in herds that had made occasional purchases from single farms and ‘low’ in herds that had not bought in any stock over the study period.

Risk of spread

The risk of spread of disease within a herd was assessed by defining associated husbandry factors and applying a score for each factor as described by Rossiter *et al* (1998). To facilitate explaining the risk factors to the farmer during the data collection process, the questionnaire was colour coded with red for high risk factors, amber for medium risk and green for low risk. A score was produced both for current husbandry practices and historic practices 5 years ago as the current level of disease will reflect historic husbandry factors due to the incubation period.

Clinical history

Investigation of clinical history allowed the herds to be divided into three groups according to their prevalence of disease. Herds with multiple (≥2) clinical cases within the last 5 years where Johne’s infection was considered endemic were designated ‘heavily infected’. Herds with an isolated case in the preceding 5 years were designated ‘single positive diagnosis in the last 5 years’ and the remainder were designated as ‘no clinical cases’.

Targeted sampling

Six animals that were considered by the farmer or vet to be potentially Johne’s disease antibody positive were targeted for sampling from each herd. These were cows suffering from secondary conditions such as unexpected weight loss, scour or poor milk production or had previously tested positive for Johnes. Both blood and milk samples were collected and submitted to BioBest Laboratories Ltd, Penicuik, UK for testing for antibodies to MAP (Paratuberculosis ELISA Kit, Institut Pourquier, Montpellier, France). Bulk milk samples were collected at the same time to assess whether bulk milk antibody titres correlated with disease prevalence (Duthie *et al* 2005). Preservative tablets were added to all milk samples (Broad Spectrum Microtabs II, D&F Control Systems, Dublin, California, US).

RESULTS

Clinical history

Four herds had experienced Johne's clinical cases in the last 12 months and had multiple diagnoses of Johne’s disease in the last 5 years. One herd had four clinical cases in 1999 and no subsequent veterinary diagnoses for Johne's disease, although it was suspected that Johne’s disease was still a problem in the herd and thus classified as heavily infected. Three herds experienced single cases of Johne's in the last 5 years in purchased animals with no confirmed homebred cases. The remaining 15 herds had no history of Johne’s disease.

Risk of introduction analysis

Table 1 gives the breakdown of herds with high, moderate and low risk of having disease, their clinical history and the results of target sampling. Of the three low risk herds, one herd had not purchased any replacements and two herds had purchased 1-2 stock bulls in the last 13 years. Nearly two-thirds (15) of the herds involved in the study were in the high risk group. Seven of these had Johne's disease diagnosed in the last 5 years and this included the five heavily infected herds. For analysis purposes herds were classified as being either “high risk” or “not high risk” (Table 2.)

Table 2. The association between “risk of introduction” group membership and occurrence of clinical disease.

Risk Group		Clinical case		Total herds
		Yes	No	
“high risk”		7	8	15
“not high risk”		2	6	8

The risk of introduction analysis showed that 7 out of a total of 9 herds with clinical disease confirmed for the disease were allocated to the high risk group equating to a sensitivity of 77% (7/9) for detection of a herd with a clinical case with a positive predictive value of 46% (7/15). This suggests that simply categorizing farms according to purchasing history may detect half the herds with clinical cases. Furthermore a herd with a high risk score is approximately three times as likely to have clinical disease compared to herds with lower risk scores (Odds Ratio 2.6), suggesting that herds with high risk scores deserve further investigation for presence of MAP.

Risk of disease spread within a herd

A breakdown of the historical risk factor scores for each of the groups; heavily infected, positive diagnosis in the preceding 5 years and no diagnosis of Johne’s disease, is given in table 3. In all herds there was potential for disease spread as it was not a normal husbandry practice to remove calves from their mothers at birth or to test cows prior to feeding waste milk or colostrum. 17 herds practised pooled colostrum feeding and 21 fed waste milk to replacement calves.

Heavily infected herds tended to be bigger (mean herd size: 242 cows) and all herds that had clinical Johne's disease had higher mean total risk factor scores for within herd spread. Herds that had no diagnosis of Johne's disease within the last 5 years had the lowest total scores for each of the management categories assessed apart from risk through the husbandry of post weaned heifers. The risk associated with the management of post weaned heifers was very low in all three groups.

Targeted sampling

The relationship between herd clinical history and target sampling results is given in Table 1. Six out of the 23 herds tested (26%) were found to be have at least one animal with a positive Johne's ELISA result in the targeted samples. Five herds in the high risk group had positive ELISA results corresponding to the herds where Johne's infection was considered endemic.

If the ELISA results are used for identifying herds with clinical disease, the sensitivity of the ELISA for detecting herds with clinical disease is 62% (5/8) with a positive predictive value of 83% (5/6) (Table 4).

Table 4. Association between ELISA seropositivity, using targeted sampling and presence of clinical disease.

		Clinical case		
ELISA positive		Yes	No	Total herds
Yes		5	1	6
No		3	14	17
		8	15	23

If the serology results are compared to risk group membership only 5 out of 15 herds in the high risk group were found to have a positive ELISA test result equating to a positive predictive value of 30% although the sensitivity was 83% (5/6) (Table 5). Thus risk status is better determined by risk analysis than by targeted sampling

Table 5. Association between "risk of introduction" group membership and ELISA seropositivity using targeted sampling.

Risk group		ELISA results		Total herds
		Yes	No	
"high risk"		5	10	15
"not high risk"		1	7	8

14 out of 15 herds with no confirmed or suspected clinical cases in the preceding 5 years had no positive samples on targeted screening. This suggests either that there was no infection present in these 14 herds or that whilst the targeted sampling regime could detect herds with clinical disease it was unable to detect herds with infection but no clinical disease. The latter explanation is most likely in view of the likely herd prevalence in the UK. Thus targeted sampling of six animals is likely to miss infection in herds without clinical disease. These are likely to be herds with relatively low rates of infection. However one herd, which had been assessed as being at low risk of having infection on the farm and with no history of Johne's disease, had a positive ELISA results in a single cow. Both serum and milk samples were positive and although the cow remains healthy 6 months after initial diagnosis but has tested positive by milk ELISA on two subsequent occasions. No positive ELISA results were obtained from the herds with isolated Johne's cases.

There was a good correlation between the individual milk and serum ELISA results overall (presented in Duthie *et al* 2005).

DISCUSSION

It was crucial for the study to gain the co-operation of farmers as collecting information about stock replacements, disease history and relevant management practices as well as identifying and sampling targeted animals involved a considerable investment of time. A number of farmers who were approached were not willing to take part primarily because they believed they were of low risk and would not benefit from the trial. Conversely, known infected herds were keen to participate as they wanted to develop their understanding and take advantage of the testing. Three of the herds with multiple cases of Johne's disease had already begun active control measures; one was undergoing a "test and cull" program and two had initiated vaccination (although none of the vaccinated heifers had entered the herd by the time of sampling). Thus this population was strongly biased towards herds with high levels of infection and presence of clinical cases.

This study suggests that systematic risk assessment and scoring is a practical on-farm procedure and could be adopted as an integral part of herd health planning. Although it is targeted towards identification of risk factors for infection with MAP, it should prove to be of value also for other diseases such as mastitis and calf scours. These results suggest that risk assessment results may be of value in triggering further investigation of MAP status by identification of herds likely to be heavily infected.

Targeted sampling of six animals was effective at identifying herds with clinical disease but only identified one herd with antibody present but no history of clinical disease. However clinical history and risk factor analysis had already identified these herds as problem herds. Thus targeted sampling of six animals would seem to be of little value in the identification of herds with infection but no clinical disease. A recent study (Grove-White 2005 unpublished) suggested that only half of MAP infected beef herds reported clinical disease implying that infection was sub-clinical in half of infected herds. This supports the findings of Kallis *et al* (1999) that paratuberculosis is sub-clinical in many dairy herds. From a control aspect, these sub-clinically infected herds are the ones that need identifying so that measures can be put in place to minimise further spread and subsequent financial loss. Effective endemic disease surveillance must accurately identify both infected and non-infected herds thus not only must positive results be meaningful (specificity) but negative results must carry a high degree of confidence that the herd is truly uninfected (sensitivity). Identification of uninfected herds is an essential component of any disease control strategy. Targeted sampling of six animals will not allow this. However computer simulation using Survey Toolbox (Angus Cameron: Australia) suggests that targeted ELISA sampling of a sub-group of 30 animals may allow identification of both infected and non infected herds with 95% confidence providing the selection procedure for the sub-group to be tested is such that the prevalence in the group is 20% i.e. a negative result would actually mean "we can be 95% confident that the prevalence in the sub-group is less than 20%". For this approach to work, the selection criteria for inclusion in the group would be of prime importance. Repeatable inclusion criteria worthy of investigation might include condition score during the dry period and previous lactational yield compared to predicted yield. This approach merits further investigation before it can be recommended as a diagnostic tool.

A combination of effective targeted sampling combined with risk factor analysis may be a cost effective health planning tool for farmers and vets to use in herds of unknown status. This may form the basis of encouraging vets and farmers to commit to official Johne's control schemes. Farmers are generally unwilling to pay for expensive testing programs if they believe the disease is not present in their herds and there is no commercial benefit to them in establishing disease status. In one herd a single targeted animal was found positive for Johnes with a low risk of presence of the disease in the herd. This has allowed us to identify the disease early in the disease process and instigate control measures likely to reduce further spread of the disease.

More robust protocols based on whole herd sampling and cull cow screens are required as part of the CHeCS accredited national control program. Environmental sampling has also been suggested as a way of monitoring infection within a herd. (Raizman *et al* 2005). These tests are viewed as costly by farmers and this limits farmer and vet involvement in Johne's control programs. With the persistence of the practice of purchasing stock of unknown health status and the trend in increasing herd size with its associated inhibition of good management practices, the risk of disease increases nationally. For a small number of herds, often pedigree beef herds where the sale of heifers generates considerable income, elite disease free status has been achieved and may be financially rewarding. Currently 193 herds in the UK are tested and validated free of MAP (HI Health, Herdcare and Premium Cattle Health Scheme). In this study, 14 herds bred their own replacements, had no history of disease and had no positive test results. With closed herds, free of MAP infection, the potential for within herd spread is largely irrelevant as long as the pathogen is kept out of the herd. The active surveillance required for these herds could be limited to monitoring of any problem cows and ensuring no changes in the purchasing strategy for the herd.

Many of the participating farmers commented on the difficulty they faced when expanding their herds due to the absence of available accredited stock. When accredited stock is unavailable, assurance is limited to veterinary certification of no confirmed cases with a defined period or sourcing stock from low risk herds (closed herds with no confirmed cases).

Interestingly three herds had confirmed clinical cases in bought in stock only. These herds were either open herds (buying in all stock) or had previously had no infection in the home herd and had unwittingly purchased stock infected with the disease. Five herds were heavily infected with Johnes (>5% clinical incidence) and were herds with over 100 cows, where the majority of stock calved indoors in calving boxes/ calving yards and where calves were fed waste milk. Some of these had multiple purchases of stock from the UK and The Netherlands.

CONCLUSIONS

All the farms that had a high incidence of clinical Johne's disease fell within the high risk group for contracting the disease according to their purchasing policy. However, nearly two-thirds of the participating farms were in this category and have a high risk of introducing the disease if stock of unknown Johne's health status continues to be traded.

All the farms in the study had calf-rearing systems which facilitate the spread of MAP. Coupled together, these findings should be of great concern to the industry as they support an increasing incidence of disease.

Farmers were unwilling to modify calf rearing practices unless there was compelling reasons to do so. Control measures such as sole use of milk replacer and more space per cow in calving areas are considered costly to implement and may have indirect economic disadvantages such as an increase in calf scour. Simpler and cheaper methods to establish herd status and assess herd risk factors such as those described in this paper are required to convince farmers of their value.

The challenge for the profession is to improve Johne's diagnostic approaches by finding ways of integrating disease history, risk factors analysis and bulk milk and targeted sampling to improve the likelihood of identifying infected herds early in the disease process.

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Table 1. Assigned likely risk of the presence of disease compared with confirmed incidence and the results of targeted sampling.

Risk of presence of disease	No. of herds	Clinical category in last 5 years	No. of herds with positive ELISA results
LOW	3	1 single case, 2 no disease	1[a]
MEDIUM	5	1 single case, 4 no disease	0
HIGH	15	5 heavily infected, 1 single case, 9 no disease	5[b]

[a] single animal in a low risk herd with no history of infection
[b] these herds were the 5 heavily infected ones

Table 3: Summary of mean risk factor scores for husbandry practices

husbandry practice (maximum possible score)	herd clinical Johne's history – last 5 years (no. of herds)		
	heavily infected (5)	single case (3)	none (15)
Calving areas	mean score	mean score	mean score
frequency of use (10)	10	10	8.7
manure build up (10)	10	10	8.7
holds older calves (10)	7	10	6
used for sick cows (10)	5	3.3	3
used for scouring cows (10)	6	6.7	3
Total	38	40	29.3
Nursing			
>24 hours with dam (10)	8	10	6.7
>4 days (10)	6	6.7	3.7
cows teats soiled (10)	4	3.3	2.1
Total	18	20	12.5
Milk/ Colostrum			
pooled colostrum fed (10)	10	10	6.1
waste milk fed (10)	10	6.7	9.3
Total	20	16.7	15.4
Pre-weaned calves contact with cows			
direct contact (10)	0	3.3	1.3
contact with adult faeces (10)	4	3.3	0.7
feeder faecal contamination (10)	3	3.3	0.3
Total	7	10	2.3
Post weaned heifers			
direct contact with cows and manure (5)	0	1.7	0.7
manure on young stock pasture (5)	0	0	0
Total	0	1.7	0.7
combined total	83	88.3	60.2
mean herd size	242	87	143