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Evaluation of PFC and AGID as flock-screening tests for OJD

National Ovine Johne's Disease Control and Evaluation Program

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TABLE OF CONTENTS

Executive Summary

A Monte Carlo simulation model was developed to estimate the sensitivity of pooled faecal culture (PFC) and the agar-gel immuno-diffusion test (AGID) as flock-screening tests for ovine Johne's disease under a range of scenarios. The flock-sensitivity of a test is the level of confidence of detecting a specified prevalence of infection. Outputs from the model are probability distributions for the flock-sensitivities of the two tests for a given scenario. The model allows direct comparison of the tests under a variety of conditions, and considers the effects of:

- Variations in animal-level sensitivity of the tests;
- Variations in flock size, sample size and prevalence of infection;
- Variations in animal-level sensitivity with type of lesion (paucibacillary or multibacillary);
- Variations in the proportions of animals with paucibacillary/multibacillary lesions; and
- Reduction in animal-level sensitivity due to pooling effects in pooled faecal culture;
- Uncertainty as to the true values of input parameters such as animal-level test sensitivity and proportion of sheep with different lesion types.

Comparison of model outputs with results from a field trial of pooled faecal culture, and with calculated estimates, confirmed that the model provides reasonable estimates of flock-sensitivity of the tests.

The model was used to estimate:

- the flock-sensitivities of current testing strategies;
- sample sizes required for pooled faecal culture and serology to provide desired levels of flock-sensitivity for surveillance and market-assurance testing; and
- the sample size required for serology to provide equivalent flock-sensitivity to pooled faecal culture under a range of scenarios.

The mean flock-sensitivities for a Check Test (sample size = 100) were 67% and 42% for PFC and AGID respectively, and for a Sample Test (sample size = 350 for PFC, 500 for AGID) were 98% and 93% respectively. When large flocks were sampled, sample sizes of 300, 350 and 450 provided a flock-sensitivity for PFC of about 95%, 98% and 99% respectively, to detect infection if present at a prevalence of 2% in the sampled population. Sample sizes for the AGID to provide equivalent sensitivity to PFC were generally 2 – 3 times the PFC sample size, depending on the assumed animal-level sensitivities of the tests.

When whole-flock testing was simulated, AGID flock-sensitivity was generally poor for smaller flock-sizes and low prevalence or animal-level sensitivities, whereas PFC flock-sensitivity remained high. The flock-sensitivity for PFC was ≥98% for all combinations of flock size, prevalence, percentage of paucibacillary lesions and animal-level sensitivities tested compared to ≥68% for the AGID.

Although the AGID appears to perform reasonably well in higher prevalence flocks, its flock-sensitivity in low prevalence or recently infected flocks is likely to be very low, unless sample sizes 2 – 3 times those used for PFC are used. This is particularly important in Australia at present, as the majority of flocks being investigated outside the Residual Zone (endemic area) are likely to be relatively recently infected and still have only a low prevalence of infection. In these circumstances, PFC should be the preferred test, and larger sample sizes or whole-flock testing should be considered to maximise flock-sensitivity.

In higher prevalence flocks, such as many of those in the Residual Zone, the AGID will provide a satisfactory flock-sensitivity and a more-rapid result than PFC, particularly if biased sampling is used to maximise animal-level sensitivity and provided sample sizes are adequate.

Recommendations

1. PFC should be the preferred screening test for surveillance and market-assurance testing for ovine Johne's disease in Australia, particularly for advancement of status.

2. The AGID may be an appropriate alternative to PFC where prevalence is likely to be high or where a rapid result is required.

3. Where the AGID is used, sample sizes should be at least 2.5 times the recommended sample size for PFC, and testing should be targeted at animals most likely to be infected (poor condition or suspect sheep).

4. Recommended sample sizes for PFC and AGID should be ≥350 and ≥850 respectively for a Sample test.

5. In suspect flocks where infection is likely to be recently introduced or prevalence is likely to be low, PFC should be the preferred test, and sample sizes ≥500, or whole-flock testing, should be considered as alternatives to Sample Testing.

6. Increasing the recommended pool-size for PFC from 50 to 100 should be considered, as a means of further reducing test costs.

Introduction

Ovine Johne's disease was first diagnosed in Australia in 1980, in the central tablelands region of New South Wales (Seaman et al., 1981). By the end of 2000, the disease had been confirmed in more than 800 flocks in all Australian States and Territories except for Queensland and the Northern Territory (Sergeant, 2001). In early 1999, agreement was reached for a 6-year national program for the control and evaluation of ovine Johne's disease in Australia, funded jointly by governments and industry.

Pooled faecal culture (PFC) was developed as a flock-level screening test for the diagnosis of ovine Johne's disease in Australia. It has the advantage of being much less expensive than serology and has been approved for use for surveillance and market-assurance testing under the national program since 1999 (Whittington et al., 2000; Sergeant, 2001).

Evaluation of PFC in pilot and field trials has shown it to be a highly sensitive and specific flock-test for detection of ovine Johne's disease infected flocks (Whittington et al., 2000). Further analysis of field trial data has demonstrated that PFC is a far more sensitive flock-test than the agar-gel immuno-diffusion test (AGID), when used on the same sheep in infected flocks, particularly in low prevalence flocks (Sergeant et al., in press). Concurrent analysis of other data from field use of pooled culture has demonstrated that it is also a highly specific test, and that flock-specificity was 100% when *M a paratuberculosis* was isolated on solid medium (Sergeant et al., in press).

The flock-sensitivity of a test is the level of confidence of detecting a specified prevalence of infection for a given sample size and animal-level sensitivity of the test. Estimation of the flock-sensitivity of PFC and AGID under various scenarios of prevalence and sample size is essential for the development of appropriate testing strategies and to ensure the optimum use of these tests in surveillance and market-assurance programs in Australia. However, the above evaluations were limited to a comparison of the PFC and AGID in the same sheep in 100 apparently infected flocks. These analyses were therefore unable to provide a direct evaluation and comparison of different testing strategies and sample sizes for the two tests under pre-determined conditions of prevalence and sample size.

The aims of this project were to provide comparative estimates of the flock-sensitivity of PFC and AGID under a range of likely scenarios, using a simulation approach. Sample sizes required to achieve equivalent performance of PFC and AGID under different conditions of prevalence and desired flock-sensitivity were also estimated, as well as the effect of variations in the assumptions on which the model was based.

Objectives

The specific objectives of this project were:

- To develop a Monte Carlo simulation model for the estimation and comparison of flock-level sensitivities of screening tests for ovine Johne's disease;
- To test and validate the model using data from the PFC field trial and by comparison with the results of alternative methods of estimation;
- To develop comparative estimates of flock-sensitivities of PFC and AGID under varying conditions of disease prevalence and sample size
- To estimate sample sizes required for equivalent flock-sensitivities for PFC and AGID under a range of scenarios; and
- To investigate the effect of varying assumed input values on the resulting flock-sensitivity estimates.

Methods

Simulation model to evaluate flock-sensitivity of PFC and AGID

A computer model to simulate the selection and testing of sheep was developed using Excel (Microsoft Corporation) and @Risk (Palisade Corporation) computer software.

For each iteration of the model the flock-sensitivity of each test was estimated as shown in Figure 1 and Table 1. Fixed input values were used for flock size, prevalence, sample size and pool size (PFC only) for each simulation. Probability distributions were used to estimate the numbers of infected sheep with paucibacillary and multibacillary lesions in the flock and in the sample, and the animal-level sensitivities of the tests for each lesion type.

Table 1: Description and formulae for variables used in a simulation model to estimate the flock-sensitivity of PFC and AGID for paratuberculosis in sheep

Description	Variable	Formulae
Flock size	N	fixed for each simulation
True prevalence of infection in the flock	TP	fixed for each simulation
Sample size	n	fixed for each simulation
Pool size	s	fixed for each simulation
Number of infected animals in flock	x	= Round(N × TP,0)
% of infected animals with paucibacillary lesions	p	= RiskBeta(α, β)*
Number of infected animals in flock with paucibacillary lesions	n(p)	= Round(x × p,0)
% of infected animals with multibacillary lesions	m	= 1 – p
Number of infected animals in flock with multibacillary lesions	n(m)	= x – n(p)
Number of animals with paucibacillary lesions in sample	x(p)	= RiskHypergeo(n, n(p), N)
Number of animals with multibacillary lesions in sample	x(m)	= RiskHypergeo(n, n(m), N)
Sensitivity in animals with paucibacillary lesions	Se(p)	= RiskBeta(α, β)*
Sensitivity in animals with multibacillary lesions	Se(m)	= RiskBeta(α, β)*
Probability ≥1 paucibacillary animal will test positive	P(p+)	= 1 – (1 – Se(p))^x(p)
Probability ≥1 multibacillary animal will test positive	P(m+)	= 1 – (1 – Se(m))^x(m)
Flock-sensitivity (Probability ≥1 animal (either paucibacillary or multibacillary) will test positive)	Se(flock)	= P(p+) + P(m+) – P(p+) × P(m+)

* α and β parameters for RiskBeta distributions for p, Se(p) and Se(m) were as shown in Table 2 for all simulations except where specified otherwise.

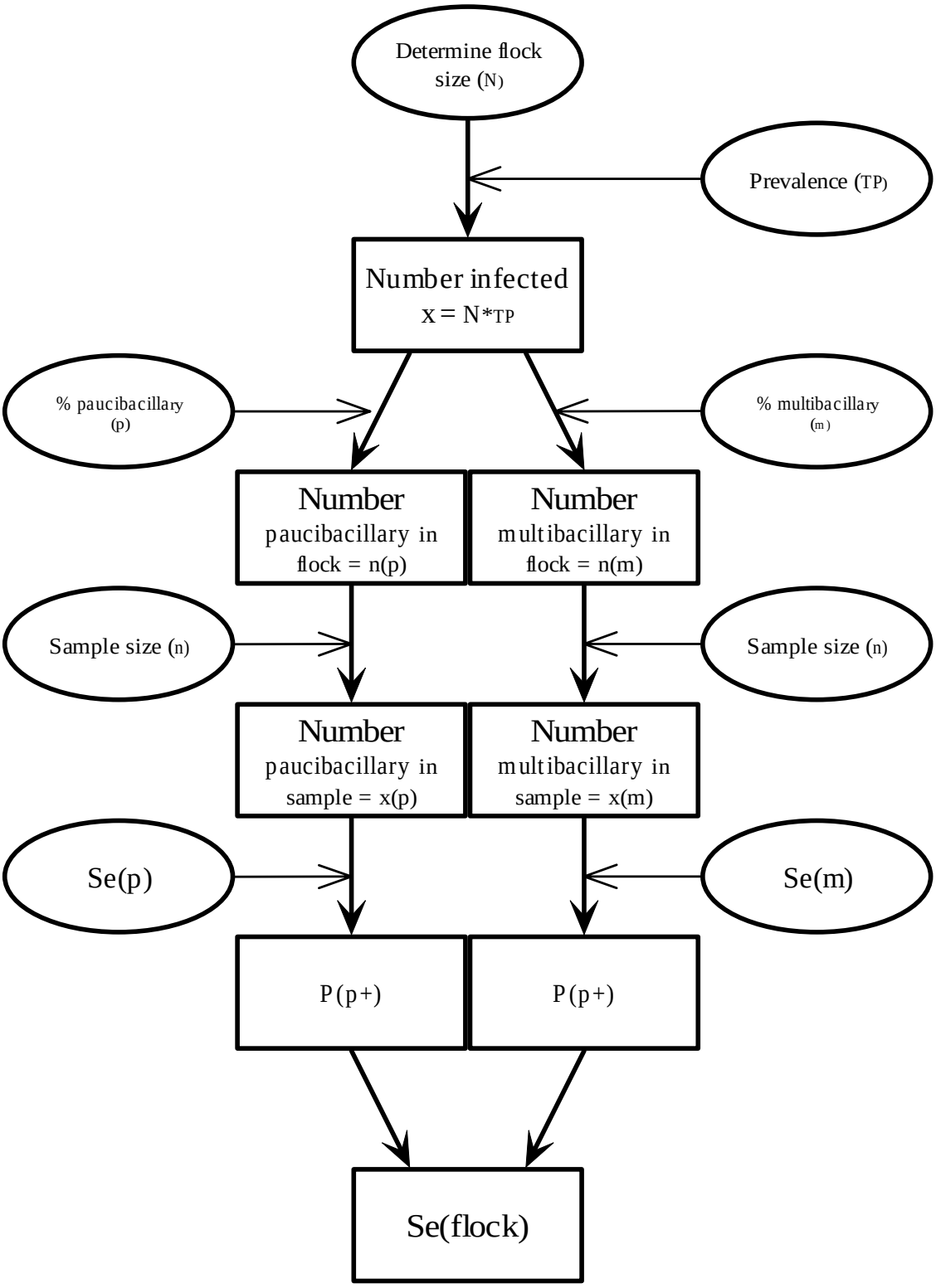
The probabilities of detecting ≥1 test-positive animal were calculated separately for sheep with paucibacillary and multibacillary lesions, and the flock-sensitivity for the test was the overall probability of detecting one or more infected animals of either lesion type. For each iteration, the flock-sensitivities of PFC and AGID were estimated assuming that the same animals were sampled for each test.

Beta distributions were used to describe the proportion of infected sheep with paucibacillary lesions, and the animal-level sensitivities of both PFC and AGID in sheep with paucibacillary and multibacillary lesions. A Beta distribution is a probability distribution commonly used to describe uncertainty about the true value of a proportion (Vose D, 1997; Vose, 2000, p 104). It is defined by the two parameters, α and β, with $\alpha = x + 1$ and $\beta = n – x + 1$, where x is the number of positive events out of n trials. As n increases, the level of uncertainty about the estimated proportion (x/n) decreases.

The estimated animal-level sensitivity of PFC used in the model was based on data from Whittington et al. (2000), except that the original estimates of sensitivity for both paucibacillary and multibacillary lesion types were adjusted downwards by reducing the observed number of positive pools at each pooling rate by two. The estimated animal-level sensitivity of the AGID and the proportion of sheep with paucibacillary lesions used in the model were based on unpublished data (J Marshall, personal communication; Marshall DJ et al., 1996). The average sensitivity of the AGID was 11.8% and 57% in sheep with paucibacillary and multibacillary lesions respectively. The majority of infected sheep had paucibacillary

lesions (69% of 224). These estimates probably over-estimate the true sensitivities, as they were not adjusted for the imperfect sensitivity of histology, which was used to determine animal infection status.

Figure 1: Scenario tree for a simulation model to estimate the flock-sensitivity of screening tests for ovine Johne's disease



The initial input parameters used for these distributions are shown in Table 2. α and β parameters were chosen to provide a mean value close to the above estimates and the distributions were relatively weak

(n was small) to allow for uncertainty about the true value of the parameters. For each simulation, the model was run for 1,000 iterations to generate output distributions for the flock-sensitivities of PFC and AGID.

Table 2: Parameters for input distributions for a simulation model to estimate flock-sensitivity of PFC and AGID as screening tests for ovine Johne's disease

	PFC					AGID				
	Point estimate (%)	x	n	α	β	Point estimate (%)	x	n	α	β
% paucibacillary	70	14	20	15	7		As for PFC			
Se(p)	40	8	20	9	13	11	4	35	5	32
Se(m)	90	18	20	19	3	57	20	35	21	16
Weighted-average	55					25				

Validation of the model

The model was validated by comparison of simulated flock-sensitivity estimates with estimates from existing data, and by comparison with point estimates calculated using a non-simulation method.

1. Comparison with existing data

Data from a field trial of PFC undertaken in New South Wales during 1998 were used as inputs for the simulation model and the results compared with the observed estimates of flock-sensitivity of PFC and AGID from this data (Sergeant et al., in press).

The selection and testing of the flocks used in this validation and the estimation of the flock-sensitivities of PFC and AGID and prevalence of ovine Johne's disease in these flocks have been described previously (Whittington et al., 2000; Sergeant et al., in press).

Briefly, 296 sheep flocks were tested between April and December 1998 as part of an ongoing surveillance program for ovine Johne's disease in Australia. Flocks were selected for testing either because of possible contact with an infected flock, or for entry to the market-assurance program. Sample sizes and strategies used to select individual sheep for testing were based on current recommendations for surveillance and assurance testing for ovine Johne's disease in Australia. In each flock, the selected sheep were tested using both the AGID and PFC, and any sero-positive animals were investigated further by postmortem examination and histology to determine their infection status. One hundred of the 296 study flocks were positive on either PFC or serology/histology or both. Flock-sensitivities of both PFC and serology/histology were calculated for these flocks using non-gold-standard methods (Staquet et al., 1981; Enøe et al., 2000). Simulated estimates of flock-sensitivity for both tests were compared with these calculated values.

For each infected flock, the true prevalence of infection was estimated after adjusting for an assumed sensitivity of the AGID of 30% (Sergeant et al., in press). The expected number of infected animals sampled from each flock was calculated as $x = n \times TP$, where x was the number of infected animals in the sample, rounded to the nearest whole number, n was the number of animals tested and TP was the estimated true-prevalence in the flock. The proportion of animals with paucibacillary lesions in each flock was estimated using a Beta probability distribution with α and β parameters as shown in Table 2 and the number of animals in the sample with paucibacillary lesions was estimated as $x(p) = x \times p$ rounded to the nearest whole number, where p was the proportion of infected animals in the flock that had paucibacillary lesions. The number of animals tested that had multibacillary lesions was estimated as $x(m) = x - x(p)$. Flock-sensitivity was estimated for each flock, and the average flock-sensitivity was calculated across all 100 flocks for each iteration. Simulations were run using the input values from Table 2, as well as using alternative values to investigate the effect of changing the assumed animal-level sensitivity on the comparison. The model was run for 1,000 iterations for each simulation to generate a probability

distribution for the estimated average flock-sensitivity, which was compared to the observed estimates of flock-sensitivity from Sergeant et al (submitted).

2. Comparison with calculated estimates of flock-sensitivity

Simulated flock-sensitivity estimates for sample sizes ranging from 100 to 1000 animals and an assumed prevalence of 2% in a flock of 2,000 sheep were compared with estimates calculated using non-simulation methods and assuming that both tests had a specificity of 100% (Martin et al., 1992).

Flock-sensitivity for each scenario was calculated as $Se_{(flock)} = 1 - (1 - TP \times Se_{(animal)})^n$, where TP was the true prevalence of infection (2%), n was the sample size and $Se_{(animal)}$ was the animal-level sensitivity of the test (Martin et al., 1992). $Se_{(animal)}$ was estimated as the weighted-average of the assumed test sensitivity in sheep with paucibacillary and multibacillary lesions used in the simulated estimates (Table 2). The calculated estimate was compared with the simulated mean flock-sensitivity for each scenario.

Estimation of flock-sensitivity of PFC and AGID

The model was used to:

- estimate the flock-sensitivities of PFC and AGID for sample sizes of 100, 350 and 500;
- estimate the sample size required for serology to provide a flock-sensitivity equivalent to sample sizes of 100, 350 and 500 using PFC;
- estimate the sample size required for both tests to provide 95%, 98% and 99% probability of detecting a prevalence of either 1% or 2%;
- estimate the flock-sensitivity of whole-flock testing using PFC and AGID for a range of input values; and
- investigate the effect of varying input values on flock-sensitivity estimates.

Where different pool sizes were used, the sensitivity of PFC was adjusted for the effect of dilution due to pooling as shown in Table 3 (adapted from Whittington et al., 2000).

Table 3: Effect of pool size on animal-level sensitivity of PFC

	Paucibacillary lesions			Multibacillary lesions		
Pool size	x	n	Se(p)	x	n	Se(m)
1	12	20	0.60	18	20	0.90
10	10	20	0.48	18	20	0.86
50	8	20	0.35	18	20	0.78
100	7	20	0.28	16	20	0.64
200	3	20	0.11	12	20	0.44

Table 4: Assumed animal-level sensitivities for three simulations to investigate the effect of changes in animal-level sensitivity on the simulated flock-sensitivity of PFC and AGID

	PFC			AGID		
	Simulation 1 (%)	Simulation 2 (%)	Simulation 3 (%)	Simulation 1 (%)	Simulation 2 (%)	Simulation 3 (%)
% paucibacillary	70	70	86	70	70	86
Se(p)	40	50	25	11	9	5
Se(m)	90	100	75	57	50	33
Se(flock)	55	65	32	25	21	9

Three simulations were run to investigate the effect of varying the sensitivities of the tests on the resulting estimates of the flock-sensitivity of PFC and AGID. All three simulations assumed a prevalence of 2% in a flock of 2,000 sheep, a pool size of 50 and sample sizes of 350, 500, 850, 1000 and 1250. The assumed values for animal-level test-sensitivities and the percentage of animals with paucibacillary lesions for each series are shown in Table 4. These values were chosen to provide a range of possible values for the animal-level sensitivity of both tests for both paucibacillary and multibacillary lesions. Simulation 1 used the same values as were used for previous simulations (Table 2). Simulation 2 used the original (unadjusted) data from Whittington et al. (2000) to estimate the animal-level sensitivity of PFC, and reduced estimates for the animal-level sensitivity of the AGID, to allow for the likely over-estimation of animal-level sensitivity of AGID in the original estimates. Simulation 3 used worst-case estimates of the animal-level sensitivity of both tests, such as could be expected in a recently infected flock. For Simulation 3, Animal-level sensitivity estimates for the AGID were based on unpublished data for Flock 5 from Marshall et al. (1996), which had a very low animal-level sensitivity and a high percentage of paucibacillary lesions. For PFC, Se(p) and Se(m) were assumed to be 25% and 75% respectively, well below the apparent values from Whittington et al. (2000).

Mean, median and 5th percentile of the output distributions for flock-sensitivity were calculated for each scenario. The median and the 5th percentile of the output distribution provide an estimate of the variability of the flock-sensitivity estimates, so that 50% of flocks will have a flock-sensitivity less than the median, and 5% of flocks will have a flock-sensitivity less than the 5th percentile.

Sensitivity analysis

Analyses were undertaken to investigate the effect of uncertainty about individual model inputs on the overall variability of flock-sensitivity estimates.

Firstly, @Risk estimated correlation coefficients for the degree of association between each input distribution and the output distributions, assuming 2% prevalence in a flock of 2000 sheep, and a sample size of 350 in pools of 50.

Additionally, simulations were run using extremely weak distributions (n=2) (very low confidence in the values used) for all inputs and with all inputs as fixed values (100% confidence in the values used) instead of probability distributions (Table 5).

Table 5: Parameters for weak input distributions and fixed-value inputs for simulations to investigate the effect of uncertainty about input values on the resulting estimates of flock-sensitivity for PFC and AGID

	PFC					AGID				
	Fixed value (%)	x	n	α	β	Fixed value (%)	x	n	α	β
% paucibacillary	70	1.4	2	2.4	1.6	As for PFC				
Se(p)	40	0.8	2	1.8	2.2	11	0.22	2	1.22	2.78
Se(m)	90	1.8	2	2.8	1.2	57	1.14	2	2.14	1.86

Results

Validation of the model

1. Comparison with existing data

Table 6 compares the results of three simulations using different input values with the observed flock-sensitivity for PFC and AGID from the field trial. The simulated mean flock-sensitivity of PFC was less than, but within the 95% CI for, the observed estimate. In contrast, the simulated flock-sensitivity of serology was substantially higher than the observed estimate and was outside the 95% CI for the observed estimate. Simulations using higher assumed values for animal-level sensitivities of PFC resulted in simulated flock-sensitivity estimates closer to the observed value, while lower animal-level sensitivities for the AGID produced simulated flock-sensitivity estimates that were closer to, but still well above, the observed estimate.

Table 6: Comparison of mean and 95% confidence intervals for simulated flock-sensitivity for PFC and AGID from three different simulations with observed estimates from a field trial in NSW during 1998

	PFC flock-sensitivity		AGID flock-sensitivity	
	Mean (%)	95% Interval (%)	Mean (%)	95% Interval (%)
Observed estimate (Sergeant et al, 2001)	92	82 - 97	61	51 - 71
PFC: Se(p)=40%, Se(m)=90% AGID: Se(p)=11%, Se(m)=57%	89	88 - 90	73	71 - 75
PFC: Se(p)=50%, Se(m)=100% AGID: Se(p)=9%, Se(m)=50%	92	91 - 93	70	68 - 72

2. Comparison with calculated estimates of flock-sensitivity

The mean of the simulated flock-sensitivity estimates corresponded closely with the estimates calculated using the non-simulation method, for both PFC and AGID for all sample sizes tested when the same input values were used (Table 7).

Table 7: Comparison of simulated mean flock-sensitivity estimates with calculated estimates for sample sizes ranging from 100 to 1000, assuming a prevalence of 2% in a flock of 2000 sheep and that 70% of infected sheep have paucibacillary lesions

	PFC flock-sensitivity [Se(p)=40%, Se(m)=90%]					AGID flock-sensitivity [Se(p)=11%, Se(m)=57%]				
Sample size	100	350	500	750	1000	100	350	500	750	1000
Se(flock) Calculated (%)	67	98	100	100	100	40	83	92	98	99
Simulated mean (%)	67	98	100	100	100	42	85	93	98	100

Estimation of flock-sensitivity of PFC and AGID

1. Estimated flock-sensitivity of current testing strategies

The output distributions for flock-sensitivity of PFC and AGID for previously recommended sample sizes are summarised in Table 8.

The mean flock-sensitivities for a Check Test (sample size = 100) were 67% and 42% for PFC and AGID respectively, and for a Sample Test (sample size = 350 for PFC, 500 for AGID) were 98% and 93% respectively. Comparison of the 5th percentiles shows that for a Sample Test, 95% of flocks would have a flock-sensitivity >89% for PFC compared to >74% for the AGID.

Table 8: Output distributions for simulated flock-sensitivity of PFC and AGID for sample sizes of 100, 350 and 500, assuming 2% prevalence in a flock of 2,000 sheep and that 70% of infected sheep have paucibacillary lesions

	PFC flock-sensitivity [Se(p)=40%, Se(m)=90%]			AGID flock-sensitivity [Se(p)=11%, Se(m)=57%]		
Sample Size	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
100	66.7	81.5	0.0	41.8	39.2	0.0
350	97.9	99.9	89.0	85.0	90.7	50.5
500	99.6	100.0	98.6	93.2	96.9	73.7

2. Sample sizes required to achieve flock-sensitivities for PFC and AGID of 95%, 98% and 99%

Table 9 shows the sample sizes required to achieve mean flock-sensitivities of 95%, 98% and 99% for each test. Sample sizes of 350 – 450 and 900 – 1000 for PFC and AGID respectively provided a flock-sensitivity 99% for a prevalence of 2%, depending on the assumed animal-level sensitivity. Sample sizes to provide the same flock-sensitivity for a prevalence of 1% were up to twice that required for a prevalence of 2%.

Table 9: approximate sample sizes required to achieve mean flock-sensitivities of 95%, 98% and 99% for PFC and AGID in flocks with 1% and 2% prevalence, and with low and high animal-level sensitivities, assuming a flock of 2,000 sheep and that 70% of infected sheep have paucibacillary lesions

	Prevalence = 2%			Prevalence = 1%		
Flock-sensitivity	95%	98%	99%	95%	98%	99%
PFC [Se(p)=50%, Se(m)=100%]	250	300	350	450	600	700
PFC [Se(p)=40%, Se(m)=90%]	300	350	450	550	700	800
AGID [Se(p)=11%, Se(m)=57%]	600	750	900	1100	1350	1600
AGID [Se(p)=9%, Se(m)=50%]	650	850	1000	1200	1600	1900

3. Sample size required for the AGID to achieve comparable flock-sensitivity to PFC

Sample sizes required for the AGID to provide equivalent mean flock-sensitivity to PFC were generally 2 – 2.5 times the PFC sample size, depending on the assumed sensitivity of the AGID (Table 10). At the higher sample sizes this also provided comparable median and 5th percentile to PFC.

Table 10: Sample sizes required for AGID testing to provide equivalent mean flock-sensitivity to PFC with sample sizes of 100, 350 and 500, assuming 2% prevalence in a flock of 2,000 sheep and that 70% of infected sheep have paucibacillary lesions

PFC flock-sensitivity [Se(p)=40%, Se(m)=90%]				AGID flock-sensitivity [Se(p)=11%, Se(m)=57%]			AGID flock-sensitivity [Se(p)=9%, Se(m)=50%]		
PFC sample size	Mean Se(flock) (%)	Median (%)	5 %ile (%)	AGID sample size	Median (%)	5 %ile (%)	AGID sample size	Median (%)	5 %ile (%)
100	67	82	0	200	73	14	250	74	19
350	98	99.9	89	750	99.5	92	850	99.4	91
500	99.6	100	99	1100	100	98.5	1300	99.9	98

4. Effect of sample size on estimated flock-sensitivity

Mean flock-sensitivity increased with increasing sample size (Table 11). Mean flock-sensitivity for PFC reached 99.5% at a sample size of 500, compared to 1000 for AGID. These sample sizes also resulted in 5th percentiles of flock-sensitivity for both PFC and AGID that were >95%, and further increases in sample size resulted in only small improvements in flock-sensitivity.

Table 11: Flock-sensitivity distributions for sample sizes ranging from 100 to 1000 head from a flock of 2,000 sheep with a prevalence of 2%.

	PFC flock-sensitivity [Se(p)=40%, Se(m)=90%]			AGID flock-sensitivity [Se(p)=11%, Se(m)=57%]		
Sample Size	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
100	66.7	81.5	0	41.8	39.2	0
200	88.9	97.6	44.4	65.9	72.2	12.4
300	96.4	99.7	79.0	80.1	86.7	33.5
350	97.9	99.9	89.0	85.0	90.7	50.5
400	98.7	100	94.3	88.5	93.7	60.2
450	99.3	100	97.2	91.2	95.7	68.0
500	99.6	100	98.6	93.2	96.9	73.7
550	99.8	100	99.2	94.7	97.8	78.3
600	99.9	100	99.7	96.0	98.5	83.1
650	99.9	100	99.8	97.0	99.0	88.3
700	100	100	99.9	97.6	99.3	89.5
750	100	100	100	98.2	99.5	91.5
800	100	100	100	98.6	99.6	93.5
900	100	100	100	99.2	99.8	96.0
1000	100	100	100	99.5	99.9	97.6

5. Effect of pool size on estimated flock-sensitivity

Mean flock-sensitivity of PFC remained high for pool sizes up to 100 pellets, but decreased substantially for a pool size of 200 pellets with a sample size of 400 (Table 12). This decrease in flock-sensitivity due to the large pool size was more than offset by increasing the sample size to 800, so that 4 pools of 200 were more sensitive than 8 pools of 50.

Table 12: Effect of sample size and pool size on simulated estimates of flock-sensitivity for PFC, assuming 2% prevalence in a flock of 2000 sheep and that Se(p)=40%, Se(m)=90% and that 70% of infected sheep have paucibacillary lesions

Sample size	400			600			800		
Pool size	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
1	99.6	100	98.5	100	100	100	100	100	100
10	99.3	100	96.9	100	100	99.9	100	100	100
50	98.7	100	94.3	99.9	100	99.7	100	100	100
100	97.9	99.8	90.0	99.7	100	98.9	100	100	99.9
200	91.0	96.0	66.2	97.2	99.2	88.0	99.0	99.9	95.5

6. Effect of flock size on estimated flock-sensitivity

Estimated flock-sensitivities of both PFC and AGID decreased slightly with increasing flock size, although the decrease for flock sizes greater than 2000 was small (Table 13). The reduction in sensitivity was smaller for PFC and for larger sample sizes.

Table 13: Effect of flock size on simulated estimates of flock-sensitivity for PFC and AGID, assuming 2% prevalence in a flock of 2000 sheep and that 70% of infected sheep have paucibacillary lesions

	PFC flock-sensitivity [Se(p)=40%, Se(m)=90%]			AGID flock-sensitivity [Se(p)=11%, Se(m)=57%]		
Flock size	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
500	99.1	99.9	96.5	88.5	91.7	66.6
1000	98.3	99.9	92.2	85.9	91.1	56.8
2000	97.9	99.9	89.0	85.0	90.7	50.5
5000	97.7	99.9	87.1	84.4	90.4	47.7
10000	97.6	99.9	86.3	83.9	90.5	43.0

7. Effect of prevalence on estimated flock-sensitivity

The flock-sensitivities of PFC and AGID decreased rapidly when the assumed prevalence was less than 2%, with AGID flock-sensitivity decreasing considerably more than that for PFC (Table 14). Generally, for equal sample sizes, the flock-sensitivity of PFC at a prevalence of 0.5% was comparable to AGID flock-sensitivity at a prevalence of 1%. A sample size of 800 using PFC provided >99% confidence of detecting infection at a prevalence of 1%, and >90% confidence of detecting a prevalence of 0.5%, compared to 90% and 68% respectively for the AGID. For an assumed prevalence ≥3%, PFC provided a flock-sensitivity ≥99.5% for all sample sizes ≥350, whereas the flock-sensitivity of the AGID was 94% and 99% for a prevalence of 3% and sample sizes of 350 and 600 respectively.

Table 14: Effect of within-flock prevalence on simulated estimates of flock-sensitivity for PFC and AGID for sample sizes of 350, 600 and 800, assuming a flock of 2000 sheep and that 70% of infected sheep have paucibacillary lesions

Sample size	350			600			800		
Prevalence	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
PFC [Se(p)=40%, Se(m)=90%]									
0.5	62.9	76.9	0	83.8	94.5	33.8	91.5	97.9	54.9
1.0	86.3	96.2	38.5	97.0	99.7	82.0	99.1	100	96.2
2.0	97.9	99.9	89.0	99.9	100	99.7	100	100	100
3.0	99.6	100	98.9	100	100	100	100	100	100
5.0	100	100	100	100	100	100	100	100	100
AGID [Se(p)=11%, Se(m)=57%]									
0.5	38.5	33.0	0	58.1	65.0	9.9	68.4	73.4	17.5
1.0	61.9	68.0	11.2	81.5	87.7	40.1	89.6	93.9	66.1
2.0	85.0	90.7	50.5	96.0	98.5	83.1	98.6	99.6	93.5
3.0	93.8	97.4	75.8	99.0	99.8	94.9	99.8	100	98.9
5.0	98.8	99.8	94.1	99.9	100	99.6	100	100	100

8. Effect of percentage of infected sheep with paucibacillary lesions on estimated flock-sensitivity

The flock-sensitivities of both tests decreased as the assumed percentage of infected sheep with paucibacillary lesions increased (Table 15). However, even assuming 90% of infected sheep had paucibacillary lesions, the mean flock-sensitivity of PFC was still >95%, compared to 73% for the AGID,

for a sample size of 350. If 90% of infected sheep were assumed to have paucibacillary lesions a sample size of 800 was required for a mean flock-sensitivity for the AGID ~95%.

Table 15: Effect of the percentage of sheep with paucibacillary lesions on simulated estimates of flock-sensitivity for PFC, assuming 2% prevalence in a flock of 2000 sheep and sample sizes of 350, 600 and 800.

Sample size	350			600			800		
% Paucibacillary lesions (%)	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
PFC [Se(p)=40%, Se(m)=90%]									
50	99.0	100	96.4	100	100	99.9	100	100	100
60	98.5	100	93.6	100	100	99.8	100	100	100
70	97.9	99.9	89.0	99.9	100	99.7	100	100	100
80	97.2	99.7	84.3	99.7	100	99.0	100	100	99.9
90	95.8	99.3	78.8	99.6	100	97.7	99.9	100	99.6
AGID [Se(p)=11%, Se(m)=57%]									
50	91.7	96.3	68.2	98.5	99.7	93.5	99.6	100	98.3
60	88.6	94.0	60.1	97.6	99.3	89.0	99.3	99.9	96.6
70	85.0	90.7	50.5	96.0	98.5	83.1	98.6	99.6	93.5
80	80.0	86.2	37.5	93.4	96.7	75.0	97.2	99.0	87.1
90	73.0	77.9	29.4	88.9	93.2	61.8	94.3	97.5	77.9

9. Effect of animal-level sensitivity of PFC

Increasing the assumed animal-level sensitivity of PFC from 40% to 50% for sheep with paucibacillary lesions and from 90% to 100% for sheep with multibacillary lesions increased the weighted-average animal-level sensitivity from 55% to 65%. However, the resulting increase in mean flock-sensitivity was only about 1% for a sample size of 350, and was negligible for larger sample sizes (Table 16). Even the worst-case scenario, with Se(p) = 25%, Se(m) = 75%, and with 85% of infected sheep with paucibacillary lesions resulted in a mean flock-sensitivity of 90% for a sample size of 350, and 96% for a sample size of 500.

Table 16: Output distributions for PFC flock-sensitivity, for different assumed values animal-level sensitivities for paucibacillary and multibacillary lesions, assuming 2% prevalence in a flock of 2000 sheep

	Simulation 1[a]			Simulation 2[b]			Simulation 3[c]		
Sample size	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
350	98	99.9	88.2	99.0	100	94.7	90.0	95.5	59.2
500	99.6	100	98.7	99.9	100	99.8	96.1	98.9	82.5
850	100	100	100	100	100	100	99.5	100	97.4
1000	100	100	100	100	100	100	99.8	100	99.1
1250	100	100	100	100	100	100	99.9	100	99.8

[a] Simulation 1: Se(p)=40%, Se(m)=90%; p = 70%
[b] Simulation 2: Se(p)=50%, Se(m)=100%; p = 70%
[c] Simulation 3: Se(p)=25%, Se(m)=75%; p = 86%

10. Effect of animal-level sensitivity of the AGID

Reducing the assumed animal-level sensitivity of the AGID from 11% to 9% and from 57% to 50% for paucibacillary and multibacillary lesions respectively reduced the weighted-average animal-level sensitivity from 25% to 21%. This resulted in a reduction of about 4% and 2.7% in the mean flock-

sensitivity for sample sizes of 350 and 500 respectively (Table 17). For the worst-case scenario, flock-sensitivity decreased to 65% and 87% for sample sizes of 500 and 1000 respectively, and sample sizes to achieve similar flock-sensitivity to PFC had to be >3 times larger.

Table 17: Output distributions for AGID flock-sensitivity, for three different scenarios of animal-level sensitivities and percentage of infected sheep with paucibacillary lesions, assuming 2% prevalence in a flock of 2000 sheep

	Simulation 1[a]			Simulation 2 [b]			Simulation 3 [c]		
Sample size	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
350	84.5	90.6	44.4	80.7	86.7	41.4	52.6	53	16.9
500	92.9	96.9	71.5	90.2	94.8	64.4	65.3	67.4	28.6
850	98.9	99.8	94.7	97.9	99.4	90.0	82.8	85.6	55.8
1000	99.5	99.9	97.6	98.9	99.8	94.7	87.1	90.1	63.9
1250	99.9	100	99.2	99.6	100	97.9	92.1	94.7	74.9
1500	100	100	99.8	99.9	100	99.2	95.1	97.1	83.0

[a] Simulation 1: Se(p)=11%, Se(m)=57%; p = 70%
[b] Simulation 2: Se(p)=9%, Se(m)=50%; p = 70%
[c] Simulation 3: Se(p)=5%, Se(m)=33%; p = 86%

Table 18: Flock-sensitivity of whole-flock testing with AGID and PFC for a range of values for flock size, prevalence, % paucibacillary and animal-level sensitivities.

				PFC flock-sensitivity			AGID flock-sensitivity		
Flock size	Prevalence (%)	% paucibacillary	Animal-level sensitivity	Mean (%)	Median (%)	5th %ile (%)	Mean (%)	Median (%)	5th %ile (%)
1000	1	70	Low[a]	99.5	99.8	98.2	81.1	82.9	59.2
1000	1	70	High[b]	100	100	100	96.1	97.3	88.6
1000	1	90	Low	98.2	99.0	94.0	68.4	69.0	44.4
1000	1	90	High	99.8	100	99.2	89.4	91.3	74.6
1000	2	70	Low	100	100	100	95.0	96.9	83.2
1000	2	70	High	100	100	100	99.7	99.9	98.7
1000	2	90	Low	99.9	100	99.5	87.2	89.5	67.1
1000	2	90	High	100	100	100	97.9	99.1	91.6
2000	1	70	Low	100	100	100	95.0	96.9	83.2
2000	1	70	High	100	100	100	99.7	99.9	98.7
2000	1	90	Low	99.9	100	99.5	87.2	89.5	67.1
2000	1	90	High	100	100	100	97.9	99.1	91.6
2000	2	70	Low	100	100	100	99.4	99.9	97.2
2000	2	70	High	100	100	100	100	100	100
2000	2	90	Low	100	100	100	97.3	98.9	89.5
2000	2	90	High	100	100	100	99.9	100	99.3
3000	1	70	Low	100	100	100	98.4	99.5	92.9
3000	1	70	High	100	100	100	100	100	99.9
3000	1	90	Low	100	100	100	94.4	96.7	81.0
3000	1	90	High	100	100	100	99.5	99.9	97.3
3000	2	70	Low	100	100	100	99.9	100	99.5
3000	2	70	High	100	100	100	100	100	100
3000	2	90	Low	100	100	100	99.3	99.9	96.4
3000	2	90	High	100	100	100	100	100	99.9

[a] Low – PFC: Se(p)=25%, Se(m)=75%; AGID: Se(p)=5%, Se(m)=33%.
[b] High – PFC: Se(p)=40%, Se(m)=90%; AGID: Se(p)=11%, Se(m)=57%.

11. Flock-sensitivity of whole-flock testing

The mean flock-sensitivity for whole-flock testing using PFC was ≥98% and the 5th percentile was ≥94% for all combinations of flock size, prevalence, percentage of paucibacillary lesions and animal-level sensitivities tested (Table 18). In contrast, the mean flock-sensitivity for the AGID ranged from 68% to 100% and was generally poor for smaller flock-sizes and low prevalence or animal-level sensitivities.

Sensitivity Analysis

1. Correlation of input variables with output distributions

The number of sheep with multibacillary lesions that were tested [x(m)] was strongly correlated (correlation coefficient >0.8) with the output distributions for flock-sensitivity for both PFC and AGID (Table 19). Other inputs were only moderately correlated (correlation coefficient ≤~0.3).

Table 19: Correlation coefficients for input variables and output distributions for PFC and AGID flock-sensitivity, assuming 2% prevalence in a flock of 2000 sheep, and a sample size of 350 sheep

Name	PFC [Se(p)=40%, Se(m)=90%]	AGID [Se(p)=11%, Se(m)=57%]
x(m)	0.826	0.883
Se(m)	0.308	0.268
% paucibacillary	-0.274	-0.311
Se(p)	0.250	0.241
x(p)	0.199	0.107

2. Effect of varying input distributions

The use of fixed input values instead of probability distributions had only a minor effect on mean and median flock-sensitivity estimates for PFC, whereas using very weak input distributions resulted in a slight decrease in the mean and median flock-sensitivity for PFC and a larger decrease in the 5th percentile (Table 20). Fixed input values also had only a minor effect on flock-sensitivity estimates for the AGID, whereas weak input distributions resulted in an increase in the mean and median flock-sensitivity for the AGID for smaller sample sizes, and a general decrease in the 5th percentile.

Table 20: Comparison of flock-sensitivity estimates for fixed input values and weak input distributions with original simulation results, assuming 2% prevalence in a flock of 2,000 sheep

	Original simulation [a]			Fixed inputs [b]			Weak input distributions [c]		
Sample size	Mean	Median	5th %ile	Mean	Median	5th %ile	Mean	Median	5th %ile
PFC									
100	66.7	81.5	0	66.8	78.4	0	65.0	78.7	0
350	97.9	99.9	89.0	98.1	99.9	92.2	95.9	99.8	76.4
500	99.6	100	98.6	99.7	100	99.2	98.4	100	92.5
750	100	100	100	100	100	100	99.5	100	98.5
1000	100	100	100	100	100	100	99.8	100	99.8
AGID									
100	41.8	39.2	0	39.7	30.4	0	52.9	56.7	0
350	85.0	90.7	50.5	83.7	90.0	45.4	89.4	96.9	48.1
500	93.2	96.9	73.7	92.8	96.6	76.6	94.5	99.5	71.9
750	98.2	99.5	91.5	98.4	99.4	93.8	97.7	100	86.2
1000	99.5	99.9	97.6	99.7	99.9	98.5	98.8	100	94.1

[a] Original simulation: PFC: Se(p)=RiskBeta(9,13), Se(m)= RiskBeta(19,3); AGID: Se(p)= RiskBeta(5,32), Se(m)= RiskBeta(21,16); p = RiskBeta(15,7)

[b] Fixed inputs: PFC: Se(p)=40%, Se(m)=90%; AGID: Se(p)=11%, Se(m)=57%; p = 70%

[c] Weak distributions: PFC: Se(p)=RiskBeta(1.8,2.2), Se(m)= RiskBeta(2.8,1.2); AGID: Se(p)= RiskBeta(1.22,2.78), Se(m)= RiskBeta(2.14,1.86); p = RiskBeta(2.4,1.6)

Discussion

This study confirms that PFC is a highly sensitive flock-test for the detection of ovine Johne's disease, and that it has a substantially higher flock-sensitivity than serology on equivalent numbers of sheep. A sample size of 2 – 3 times larger was required for the AGID to provide equivalent flock-sensitivity to PFC under the scenarios examined.

The simulation method described here provided realistic and accurate estimates of the flock-sensitivity of both tests when compared to calculated estimates based on the same assumptions. Simulated estimates for the flock-sensitivity of PFC were also comparable to the observed estimate based on field trial data. However, simulated estimates of AGID flock-sensitivity were higher than the observed estimate. This might be because the input values that were used over-estimated the animal-level sensitivity of the AGID, or alternatively because the true-prevalence estimates for the flocks in the field trial over-estimated the true values.

Although the simulation approach allowed for uncertainty about input values, the main factor affecting the flock-sensitivity distributions was the number of multibacillary animals tested — which was determined by the percentage of paucibacillary lesions, the sample size and sampling variation. For low animal-level sensitivities and small sample sizes the 5th percentile was very low, increasing rapidly with sample size and test sensitivity. Thus, increased sample size and targeting sample selection to increase the probability of including infected animals will reduce the variability of flock-sensitivity and provide an overall improvement in test performance at a flock level.

Importantly, using larger sample sizes for the AGID greatly reduces the variability of the estimated flock-sensitivity, as well as improving the mean flock-sensitivity for the test. This means that a much lower proportion of flocks will have an unacceptable flock-sensitivity than would otherwise be the case. In fact, for current recommended sample sizes for the Check Test and Sample Test, the 5th percentile for the AGID is higher than that for PFC for the corresponding sample size.

Although increasing the sample size for AGID testing overcomes much of the disparity between the tests, the two tests are still not truly equivalent, because of PFC's ability to detect individual cases much earlier than serology (Whittington and Sergeant, 2001; Chaitaweesub et al., 1999). In recently infected flocks the percentage of early paucibacillary cases is likely to be high, and the animal-level sensitivity of both tests is likely to be reduced. However, the sensitivity of PFC is likely to remain substantially better than that for the AGID, because of the earlier detection of cases. Evaluation of such a scenario showed that under such conditions, sample sizes for the AGID may need to be >3 times larger than those for PFC to provide equivalent flock-sensitivity in recently infected flocks. Because the disparity between the two tests is likely to become progressively greater in earlier cases, the equivalent sample size for the AGID will continue to increase as animal-level sensitivities of the tests decrease.

The simulation results presented here are dependent on the assumptions used, and in particular on the assumed values of the sensitivities of PFC and AGID for sheep with paucibacillary and multibacillary lesions. Because of the apparent difference between the two tests, deliberately conservative (reduced) values for the sensitivity of PFC were used, compared to the actual estimates from the original pooling experiments (Whittington et al., 2000). At the same time, the animal-level sensitivity estimates used for the AGID were based on real data and while lower than previous assumed values, were probably higher than the true animal-level sensitivity in many flocks (unpublished data). Comparison with the estimated flock-sensitivity of serology from the PFC field trial also suggests that the assumed animal-level values used in this simulation are more likely to over-estimate than under-estimate the true sensitivity of the AGID. Therefore, the estimates presented here may still over-estimate the true flock-sensitivity of the AGID in many flocks.

Conclusion

Although the AGID appears to perform reasonably well in higher prevalence flocks, its flock-sensitivity in low prevalence or recently infected flocks is likely to be very low, unless sample sizes 2 – 3 times those used for PFC are used. This is particularly important in Australia at present, as the majority of flocks being investigated outside the Residual Zone (endemic area) are likely to be relatively recently infected and still at low prevalence (Whittington and Sergeant, 2001; Sergeant and Baldock, submitted). In these circumstances, PFC should be the preferred test, and larger sample sizes or whole-flock testing should be considered to maximise flock-sensitivity.

In higher prevalence flocks the AGID will provide a satisfactory flock-sensitivity and a more-rapid result than PFC, particularly if biased sampling is used to maximise animal-level sensitivity and provided sample sizes are adequate.

Outcomes

The main outcomes of this project were:

- A better understanding of the relative performance of PFC and AGID as flock-screening tests for ovine Johne's disease.
- Quantitative estimates of the flock-level sensitivity of PFC and AGID under a range of scenarios of prevalence and animal-level test sensitivity.
- Confirmation of recommendations for sample sizes required to achieve the desired performance of these tests in assurance and surveillance testing.
- A generalised modelling framework suitable for the evaluation of new tests applicable to most diseases including bovine Johne's disease.

This work provides more detailed analysis of the flock-sensitivity of PFC than previous reports, and has finalised the evaluation of pooled faecal culture as a flock-screening test for ovine Johne's disease in Australia.

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