

Is targeted milk sampling an effective means of detecting Johne's disease in dairy herds?

Hanks, J.D.¹, Taylor, N.M.², Kossaibati, M.A.³,

¹PAN Livestock Services Limited, SAPD, Earley Gate, P.O.Box 237, Reading, RG6 6AR

²Veterinary Epidemiology and Economics Research Unit (VEERU), University of Reading, SAPD, Earley Gate, P.O.Box 237, Reading, RG6 6AR

³225 Cassiobury Drive, Watford, WD17 3AN

ABSTRACT

Testing of milk samples for Johne's disease (JD) antibodies is increasingly common in routine milk recording. This study used data from 69,798 cows in 385 herds that had routinely tested all cows for JD every 90 days for at least 12 months.

To encourage testing, farmers are commonly offered the cheaper option of a preliminary screen of just 30 cows. Uncertainty exists about the reliability of this approach, specifically with respect to interpretation of negative screening results.

The purpose of this work was to find empirical associations between health, production and fertility parameters and the JD test status of cows to identify valid criteria on which to base targeted selection of cows for screening that could increase detection sensitivity.

The JD status of individual cows was compared with other parameters from routine milk records. Strong associations were found between JD status and the cow's lactation number, current and recent somatic cell counts and milk yield.

Targeting the 30 cow selection increased the probability of detecting positive cows to levels comparable with random sampling of 60 cows and picked out at least one positive cow in 95% of the 385 study herds, compared with 84% if cows were selected at random.

INTRODUCTION

Johne's Disease (JD), otherwise known as paratuberculosis, is a chronic mycobacterial infection in cattle (Chiodini and others 1984). The disease is common in traditional dairy countries (Nielsen and others 2000). Early control programmes were based on vaccination, for example in France (Vallee and others 1934) and Great Britain (Wilesmith 1982). However, vaccination has not been widely adopted because it does not provide sterile immunity in all cases and can interfere with testing programmes for bovine tuberculosis (Benedictus and others 2000). More recently, control programmes have focussed on testing to identify infected animals and herds, linked to hygiene and sanitary measures. Australia has emphasised the control of animal movement and the use of serology in its programme for cattle and sheep (Kennedy and Neumann 1996, Jubb and Galvin 2004a, Jubb and Galvin 2004b). In Ontario, Canada, a control programme was introduced in 2005 that included the use of an antibody milk ELISA to routinely test milking cows (Sorge and others 2011).

Cost is an important consideration when a diagnostic test is to be used for screening large numbers of animals. Acceptable accuracy at least cost is often achieved by antibody detection using enzyme-linked immunosorbent assay (ELISA) technology because of its low cost and high-throughput potential through automation. Several ELISA kits based on serum antibody detection are available, and some companies have adopted this technology for milk samples (Collins and others 2005).

Since 2010 National Milk Records (NMR) has offered

ELISA testing for JD antibodies in routine milk samples as part of the "Herdwise" programme. Encouraging more dairy farmers to test for JD in their herds is essential if the disease is to be tackled effectively in the national herd, but many farmers are discouraged by the perceived high costs involved. The initial cost of detecting JD in the herd could be reduced if testing a sample of cows, rather than the entire herd, can be demonstrated to give a reliable result over a range of herd sizes and levels of prevalence of milk antibody positive cows.

NMR has offered an initial herd screen of 30 cows on a trial basis, selecting the sample of cows for milk testing according to age and general perceived health. Once positive cows are detected, farmers often move on to regular testing of the whole milking herd, linked to interventions to control further spread of infection in the herd. However, when no positive cows are detected in the 30 cow screen there remains doubt as to whether the herd was infected but positive cows were missed in the sample selection, especially when the prevalence of positive cows could be low. The sensitivity of the 30 cow screening approach is uncertain requiring a better understanding of the likelihood of detecting positive cows in herds with different prevalences of positive cows. Furthermore, a valid protocol for targeting the selection of cows for screening, that increased detection sensitivity, would reduce the uncertainty of a negative screening result. The objective of this work was to find empirical associations between health, production and fertility parameters and the likelihood that cows are positive. The principal aim was to identify valid criteria

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on which to base a targeted selection of cows for milk test screening and to quantify any increase in detection sensitivity.

The increased uptake of 90 day whole herd milk testing has provided a large dataset in which to explore the potential sensitivity of a 30 cow screen and the efficacy of a more structured protocol to target the selection of cows for testing.

MATERIALS AND METHODS

From the NMR "Herdwise" programme 385 herds were identified that had carried out routine 90 day testing for JD antibodies in milk for at least 12 months. These herds all had at least one positive animal at the herd's latest test date ('reference date'), which was between January and March 2012. In total 68,798 cows were tested on the reference date in the 385 herds.

The JD milk antibody ELISA results (positive or negative) of the individual cows on the reference date were collated in a dataset that also included key measures of production and health obtained from the milk recording data of each animal. An overall prevalence of cows with a positive ELISA test result for JD antibodies in the milk (hereafter referred to as 'positive cows') was calculated for each herd on the herd's reference date. In addition, the proportions of positive cows were derived for different groupings according to a range of measures. These included:

1. The lactation number of a cow on the reference date.
2. The somatic cell count (SCC) in the milk sample on the reference date. Cows were allocated to one of three categories according to their SCC, namely: below 200,000 cells/ml milk; 200,000 to 499,000 cells/ml milk, and; $\geq 500,000$ cells/ml milk. The figure of 200,000 cells/ml is commonly used as a threshold to indicate a high likelihood of bacterial infection of the udder. A higher value of 500,000 cells/ml is also used as indicative of longer established chronic infection (Hanks and Biggs 2008).
3. The number of high SCC ($\geq 200,000$ cells/ml) milk samples for the cow in the latest six milk recordings, up to and including the reference date. Only cows with six latest milk recordings were included (these milk recordings could cover more than one lactation).
4. The actual milk yield recorded on the reference date compared to the 'adjusted herd average'. This adjusted average is calculated for each herd within the InterHerd+ program based on past lactation records for cows in that herd adjusted for lactation number, days in milk and month of calving, as described by Tena-Martinez and others (2009). The difference between the actual yield and the adjusted herd average was then assigned to one of six categories: (1) lower than 50% less than the adjusted average yield; (2) between 25% and 50% less than the adjusted average yield; (3) between average and 25% less than the adjusted average yield; (4) up to 25% more than the adjusted average yield; (5) between 25% and 50% more than the adjusted average yield; (6) higher than 50% more than the adjusted average yield. Cows more than 305 days after calving and cows beyond their tenth lactation were omitted from this analysis because the average yield adjustment is less reliable in these situations.
5. Total services within 150 days of calving in the cow's current lactation. Only cows that were at least 150 days after calving on the reference date were included. Cows with zero services were excluded.
6. Total services within 150 days of calving in the cow's previous lactation. Only cows that were in second lactation or later were included. Cows with zero services were excluded.

The proportion of positive cows in each of these groups was compared in bar charts. Where distinct associations between the measure and proportion of positive cows were apparent, these associations were examined further by aggregating cows into two groups for comparison by calculating relative risk of being positive. The 95% confidence intervals for the relative risks were calculated using a standard formula (for example, as provided by Gardner and Altman 1994).

The measures most strongly associated with the proportion of positive cows were then used in a scoring system aimed at identifying cows most likely to be positive. The principles of the scoring system were that the levels of a measure associated with a higher proportion of positive cows would be allocated higher scores. Scores were weighted so that bigger differences in the proportion of positive cows between levels were reflected in bigger differences in score. Furthermore, weighting took account of the likely impact of the differences in the proportion of positive cows on the probability of detection by screening: for example, a doubling from 2% to 4% positive cows could be expected to increase the probability of detecting one positive in a sample of 30 cows from 50% to 75%, while a further doubling from 4% to 8% positive cows (twice the absolute increase) could be expected to increase the probability of detecting one positive in a sample of 30 cows from 75% to 95% (a smaller increase in probability of detection).

Every cow within each of the 385 herds in the dataset was given a total score based on their lactation number, their SCC history and the comparison between actual yield and herd adjusted yield on the reference date. To simulate a targeted 30 cow screening process, the cows with the 30 highest scores in each herd were selected within the dataset. Where there was a tie for 30th position, later lactation cows were preferred over early lactation animals.

To assess the efficacy of this targeted selection system for identifying cows most likely to be positive, the prevalence of positive cows in the targeted selection was compared with the prevalence among non-targeted cows by calculating relative risk (with 95% confidence interval). This comparison was performed over all cows

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and for cows grouped in categories of different herd sizes and different within-herd prevalences of positive cows.

The cows selected from each of the 385 herds were examined to assess the ability of the 30 cow targeted screening protocol to detect positive herds.

To assess the improvement in effectiveness of targeted over random selection of cows the probability of detecting at least one positive cow in a random sample of 30 cows was calculated based on a standard formula: $p=1-((1-(d/(N-((n-1)/2))))^n)$, where: p=probability to detect at least one positive in a random sample); N=herd size; n=sample size; d=the number diseased in the herd (Cannon and Roe 1982). The calculation was carried out using the known herd size and overall prevalence of positive cows in each of the 385 herds.

As the result of this calculation is a probability for each herd, the detection of herds varies depending on the random selection. To account for this, a Monte Carlo simulation of random sampling in the 385 herds was repeated 100 times. In each iteration of the simulation the proportion of herds in which at least one positive cow was detected was recorded. The median proportion detected (of the 100 simulations) was compared with the performance of the targeted selection.

This comparison was performed over all cows and for cows grouped in categories of different herd sizes and different within-herd prevalences of positive cows.

RESULTS

Factors associated with JD milk ELISA positive status

Of 69,798 cows tested for JD antibodies in the milk, 5,539 (8.1%) were positive at the reference date. It should be noted that the low sensitivity of the ELISA test (Lombard and others 2003, Jubb and others 2004) means that this will be an under-estimate of the true prevalence of JD infected cows. Most cows in the sample had a number of milk tests over the previous 12 months. There were cows that were negative on the reference date that had positive results at earlier recordings. The low sensitivity of the milk ELISA test, along with possible variation in levels of specific antibody in serum and milk related to stress and stage of lactation, are possible explanations for the negative result on the reference data. These cows can be assumed to remain infected. The prevalence of cows that had been positive to a milk ELISA test at any time in the previous 12 months was 15.8% of all cows tested on the reference date.

The median within-herd prevalence of positive cows on the reference date across the 385 herds was 7.0% of cows tested, with an interquartile range between 4.2% and 11.1%. Therefore, half the herds had 7% or fewer of their cows testing positive to the JD milk ELISA on the reference date, and a quarter of herds had 4.2% or fewer of their cows testing positive to the JD milk ELISA on the reference date.

The percentage of positive cows (according to the reference date test only) within each of the examined categories is shown in Figure 1 to Figure 6. Table 1 summarises the associations between these measures and JD milk ELISA status in terms of relative risk when cows are aggregated into two comparison groups. The

Figure 1. Percentage of JD milk ELISA positive cows tested on the reference date, grouped by lactation number.

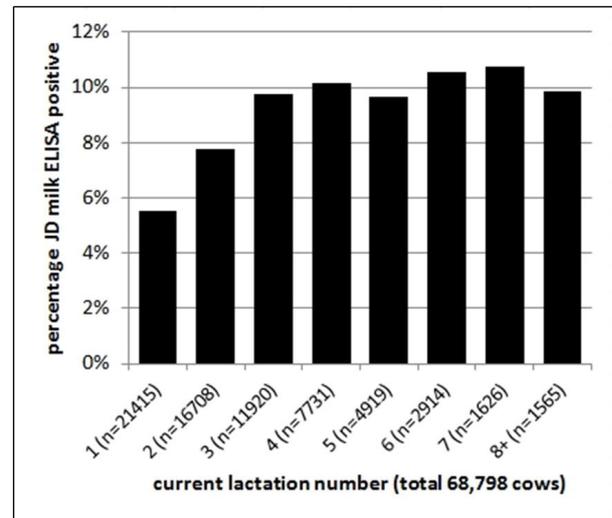


Figure 2. Percentage of JD milk ELISA positive cows tested on the reference date, grouped by the latest somatic cell count.

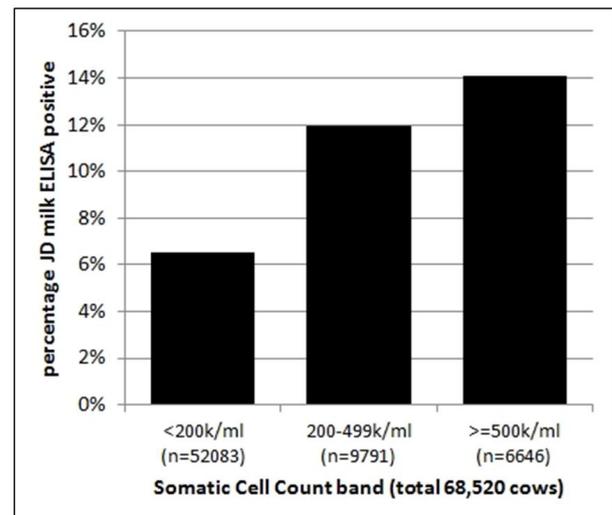
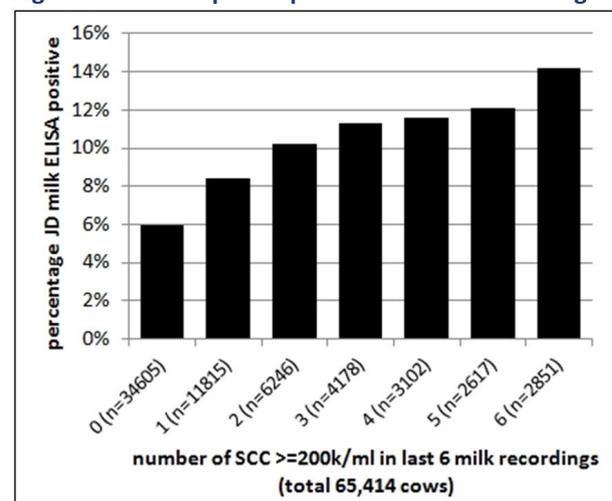


Figure 3. Percentage of JD milk ELISA positive cows tested on the reference date, grouped by the number of high SCC milk samples in previous six milk recordings.



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Figure 4. Percentage of JD milk ELISA positive cows tested on the reference date, grouped by total services recorded in the current lactation by 150 days after calving (excluding cows less than 150 days after calving on the reference date and cows with zero services).

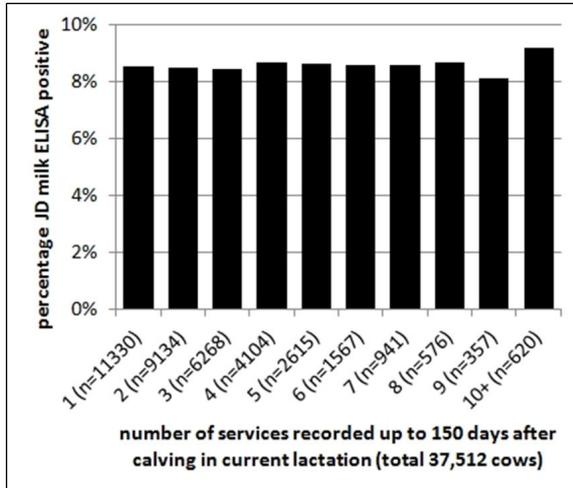
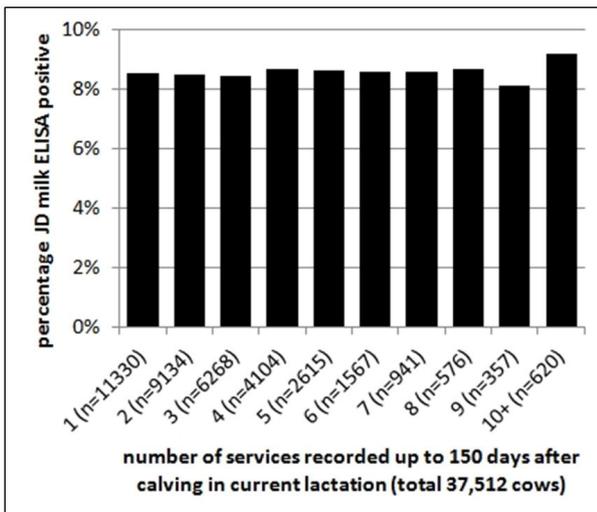


Figure 5. Percentage of JD milk ELISA positive cows tested on the reference date, grouped by total services in the previous lactation by 150 days after calving (first lactation and cows with zero services recorded are excluded).

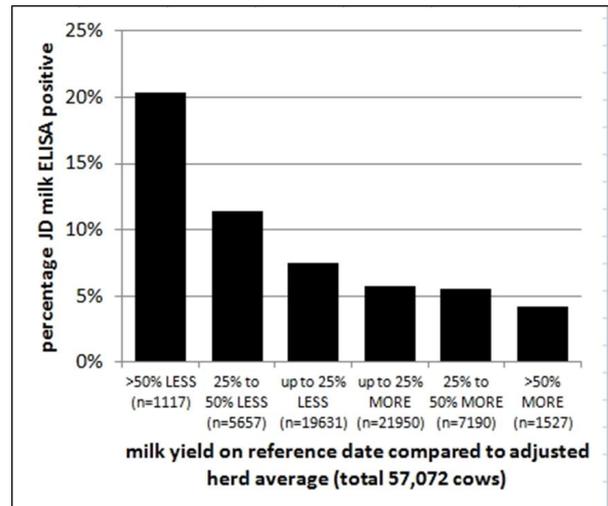


relative risk describes the difference in likelihood of cows in one group being positive compared to those in another group. A relative risk value of 1 indicates no association, while 2 would indicate a doubling of likelihood. The difference in relative risk from 1 indicates the strength of association between the parameter and the JD milk ELISA status. The associated 95% confidence interval is an indication of the statistical significance of that relative risk. A 95% confidence interval that does not include 1 indicates statistical significance with $p < 0.05$.

Table 1 shows apparent strong associations between JD milk ELISA status and lactation number, SCCs and milk yield, as follows:

1. Lactation number: Cows in lactation 3 and above are 1.5 times as likely to be positive than cows in lactations 1 and 2.

Figure 6. Percentage of JD milk ELISA positive cows tested on the reference date, grouped by the difference between actual and herd adjusted average milk yield (excluding cows over 305 days after calving and cows in over tenth lactation).



2. High SCC: Cows with SCCs over 200,000 cells/ml on the day of testing are 2 times as likely to be positive than lower SCC cows. Cows with 2 or more high SCC in the last 6 milk recordings are 1.8 times more likely to be positive cows. Figure 3 shows that the percentage of positive cows increases steadily with the number of high SCC recordings.
3. Milk yield: Cows giving 25% below the adjusted herd average yield, or lower, (adjusted for the cow's lactation, month of calving and days in milk) are 2 times as likely to be positive cows.

It was hypothesised that if JD infection negatively impacts fertility of cows, then returns to service would be more frequent in JD positive cows. This could be expected to result in a higher percentage of positive cows among those with higher numbers of services in the 150 days after calving. However, when cows that were over 150 days into their latest lactation were categorised according to the number of services by day 150 after calving, the percentage of positive cows was similar in all groups (Figure 4). Where the service data are available there is no clear association between the number of services and JD status. A similar analysis using the total services by day 150 in the previous lactation also revealed no clear association between the number of services and JD milk ELISA status (Figure 5).

Using the associations to actively select a sample of 30 cows for screening

Based on the stepwise associations between different factors and the proportions of positive cows (Figures 1 to 6), a scoring system for targeted selection was devised using the principles described in materials and methods. The resulting scoring system is shown in Table 2.

All cows in each of the 385 herds were assigned an aggregate score by summing the three components. The

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Table 1. Comparison of JD milk ELISA status between cows aggregated into two comparison groups for each of four measures.

		number cows tested	number positive	(% pos)	Relative Risk (RR)	95% confidence limits of RR
Lactation number	3rd lactation or above	30,675	3062	(10.0%)	1.5	(1.46 - 1.62)
	1st or 2nd lactation	38,123	2477	(6.5%)		
SCC on reference date	>=200,000 cells/ml	16,437	2106	(12.8%)	2.0	(1.86 - 2.06)
	<200,000 cells/ml	52,083	3416	(6.6%)		
Number of High SCC in last 6 records	>= 2 high SCC	18,994	2194	(11.6%)	1.8	(1.67 - 1.85)
	< 2 high SCC	46,420	3052	(6.6%)		
Yield on reference date compared with adjusted herd average	Yield 25% below aver- age, or lower	6774	870	(12.8%)	2.0	(1.88 - 2.17)
	Yield not more than 25% below average	50298	3195	(6.4%)		

Table 2. Scoring system used as the basis for targeted selection of 30 highest scoring cows in each herd.

Lactation number	Score
1	-6
2	0
3+	5
Number of SCC>=200k in last 6 MRs	Score
0	-7
1	1
2	4
3	5
4	5
5	6
6	7
Yield compared to herd average*	Score
>=50% more than average	-10
25% to <50% more than average	0
0% to <25% more than average	0
>0% to 25% less than average	6
>25% to 50% less than average	13
>50% less than average	15

*herd average adjusted for parity, month of calving and days in milk

30 highest scoring cows in each herd were selected for the 'virtual' targeted screening. Where there was a tie for 30th position, later lactation cows were preferred over early lactation animals.

The JD milk ELISA status at the reference date was checked for targeted and non-targeted cows. Table 3 shows that the targeted selection concentrated the Johne's positive cows to a significant degree. The relative risk (2.1) shows that the weighted scoring protocol selected a sub-population within which animals are more than twice as likely to be positive than the unselected cows. Table 4 shows that this doubling of proportion of positive cows was consistent across the range of within-herd prevalence and herd sizes.

The ability of the targeted 30 cow screen to detect herds with positive animals

While Table 3 and Table 4 show that the selection of cows by targeting according to performance can double the proportion of positive cows in the screened sample (compared to the non-targeted sub-population) the key question is how well the targeted selection of a 30 cow sample can detect herds with positive cows, compared with a random selection of 30 cows.

Table 5 compares the detection efficacy of the targeted 30 cow screen with screening of 30 cows selected by simple random selection, among herds grouped according to within-herd prevalence of positive cows.

Over all herds in the dataset, the targeted selection improves the likelihood of picking up a positive cow in a herd from 84% in a random sample of 30 cows to 95%

Table 3. Percentage of JD milk ELISA positive cows among the cows selected for the 30 cow sample in the 385 herds compared to non-selected cows.

	number cows tested	number	(%) positive	Relative Risk (RR)	95% confidence limits of RR
INSIDE the 30 cow sample	11,529	1,669	(14.5%)	2.1	(2.03 – 2.26)
OUTSIDE the 30 cow sample	57,269	3,870	(6.8%)		
ALL cows	68,798	5,539	(8.1%)		

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Table 4. Percentage of JD milk ELISA positive cows among the cows selected for the 30 cow sample compared to non-selected cows, in herds grouped according to within-herd prevalence and herd size.

	n herds	(n cows)	% positive among targeted cows	% positive among non-targeted cows	RR (targeted compared with not targeted)	lower 95% CI	upper 95% CI
Overall	385	(68,798)	14.5%	6.8%	2.14	2.03	2.26
By category of within-herd prevalence of positive cows							
<3%	60	(10,929)	3.6%	1.7%	2.09	1.57	2.77
3% to <5%	71	(15,364)	8.1%	3.5%	2.34	1.97	2.77
5% to <7%	61	(11,611)	11.2%	5.1%	2.21	1.89	2.58
7% to <10%	82	(15,578)	15.1%	7.4%	2.03	1.82	2.27
>=10%	111	(15,316)	25.7%	14.9%	1.73	1.61	1.86
By herd size category							
0-99	89	(6,426)	14.6%	9.0%	1.62	1.42	1.86
100-199	177	(25,929)	14.5%	7.4%	1.97	1.82	2.14
200-299	74	(17,809)	15.2%	6.3%	2.42	2.16	2.72
300+	45	(18,634)	13.0%	6.0%	2.17	1.86	2.52

Table 5. Percentage of the known positive herds detected by a 30 cow screening based on targeted and random selection, compared in different within-herd prevalence categories.

within-herd prevalence of JD milk ELISA positive cows	n herds	Cumulative % of all herds	% herds where positive cow(s) were detected using:	
			Targeted selection of 30 cows	Random selection of 30 cows*
<1%	5	1%	40%	23%
1% to <2%	24	8%	79%	43%
2% to <3%	31	16%	77%	57%
3% to <4%	32	24%	97%	70%
4% to <5%	39	34%	97%	79%
5% to <6%	28	41%	93%	85%
6% to <7%	33	50%	100%	90%
7% to <8%	25	56%	100%	93%
8% to <9%	24	63%	100%	95%
9% to <10%	33	71%	100%	97%
>=10%	111	100%	99%	99%
ALL herds	385		95%	84%

* median outcome of 100 Monte-Carlo simulations

by targeted screening. This improvement is apparent at all levels of within-herd prevalence, and is most dramatic in herds with a low prevalence of positive cows: of the 92 herds with less than 4% positive cows on the reference date, the targeted 30 cow sample would have detected positives in 76 (83%) compared with, on average, only 51 or 52 (56%) if the sampling were random.

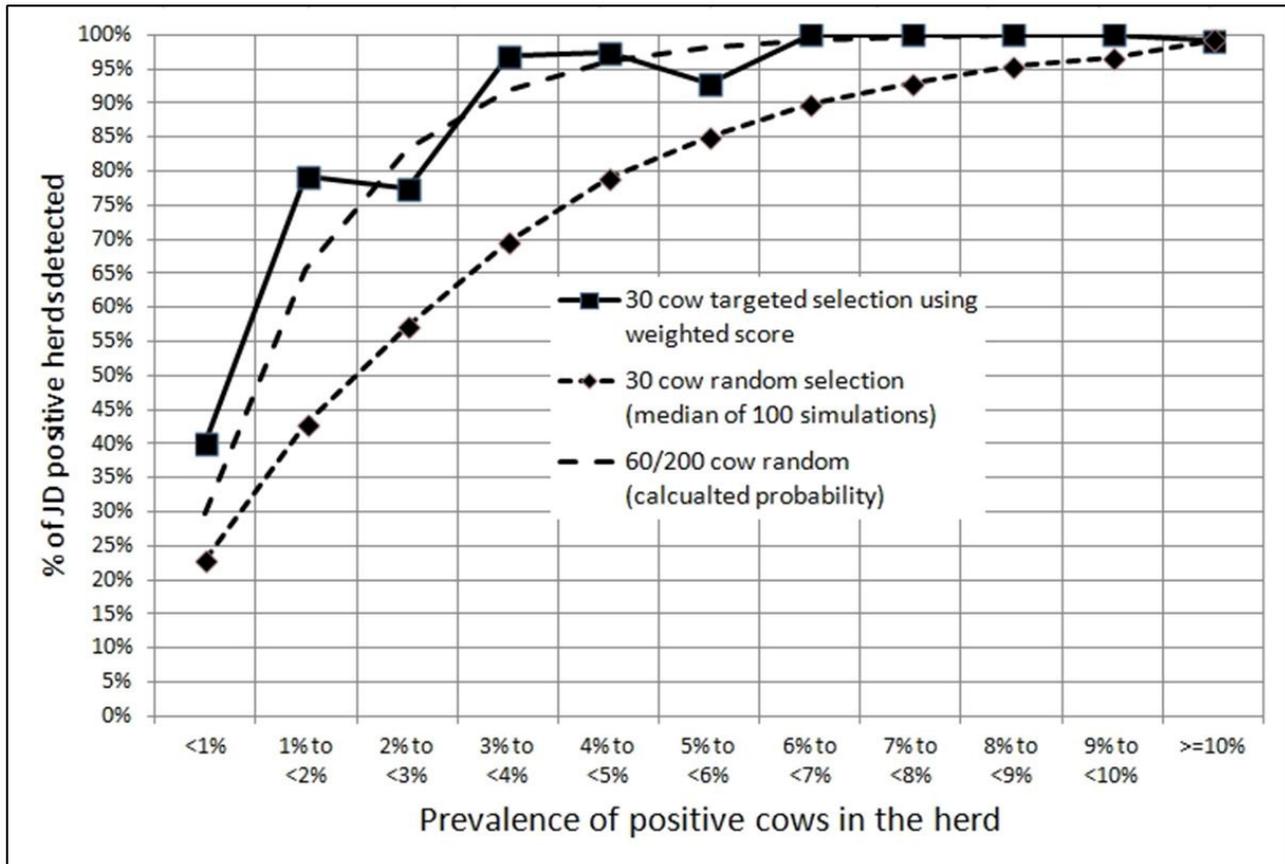
Figure 7 is a graphic representation of the data in Table 5. A further line is added to the chart illustrating the calculated probabilities of detecting at least one positive in a random sample of 60 cows from a herd of 200 cows. The figure shows that the targeted selection of 30 cows has a performance in detecting positive herds that is comparable with random sampling of 60 cows.

DISCUSSION

An important step towards proactive health management in dairy herds is early detection of infections to allow timely decisions on control strategies. Nielsen and others (2002) discussed the potential deficiencies in using ELISA for JD antibodies in milk as a tool to detect JD infected cows. They pointed out that the chronicity of the infection, associated with varying sequences of immune response and shedding of the organism over the course of infection, complicates diagnosis. They particularly noted that the sensitivity of the ELISA is not optimal in cows until their 2nd or 3rd parity due to the chronic nature of the disease and the time taken for an immune response to develop. They also suggest that changes may occur across lactation because the infected cow concentrates antibodies at certain times in lactation.

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Figure 7. Comparison of targeted selection against random samples of 30 and 60 cows for detecting positive herds at different prevalence levels.



Nevertheless, the use of ELISA for antibody demonstration is routine in growing numbers of countries, including the United Kingdom. Developments in ELISA testing allow farmers to have routine milk samples tested for evidence of many infections, making this a cost-effective, if imperfect, first approach to identifying infection in the herd. In the case of JD, the main argument favouring more expensive whole herd testing as a first step in herds of unknown status, is the risk of missing positive cows in a small sample. The challenge of detecting a positive cow in a sample of as few as 30 cows is greater where the proportion of positive cows in the herd is very low. For example, if the actual proportion of positive cows is less than 4% then the chance of detecting a positive animal in a screening sample of 30 randomly tested cows is less than 75%. A quarter of the 385 herds in this dataset contained below 4% positive cows. Detection sensitivity can be increased by increasing the sample size, but this also increases cost.

The purpose of this work was to find empirical associations between health, production and fertility parameters and the likelihood that cows are positive. The principal aim was to identify valid criteria on which to base targeted selection of cows for milk test screening and to quantify any increase in detection sensitivity.

Within the large dataset of 385 known JD positive herds the likelihood of a cow being positive was strongly associated with increasing age (as indicated by lactation number), high SCC and depressed milk yield. The present

study did not demonstrate an association with fertility, as indicated by total services between calving and 150 days after calving. This does not necessarily mean that JD infection has no effect on fertility as the number of services recorded is a very crude indicator of fertility performance and is influenced by many management factors. Furthermore, the accuracy with which farmers record services and pass on the information to the NMR recorder can be highly variable.

When the strongly associated parameters were combined using a weighted scoring system, the resulting targeted selection of 30 cows from each herd produced a sample with twice the proportion of positive cows, compared with the unselected remainder. Taken over the whole dataset, this result is in large part a self-fulfilling prophecy, in that the targeting was based upon pre-existing knowledge of the association between the selection factors and JD status in the dataset. However, the fact that the two times 'concentration' of JD positive cows within the targeted 30 cow selection was consistent within sub-groups of herds categorised by within-herd prevalence and herd size suggests that the targeting criteria would be widely applicable, and likely to remain valid outside the current dataset.

A doubling of the probability that each of the cows in the 30 cow sample is positive does not translate to a doubling of sensitivity to detect an infected herd, because detection requires only one of the 30 cows to be positive. The application of targeting criteria to the dataset and comparison with calculated probabilities

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of randomly selecting at least one positive cow in a 30 cow screen showed that using targeted selection would detect the disease in 95% of the herds, compared with 84% by random sampling of 30 cows per herd.

The improvement achieved by targeted sampling was greater among herds with lower proportions of positive cows. This result indicates that targeted screening has a larger competitive advantage in terms of detecting herds earlier, before infection has become widespread, allowing a control strategy to be put in place sooner rather than later.

It is important to acknowledge that even whole herd testing will fail to detect some infected herds, due to low test sensitivity. Routine repeat testing is recommended in all herds so a targeted 30 cow selection is clearly a financially attractive option for herds where the disease was undetected at earlier tests.

This analysis has demonstrated that, at least within the herds in this large dataset, a targeted 30 cow screening for JD antibodies in milk detects infected herds with a similar overall sensitivity to a random sample of 60 cows, which would have twice the cost. In herds with more than 3% cows positive the detection probability with targeted selection was very close to 100%, making this a very cost-effective initial approach compared to whole herd screening. The targeting of the sample uses existing data from routine milk recording and so entails very little additional cost.

In conclusion, the approach to targeted sample selection for herd health screening demonstrated in this study could be applied to other infections that are associated with consistent changes in production parameters and where tests are based on milk samples. It is highly likely that further analysis would refine the selection process for each disease, making routine health screening increasingly cost-effective, requiring fewer samples to maintain the required level of detection sensitivity.

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REFERENCES

- Benedictus, G., Verhoeff, J., Schukken, Y.H., Hesslink, J.W.** (2000) Dutch paratuberculosis programme history, principles and development. *Veterinary Microbiology* **77**: 399-413
- Cannon, R.M., Roe, R.T.** (1982) *Livestock disease surveys. A field manual for veterinarians.* Page 30. Department of Primary Industry, Bureau of Rural Science. Australian Government Publishing Service, Canberra 1982.
- Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S.** (1984) Ruminant paratuberculosis (Johne's disease): The current status and future prospects. *Cornell Vet.* **74**: 218-262
- Collins, M.T., Wells, S.J., Petrini, K.R., Collins, J.E., Schultz, R.D., Whitlock, R.H.** (2005) Evaluation of Five Antibody Detection Tests for Diagnosis of Bovine Paratuberculosis. *Clinical and Diagnostic Laboratory Immunology*, **12**(6): 685-692
- Gardner, M.J., Altman, D.G.** (1994) *Statistics with confidence.* BMJ publications. Reprint 1994 p51-52.
- Hanks, J.D., Biggs, A.M.** (2008) Chronic high somatic cell counts in the national dairy herd. *UK Vet.* **13**(3):
- Jubb, T. F., Galvin, J.W.** (2004a). Effect of a test and control programme for Johne's disease in Victorian beef herds 1992-2002. *Australian Veterinary Journal.* **82**(3): 164-166
- Jubb, T.F., Galvin, J.W.** (2004b) Effect of a test and control programme for bovine Johne's disease in Victorian dairy herds 1992-2002. *Australian Veterinary Journal.* **82**(4): 228-232
- Jubb, T.F., Sergeant, E.S.G., Callinan, A.P.L., Galvin, J.W.** (2004) Estimate of the sensitivity of an ELISA used to detect Johne's disease in Victorian dairy cattle herds. *Australian Veterinary Journal*; 2004. **82**(9): 569-573
- Kennedy, D.J., Neumann, G.B.** (1996) The Australian National Johne's Disease Market Assurance Program. In: *Proceedings of the Fifth International Colloquium on Paratuberculosis*, Madison, WI, pp. 121-131
- Lombard, J.E., Byrem, T., McCluskey, B.J.** (2003) Comparison of a milk ELISA test for Johne's disease to traditional testing modalities in dairy cattle. *Proceedings of the 10th International Symposium on Veterinary Epidemiology and Economics*, 2003. www.sciquest.org.nz
- Nielsen, S.S., Thamsborg, S.M., Houe, H., Bitsch, V.** (2000) Bulk tank milk ELISA antibodies for estimating the prevalence of paratuberculosis in Danish dairy herds. *Prev. Vet. Med.* **44**: 1-7
- Nielsen, S.S., Grohn, Y.T., Enevoldsen, N.C.** (2002) Variation of the Milk Antibody Response to Paratuberculosis in Naturally Infected Dairy Cows. *J. Dairy Sci.* **85**: 2795-2802
- Sorge, U.S., Lissemore, K., Godkin, A., Jansen, J., Hendrick, S., Wells, S., Kelton, D.F.** (2011) Changes in management practices and apparent prevalence on Canadian dairy farms participating in a voluntary risk assessment-based Johne's disease control program. *J. Dairy Sci.* **94**: 227-5237
- Tena-Martinez, M.J., Val-Arreola, D., Hanks, J.D., Taylor, N.M.** (2009) The use of early lactation milk protein content to predict subsequent fertility performance and likelihood of culling in commercial dairy cows. *Journal of Animal and Feed Sciences* **18**: 209-220
- Vallee, H., Rinjard, P., Vallee, M.** (1934) Sur la premunition de l'enterite paratuberculeuse des bovidés. *Rev. Gen. Med. Vet.* **43**: 777-779
- Wilesmith, J.W.** (1982) Johne's disease: a retrospective study of vaccinated herds in Great Britain. *Br. Vet. J.* **138**: 321-331