3.5 Objective 5: Epidemiology

Objective title Epidemiology
Objective text Development of control strategies to be used by deer, cattle and/or sheep farmers. This will be achieved through a better understanding of the epidemiology of the disease and economic impacts
Objective achievement measure
More robust understanding of Johne's disease in farmed livestock has been documented, and simulation models are available that include epidemiological and other information to assist farmers in reducing the impact of Johne's disease.

3.5.1 Milestone 5.1 Baseline data and Milestone 5.2 Modeling

Description 5.1	Determination of herd/flock prevalence of infection in single- and multi-species herds, risk factors for and herd/flock economic performance associated with the disease. Evaluation of the likelihood of transmission between species within herds.
Alignment with JDRC Strategy	Tool: Providing evidence to support Herd/Flock tools and management techniques to control JD
Status	Final year of project. On target, achieved contracted milestones 2008-2011. Note: This report includes details of results from Landcorp funded extension studies (proposed Milestone 5.1.1)

Description 5.2	Development of statistical and mathematical models to estimate determinants of herd/flock prevalence and economic outcomes of disease control measures.
Alignment with JDRC Strategy	Tool: Providing evidence to support Herd/Flock tools and management techniques to control JD
Status	Ongoing project (years 1-5). On target, achieved contracted milestones 2008-2011

JDRC Annual Science Review report

Year 3

(July 2010 to June 2011)

JDRC Platform: Epidemiology and Herd Control

Date: 22 March 2011

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1. Executive Summary

Activities scheduled under the 3-year plan of the epidemiology Objective are on target. A large mail survey (1,940 responding farms) has been completed, analysed and reported[8]. A stratified-random subset of 238 of the responding farms have been visited, sampled, and samples tested by culture and serology (Elisa, Paralisa©). The data were subjected to stochastic analysis evaluating the estimated true MAP infection prevalence of subsets of species/farm-combinations for beef cattle, sheep and deer. Estimates were adjusted for lack of sensitivity and specificity of the tests employed and for sampling fractions to reflect population prevalence. These two surveys (postal, sampling) informed JDRC about the clinical incidence of JD and the prevalence of MAP infection. Further analysis of these data allowed an estimate of the between species transmission (within farm) that is likely to occur through the joint use of pasture. In addition, results about the association between MAP infection or clinical JD with farm productivity parameters will be available until the end of year 3. A database of livestock farms with known infection status, disease incidence and production performance was established.

[8] Survey report 1: Livestock Survey about Johne's disease and leptospirosis, preliminary results; Survey report 2: Research into Johne's disease and Leptospirosis

In addition to these 238 commercial farms, 62 mixed species (S,B,D) and 35 dairy farms from Landcorp Ltd. (LC) were sampled in 2010, samples tested and results received. These data are currently analysed (March/April 2011).

Strain typing (ST) of 154 isolates was reported back to Massey on 22 February 2011. Molecular analysis of strain diversity and associations with phenotypes (clinical JD, species, farm, farm type) is currently being undertaken. First descriptive results showed that both Type-C and Type-S strains were isolated from all host species. The data also suggest that at least one ST was more associated than another ST with the reported occurrence of clinical JD. Further analysis will explore inter-species transmission, farm dependence and the hypothesis of a strain dependent virulence of MAP.

Modeling has advanced to the extent that, firstly, a diagnostic model has been completed and reported, evaluating the accuracy of faecal culture and Paralisa in deer. Secondly, a two-species (cattle, sheep) biological MAP- infection prevalence model has been developed as a prototype for multi-species simulation of the effect of interventions for JD control (e.g. grazing management, vaccination, test-and-cull, use of biomarkers).

Research during JDRC years 4 and 5 will be directed towards the evaluation of interventions strategies for the control of negative impacts of JD on farm productivity. In view of the short time frame for measuring intervention effects and uncertainties about available funding, it is recommended to use available resources for quantifying JD dependent sub-clinical production effects and validating the efficiency of intervention tools. Parallel development of simulation models is likely to achieve the overall goal of JDRC, the development and evaluation of herd control options for Johne's disease.

2. Introduction

Based on the justification of the overall JDRC science plan, the first three years of the Epidemiology Objective implemented two large scale surveys that aimed to describe the distribution, prevalence and incidence of MAP/JD in the target population of cattle, sheep and deer. Surveys were also designed to inform about interspecies transmission of MAP and associations between MAP/clinical JD and production parameters of herds and flocks. Strain typing information from MAP isolates generated from sampling multi-species mobs from commercial and Landcorp Ltd. farms contributed to information about transmission between species and allowed the testing of the hypothesis of strain dependent virulence variation.
This information is a key requisite for the next phase of epidemiological studies under JDRC. The data will inform the selection of candidate farms for field studies and the evaluation of economic effects of intervention to control JD.

3. Methodology

Baseline data (5.1)

3.1 A mail survey of 7,998 clients of 28 collaborating veterinary service clinics in Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui, Marlborough, Canterbury and Southland resulted in a database of 1,936 responses. The data informed about the clinical incidence Johne's disease (JD) and the risk of clinical JD on single- *vs.* multi-species properties.

3.2 A stratified-random sample of 300 farms from this database was targeted for sampling. The farm sample was stratified for reported clinical JD and species present on-farm. In 2009/2010, faeces and blood from 20 animals per species mob (sheep, deer, beef) of 238 of these farms were collected, tested by pooled faecal culture and serum-antibody, and subjected to analysis. Faecal samples were pooled (PFC) at reception and forwarded fresh

for Bactec culture; one aliquot was stored at -80 degrees for reference. All isolates were stored for strain typing. Blood samples were frozen and tested if the PFC of a species mob returned a negative culture result. A faeces and serum bank was established at Massey. Farm productivity data were collected again at the time of sample collection as a one-year follow-up of the postal survey data, as a second production cycle and validation of the previous postal data.

3.3 Under the agreement between JDRC and Landcorp NZ Ltd. samples were collected in identical fashion to 3.1 from all Landcorp farms, but also including dairy farms, to determine their MAP/JD status and estimating the infection prevalence of dairy farms.

3.4 Isolates from faecal cultures from commercial and Landcorp farms were subjected to genotyping by methods developed under JDRC by AgResearch at Wallaceville (methods described by Obj.3.2 Pathobiology). Results were received on 22 February 2011 and are currently analysed. The analysis describes the relatedness of strains from different sources using Bionumerics©, and explores associations between genotype and phenotype data collected through the survey using conventional statistical analysis and the specific software Structure©.

Modeling (5.2)

3.5 Faeces and blood samples were collected from individual deer from 20 herds in the South Island and 18 herds in the North Island with an approximately equal representation of farms with/without observed clinical JD. Samples were tested by individual faecal culture and Paralisa©. A diagnostic model for deer was developed to evaluate the accuracy of faecal culture and Paralisa© as tests for MAP infection in apparently healthy 1+ year-old deer. A latent class diagnostic model was developed in WinBugs through collaboration with the University of California, US, and University of Warwick, UK.

3.6 Survey data described under 3.1-3.3 were subjected to latent class prevalence models, one for each species (sheep, deer, beef cattle). Outcome parameters were true herd prevalence by island and farm type (i.e. species composition).

3.7 MAP farm prevalence and clinical incidence data of observed JD were evaluated to demonstrate interactions between co-grazing species.

3.8 A prototype of a mathematical, single-farm, multi-species animal prevalence model was developed to reflect changes in infection pressure and clinical disease in association with changes in the environment, host resistance and pathogen virulence. Environmental changes included human interventions for JD control.

4. Results

Baseline data (5.1)

4.1 The mail survey returned 1,940 responses (24.3%). Survey data analysis resulted in estimates of the regional distribution of observed and confirmed, annual incidence of clinical JD, and an association between clinical JD incidence and contact between species through co-grazing. Two reports (http://www.jdrc.co.nz/news.html#fnews) were distributed to 28 collaborating practices and their associated client farms providing the data.

4.2 Samples and test results from 238 commercial farms including 375 species mobs and 7,500 animals have been received and processed. Culture results are available from all farms. Results were reported back to collaborating veterinary practices and farmers and are available in a database. Figure 1 shows the location of JD positive and negative survey farms, in that a positive farm had at least one species test positive.

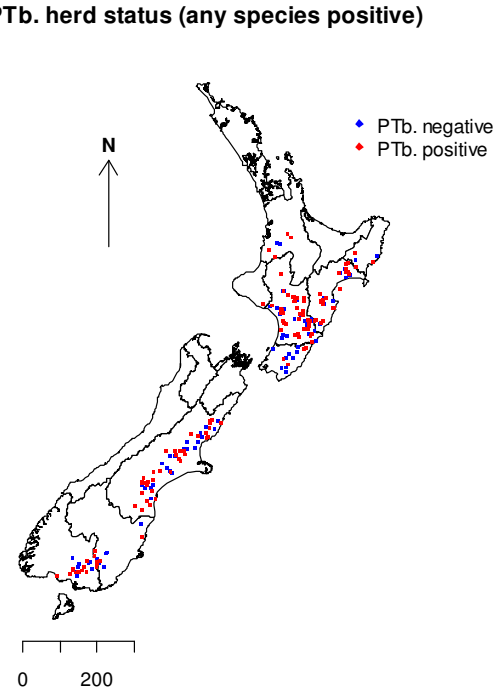

Figure 1: Location of JD positive and negative farms of which beef cattle, sheep and deer were sampled 2009/10.

4.2.1 **Prevalence:** The population of sheep breeding flocks were most highly infected by MAP (80%) followed by deer (51%) and beef herds (41%). The infection prevalence of deer was more than twice as high in the South Island than in the North Island. Table 1 shows the population prevalence of farms and species mobs in the two islands and at national level.

The results were adjusted for sampling fractions considering the stratified-random sampling design, and for the lack of test accuracy of Elisa, Paralisa and pooled faecal culture. The positive or negative differences between test and estimated true prevalence are attributable to the differences in Elisa or Paralisa prevalence and the associated misclassification as false negative and/or false positive.

Table 1: Herd and flock results of observed (Test%) and estimated true prevalence (ETP%) of MAP infection (2009/10). The rates were adjusted for lack of test accuracy of Elisa, Paralisa and pooled faecal culture, and for fractions of sampled strata.

	Farms	Sheep	Beef	Deer
North Island				
n	136	96	86	41
pos		73	27	20
Test%		76%	31%	49%
ETP (%)		74%	33%	42%
South Island				
n	102	66	30	58
pos		42	7	36
Test%		64%	23%	62%
ETP (%)		60%	27%	73%
New Zealand				
n	238	162	116	99
pos		115	34	56
Test%		71%	29%	57%
ETP (%)		68%	31%	60%

4.2.2 **Species interactions:** Co-grazing effects on MAP infection status and farmer-observed, clinical disease incidence of sheep flocks, beef and deer herds were evaluated by adjusting for confounding effects of misclassification due to the use of imperfect diagnostic tests and for incomplete testing of animals present on farm. Results are summarised as follows:

Sheep: Co-grazing with deer did not alter the risk of MAP infection but was associated with a lower incidence of clinical JD in sheep. Contact between sheep and beef cattle increased the risk for MAP infection in sheep, but did not affect the incidence of clinical JD in sheep. However, co-grazing sheep with both, cattle and deer, increased the expression of disease in sheep whereas MAP infection was not affected. The results suggest that farms co-grazing sheep, deer and beef with contact among all species are particularly prone to report clinical disease in sheep, possibly due to either farming system specific management factors or a biased awareness.

Beef cattle: Co-grazing with sheep increased both, the MAP infection risk and the reported incidence of clinical JD in beef cattle. In contrast, co-grazing beef cattle with deer tended to reduce the infection risk and clinical disease in beef cattle.

Deer: Contact with beef cattle increased the risk of MAP infection and occurrence of clinical JD in deer. Co-grazing deer with sheep did not alter the infection risk, but reduced the rate of clinical disease in deer. Similar results were reported from an earlier case-control study of deer herds (Glossop et al. 2008).

As a general trend, deer benefitted from co-grazing with sheep but experienced higher infection and clinical disease rates when in contact with beef cattle. Beef cattle also increased the risk for sheep, and were themselves at higher risk when in contact with sheep

than when grazed in isolation. But both, beef cattle and sheep, benefitted from the presence of deer. The results suggest that MAP is often transmitted between livestock species by either direct contact or contamination of pasture.

4.3 Sampling of Landcorp farms has been completed by December 2010. Results from faecal culture are available, sera from negative farms are waiting to be tested.

4.4 Descriptive results of strain typing were reported by the JDRC Objective 3.2 Pathobiology (Des Collins/AgRes) who processed isolates collected by the Epidemiology Objective. Initial descriptive results are available and more specific analyses are ongoing, notably a description of the relatedness and associations with phenotypic data. Some initial findings are:

4.4.1 A total of 168 strain types (ST) from 154 isolates were obtained from 96 farms, 26 from beef cattle (20 farms, 13 Type-C, 13 Type-S), 67 from deer (31 farms, 64 Type-C, 3 Type-S), and 75 from sheep (57 farms, 12 Type-C, 63 Type-S). This included 39 STs from Landcorp Ltd. farms. In the classification sequence, numbers indicate tandem repeats of VNTR loci 292-25-X3-7-3 and SSR8. Different numbers within the same locus were regarded as different STs from the same sample/isolate.

Two deer farms where 20 individual animals were sampled returned 7 and 13 FC positive animals, respectively. All isolates on both farms were of the same strain type (ST 432224).

The cross-tabulation of strain types by clinical JD cases showed that one strain (ST 432224) was isolated from 45 farms all of which reported clinical JD whereas this type did not occur on a single farm without disease. Given that 35/129 (27%) farms did not report clinical JD, this strongly suggests an association between ST 432224 and clinical JD. In contrast, ST 431113 was isolated from 52% farms without and 48% farms with clinical JD, suggesting a negative association, as 73% of all farms reported clinical JD (Table 2).

The majority of the 62 isolates of the ST 432224 (disease associated) were from deer: 49 from deer *vs.* 6 from sheep and 7 from beef cattle. Almost all (97%) bovine Type-C isolates were from farms reporting clinical JD whereas only 46% ovine Type-S were from farms with clinical JD. However, both types were isolated from all livestock hosts: 50% isolates from cattle, 16% isolates from sheep and 96% isolates from deer were MAP Type-C.

Table 2: Association between MAP strain types and the number of observed clinical JD cases over the past four years: entries in the table are the number of farms (excluding 39 Landcorp farms of which information about clinical JD has not yet been received).

	Observed clinical cases of JD over past 4 years					
Strain type	0	1	2	>=3	Total	% clin.JD
3320.525			3	0	3	100%
331113	2	2		1	5	60%
331213				1	1	100%
332223				0	0	
332224			1	1	2	100%
332225	2	4	6	0	12	83%
431113	25	9	1	13	48	48%
431213	2	1		2	5	60%
432214		1		0	1	100%
432224		1	41	3	45	100%
432225			1	0	1	100%
432234		1		0	1	100%
531113	2			0	2	0%
631113	1			0	1	0%
731113	1		1	0	2	50%
Total isolates	35	19	54	21	129	

Even though only a small and highly variable number of isolates was obtained from an individual farm, the tendency of the same strain type to be repeatedly found on the same farm was examined (Figure 2). The general pattern followed the expectation that every isolate from a farm was different from other isolates from the same farm. However, isolates from two farms, each with a larger number of isolates from deer, were all of the same strain type (432224).

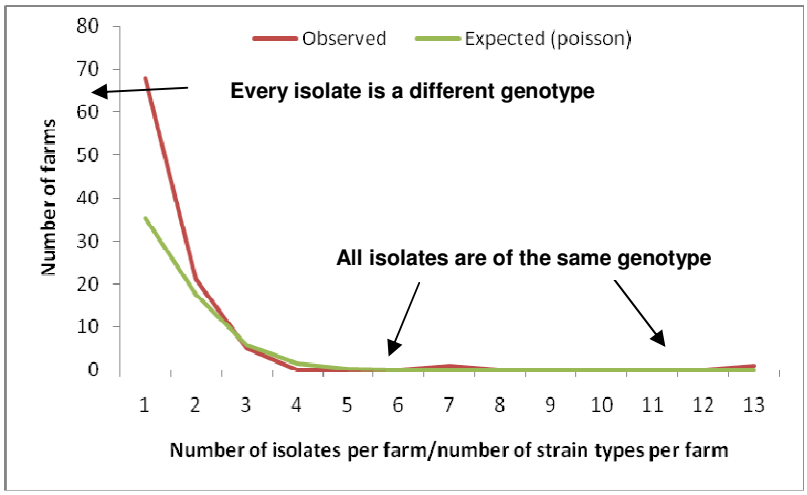

Figure 2: Number of different strain types from single farms: 1 = every isolate is a different strain; 13 = all 13 isolates were of the same strain-type (red line). The green line is the expected count of farms assuming that every isolate from one farm is a different strain (average count = 1)

Further analysis will adjust these rates for confounding effects of location, species, clinical disease incidence and co-grazing between species.

Modeling (5.2)

4.5 Paired faecal and serum samples were collected, between July 2009 and April 2010, from 20 individual yearling (12-24 month old) deer in each of 20 South Island (SI) and 17 North Island (NI) herds and subjected to culture and the Paralisa test, respectively. Two faecal samples and 15 serum samples from 336 NI deer, and 55 faecal and 37 serum samples from 401 South Island deer, were positive. Bayesian latent class models were developed according to the code of conduct for the validation of diagnostic tests recommended by the OIE. The estimate of IFC sensitivity was 77% (95% CI 61-92%) with specificity 99% (95% CI 98-99.6%). The Paralisa sensitivity estimate was 19% (95% CI 10-30%), with specificity 94% (95% CI 93-96%). All estimates were robust to variation of priors and assumptions tested in a sensitivity analysis. These data inform the use of the tests in determining infection status at the individual and herd level. They may therefore be applied to developing herd classification programmes and to monitor the effects of control interventions in New Zealand. Results and inferences were reported in detail to JDRC and can be viewed http://www.jdrc.co.nz/members_area/sciencereview.html.

4.6 The results of latent class modelling are presented above (4.2.1).

4.7 Results of Bayesian models evaluating interactions between livestock species and MAP infection prevalence and reported clinical disease incidence are briefly described above (4.2.2).

4.8 A biological MAP infection prevalence model initially considered the approach described by Mitchell et al. (2008) for dairy herds. A second species model was added and transmission between these species was facilitated through the joint use of pasture. Transmission through the environment considered the amount of shedding at different stages of infection, the number of animals shedding, the stocking density and the amount of grass ingested per day. The model was positively reviewed by an external collaborator from Cornell University. However, more beta-testing under different scenarios is required for further adjustments to improve the robustness of this prototype (May to August 2011).
The model constitutes the basis for simulating intervention effects (e.g. multi-species grazing management) on the prevalence of MAP infection and the incidence of clinical JD. The final step is to link this biological model with an economic module that converts prevalence and incidence to production loss, and considering the cost of control efforts, to cost-benefits discounted over time.

5. Discussion

Robust information is now available about the distribution of farms and adult herds and flocks of sheep, deer and beef cattle infected with *Mycobacterium avium* subtype *paratuberculosis* (MAP) as well as about the incidence of clinical disease in these species. However, a bias in the data about clinical disease cannot be ruled out: deer farmers might have overestimated the occurrence of JD in their herds (clinical rates in the North Island were higher than infection rates), whereas

sheep and beef breeders might have overlooked (and rarely tested for) JD when the incidence was low, and therefore underreported the disease. The herd/flock infection prevalence was highest in sheep and deer (68% and 60%, resp.) and only about half as high in beef cattle (31%). The low rates in beef cattle, as opposed to a much higher (presumed) infection rate of dairy herds, may be attributable to a lower production intensity, notably to a much lower stocking density and less chance of contact between young (susceptible) and adult (shedding) animals. If this deduction is correct, it suggests that most infections are transmitted through the environment, i.e. pasture in the New Zealand systems. Since beef calves usually spend 4-7 months with their dams before being separated, it also suggests that the cow-calf transmission route, including vertical and pseudo-vertical transmissions, is of lesser importance than infection facilitated through pasture. Pasture dependent infection is therefore an important transmission route for the consideration in herd control interventions. Mathematical models of infectious disease for New Zealand's pasture based production systems (as opposed to housing systems typical for Northern hemisphere systems where previous models were developed) need to adequately accommodate this transmission pathway.

The prevalence data generated in 2010 revealed significant associations between the risk of MAP infection and the mix of species on farm. Results suggest that contact through the joint use of pasture (co-grazing) facilitates transmission across species. Moreover, the incidence of farmer-observed clinical JD also depended on the presence/absence of other co-grazing species. However, the risk of infection or clinical JD was not uniform. For example, both sheep and deer were more likely to be infected when co-grazing with beef cattle than when grazing separately or on single-species farms. Co-grazing with beef cattle also increased the clinical JD incidence in deer, whereas co-grazing deer and sheep decreased clinical JD in both species. This finding confirms earlier observations in deer (Hunnam et al, 2011). It further suggests that reporting bias of farmers – i.e. one farmer observing clinical JD in one species might be more likely to also observe JD in another species – might have been low, else finding both positive and negative associations between species were unlikely. These findings propose that JD control may be supported, if not feasible on its own, by managing the co-grazing of different species, i.e. keeping contact between deer/sheep and cattle at a minimum, and increasing joint grazing of sheep with deer. Such a control option, on farms where this can be managed, may be more cost effective than expensive test-and-cull strategies. Extra effects like the control of worm burden may be brought into consideration when appreciating the effect of managing the co-grazing of different species.

The participation of Landcorp NZ Ltd. in the survey of sub-clinical prevalence of MAP-infection (extra cost funded by Landcorp) has enhanced the statistical power of the analysis of effects described above. While data collection is almost complete and all farms and most samples were tested, comprehensive analysis is still due to be performed. In addition, the Landcorp data will allow estimating the MAP infection rate, clinical JD incidence, and the association of MAP/JD with production performance of dairy farms.

Modeling has delivered several anticipated outcomes. Firstly, a validation of the two tests of MAP/JD most frequently used in New Zealand deer at present (faecal culture, Paralisa©). The results suggested that the use of the Paralisa© for the classification of normal, unaffected yearling deer as MAP-infected has low accuracy (20% sensitivity, 94% specificity). It is appreciated however, that the Paralisa© appears to perform much better when deer are clinically affected or are strong shedders. If used for test-and-cull in herds with a low MAP prevalence, the low sensitivity may not be as much a constraint as the 6% false positive rate, because most animals would be non-infected on such farms, hence affected by this false positive rate and be unreasonably culled. Consequently, this test may be useful for a test-and-cull intervention of severely affected herds, i.e. deer with a high infection rate, high incidence of (confirmed) clinical JD and a likely high proportion of shedders. The logical next step is to validate the test for such herds with the aim to predictive the level of shedding and the risk of future clinical disease.

Another important outcome of modelling is a prototype of an infectious disease model to be used as a basis for simulating interventions. Collaborative agreements have been set up with the University of Sydney and the University of Cornell for further developing this model. A new PhD candidate has started working on this project (Dec 2010).

6. Conclusions

At reporting date (22 Mar 2010), the epidemiology Objective has achieved most of the anticipated targets for year 3 (Jul'10 – Jun'11). Robust estimates of the MAP infection prevalence were established for sheep, deer and beef breeding herds, studies hypothesizing species interactions have provided strong evidence for both, transmission of MAP between species and the consequence of clinical JD. First results of associating MAP VNTR/SSR strain types (ST) with phenotypic data suggested that one ST was more, another one less likely to be found on farms reporting clinical JD. More results, including a comparison of production performance of farms infected/not infected with MAP, will be available by the end of year 3. Year 3 has also established a database of farms infected with MAP and affected by JD. , setting a basis for studies in years 4+5.

Years 4+5 will focus more directly on the evaluation of interventions for the control of negative impacts of MAP/JD on infection prevalence, disease incidence and farm economics. Considerable thought has been given to the design and ramifications of intervention studies. It was found that, if properly executed, such studies would require considerably more time than two years. Moreover, no clear funding guidelines are yet available, thus limited funding may further jeopardize on-farm intervention studies. It is therefore strongly recommended to prioritise the validation of intervention tools (tests, gene markers) and quantification of sub-clinical effects of MAP on production performance in individually monitored animals.

As already pointed out in the annual science report 2010, we propose the implementation of longitudinal studies in dairy cattle, sheep and deer, and the continued development of mathematical models to evaluate on-farm interventions. Our findings have shown that such interventions are not limited to test-and-cull, the use of biomarkers and vaccination, but may also include adequate pasture management.

7. Acknowledgement

Our studies in 2010 would not have been feasible without the help of hundreds of farmers and 28 contracted veterinary practitioners who facilitated and implemented sample collection. Their support is equally appreciated. We also like to express our gratitude to Landcorp Ltd., notably Gordon Williams, for supporting the JDRC epidemiology Objective with the contribution of all farms for sampling and for funding these activities.
We also like to thank Geoff DeLisle and Des Collins, AgResearch Wallaceville, for testing faecal samples by culture and strain typing of isolates as well as for valuable advice on interpreting the test findings. Thanks also to Raewynne Pearson, NZVP Palmerston North, Simon Ligget and Frank Griffin, DRL Dunedin, University of Otago, for testing serum samples by Elisa/Paralisa, and Rory O'Brian for pilot testing of faecal samples by RT-PCR.

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