Estimation of sensitivity and flock-sensitivity of pooled faecal culture for *Mycobacterium avium* subsp. *paratuberculosis* in sheep


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Abstract

Pooled faecal culture (PFC) is a widely used test in ovine Johne’s disease (OJD) control programmes in Australia but information about its characteristics is limited. We conducted this study to estimate sensitivity and flock-sensitivity of PFC in sheep with different OJD histopathological lesions in simulated flocks with a range of infection prevalence levels. Initially, a known quantity of faeces from sheep with pauci- or multi-bacillary lesions was pooled with uncontaminated faeces from confirmed non-infected sheep and cultured using PFC technique. PFC sensitivity, calculated as a proportion of the pools of a particular size that tested positive, was determined to be 90% in sheep with the multibacillary form of the disease but varied with pool size in sheep with the paucibacillary form of OJD. Subsequently, probabilistic models were developed to estimate overall pool-sensitivity achieved in a flock (Seₖ) and flock-sensitivity of PFC (FSe) in various simulated scenarios. In flocks with a given ratio of multi- to pauci-bacillary sheep and with low to moderate infection prevalence level, Seₖ decreased with increase in pool size, but increased with pool size in flocks with ≥ 10% prevalence. FSe, in contrast, increased with pool size in all the tested scenarios. Both Seₖ and FSe increased with infection prevalence, ratio of multi- to pauci-bacillary sheep and the number of pools sourced from flocks. Sensitivity analyses indicated that the estimates and trends were robust to moderate changes in input parameters. The results suggest that the current testing of seven pools of size 50 is adequate for most scenarios, however, for very
low prevalence flocks, a gain in FSe can be made by increasing the number of pools tested and a higher Se, can be achieved by reducing pool sizes.

**Keywords**: pooling, paratuberculosis, ovine Johne’s disease, Australia, Mycobacterium, epidemiology.

1. **Introduction**

Ovine Johne’s disease (OJD), a chronic debilitating disease of sheep caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is prevalent in several countries of the world including Australia and causes substantial economic losses. Many countries have initiated disease control programmes to minimise losses from the disease but success of a control programme depends heavily on the availability of a sensitive and specific diagnostic test that is also economical to use on a large scale. Therefore, the pooled faecal culture (PFC) technique developed by Whittington et al. (2000), being substantially cheaper than individual faecal culture and more sensitive and specific than serology, has been adopted as a standard test for OJD control in Australia. Besides control programmes, this pooled test is commonly employed in OJD research, primarily to reduce costs of testing sheep (Abbott et al., 2004; Reddacliff et al., 2006; Dhand et al., 2007, 2009). However, the sensitivity of PFC is known only for certain pool sizes (number of faecal pellets constituting a pool) (Whittington et al., 2000) whereas in practice often varied sizes of faecal pools are collected due to the requirements of sample size, availability of funds and logistics of sample collection. The sensitivity of PFC for variable pool sizes needs to be established.

Further, the sensitivity of PFC varies according to the disease pathology. Two major types of pathological entities are associated with OJD: the multibacillary and paucibacillary forms of the disease. In the former, a large number of MAP are present in the lesions and, therefore, shed in the faeces, whereas in the paucibacillary form, very few or no organisms are detectable in the lesions, and consequently, few are excreted in the faeces. Obviously, the sensitivity of PFC is substantially higher for sheep with the multibacillary form of OJD than those with the paucibacillary form (Whittington et al., 2000). However, there is very limited information about the effects of proportion of multibacillary sheep and pool size on overall pool sensitivity achieved in a flock.

Similarly, the flock-sensitivity of PFC is also likely to vary with pool size and the proportion of multibacillary cases among infected sheep. Although estimated during comparison of PFC with serology (Sergeant et al., 2002), flock-sensitivity has never been evaluated taking these factors into account.

Information about characteristics of this widely used test is crucial in field situations for making farm level decisions in disease control programmes. The present study was therefore conducted with the objectives: (1) to evaluate PFC sensitivity in sheep with different types of histopathological lesions and at a range of pool sizes; and (2) to assess the effects of prevalence, of the proportion of infected sheep that are multibacillary, and of pool size on both overall pool sensitivity achieved in a flock and flock-sensitivity.

First, we will discuss the laboratory study conducted to achieve the first objective and then the simulation approach to achieve the second.

2. **Laboratory study**

2.1. **Methods**

2.1.1. **Faecal samples**

Faecal samples from sheep of known infection status determined on the basis of individual faecal culture and histopathology were available from experimentally infected sheep. The samples had been individually cultured using a modified BACTEC radiometric method.
(Whittington et al., 1998; Whittington et al., 1999). Histopathological lesions in the samples collected from terminal ileum, ileocecal lymph nodes and jejunal lymph nodes from these animals had been graded according to Perez et al (1996), with no lesion as ‘0’; paucibacillary as 1, 2, 3a and 3c; and multibacillary as early and late 3b. Similarly, confirmed culture negative faecal samples collected from uninfected animals were available for creation of pools.

2.1.2. Pooling of samples
Culture positive faecal samples from 23 sheep with specific histopathological lesions (10 sheep with the multi- and 13 with the pauci-bacillary form of the disease) were selected for pooling with culture negative faecal samples from confirmed negative sheep. Six aliquots of 0.25 g of faeces from each of the 23 infected sheep were pooled with respectively 1, 2.25, 4.75, 7.25, 9.75 and 12.25 g of faeces from tested MAP-negative animals to create six pools per infected sheep. Thus, these pools contained faeces from infected and uninfected sheep in the ratio of 1:4, 1:9, 1:19, 1:39 and 1: 49, respectively, which is equivalent to creating pools of size 5, 10, 20, 30, 40 and 50, respectively, by mixing one pellet from infected sheep with 4, 9, 19, 39 and 49 pellets, respectively, from uninfected sheep.

Before pooling, faeces from each infected sheep were mixed thoroughly using a blender. After pooling with faeces from uninfected sheep, the faeces were mixed using a wooden applicator stick for about 1-3 min (depending on the quantity).

2.1.3. Pooled faecal culture
Pooled faeces were cultured using a faecal culture technique (Whittington et al., 2000). Briefly, the sample was decontaminated by the double incubation method using HPC and vancomycin, nalidixic acid and amphotericin B (VAN) prior to culture (Whitlock and Rosenberger, 1990). Radiometric BACTEC 12B media supplemented with PANTA PLUS, mycobactin J and egg yolk was used for culturing. BACTEC broth from the largest pool size showing growth for each sample and from pools that took ≥ 9 weeks to grow were further tested using PCR to confirm the presence of IS900 (Whittington et al., 1998).

2.1.4. Data analysis
PFC sensitivity for paucibacillary sheep (with histopathological classification of 3a, 3a-3b and 3a-3c) and multibacillary sheep (3b early and 3b) at a given pool size was calculated as a proportion of pools of that size and histopathological classification that cultured positive. Exact confidence intervals were calculated using FREQ procedure in SAS statistical programme (release 9.1.3, © 2002-2003, SAS Institute Inc., Cary, NC, USA). Impact of pool size on PFC sensitivity for pauci- and multi-bacillary sheep was evaluated using exact conditional logistic regression (conditional on infected sheep) employing the SAS statistical programme.

2.2. Results
In total, 137 pools (1 pool for each pool size for each of the 23 sheep; also see footnote (b) for Table 1) were constructed from sheep with varying histopathological lesions. Of the 10 sheep with the multibacillary lesions, seven had full-blown Perez 3b histological lesions whereas three had early 3b lesions. All of the 42 pools constructed by pooling faeces from sheep with advanced Perez 3b histological lesions were detected by the PFC test as against only 67% of the pools (12/18) constructed by pooling faeces from sheep with early 3b lesions. Of the 13 with the paucibacillary form of the disease, eight had typical Perez 3a lesions whereas five had intermediate lesions. Of the 48 pools constructed by pooling faeces from sheep with typical Perez 3a lesions, PFC could detect only seven pools (14.6%). Similarly, of the 30 pools constructed by pooling faeces from sheep with intermediate lesions, only seven pools 23.3%) were positive in PFC.
The results of PFC sensitivity at various pool sizes for pauci- and multi-bacillary sheep are presented in Table 1. The observed PFC sensitivity for multibacillary sheep was not significantly different across pool sizes tested ($b$: -0.035; odds ratios 0.97; 95% CI of odds ratios: 0.89, 1.04; $p = 0.38$) whereas PFC sensitivity for paucibacillary sheep varied according to pool size ($b$: -0.12; odds ratios 0.89; 95% CI of odds ratios: 0.79, 0.96; $p = 0.0002$).

### Table 1. Sensitivity of pooled faecal culture (PFC Se) at different pool sizes in experimental sheep exhibiting pauci- and multi-bacillary lesions.

<table>
<thead>
<tr>
<th>Pool Size</th>
<th>Paucibacillary</th>
<th></th>
<th></th>
<th></th>
<th>Multibacillary</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Num Positive/tested</td>
<td>PFC Se</td>
<td>Exact 95% CL</td>
<td>Num Positive/tested</td>
<td>PFC Se</td>
<td>Exact 95% CL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5/13</td>
<td>0.38</td>
<td>0.14</td>
<td>0.68</td>
<td>9/10</td>
<td>0.90</td>
<td>0.56</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>4/13</td>
<td>0.31</td>
<td>0.09</td>
<td>0.61</td>
<td>10/10</td>
<td>1.00</td>
<td>0.69</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>3/13</td>
<td>0.23</td>
<td>0.05</td>
<td>0.54</td>
<td>9/10</td>
<td>0.90</td>
<td>0.56</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>1/13</td>
<td>0.08</td>
<td>0.00</td>
<td>0.36</td>
<td>8/10</td>
<td>0.80</td>
<td>0.44</td>
<td>0.97</td>
</tr>
<tr>
<td>40</td>
<td>1/13</td>
<td>0.08</td>
<td>0.00</td>
<td>0.36</td>
<td>10/10</td>
<td>1.00</td>
<td>0.69</td>
<td>1.00</td>
</tr>
<tr>
<td>50</td>
<td>0/13</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
<td>8/10</td>
<td>0.80</td>
<td>0.44</td>
<td>0.97</td>
</tr>
</tbody>
</table>

a. All pools had a given proportion of confirmed MAP positive faeces.

b. A pool of size 50 could not be constructed for one animal but this was assumed negative because all other pools of size 10 - 40 for this animal were negative.

### 3. Probabilistic models

#### 3.1. Methods

Employing experimental estimates of PFC sensitivity for pauci- and multi-bacillary sheep discussed above, overall pool sensitivity achieved in a flock ($Se_k$) and flock sensitivity ($FSe$) were calculated at various combinations of infection prevalence, pool size, and multi- to pauci-bacillary ratio using two models programmed in R-language (version 2.5.0, © 2007 The R foundation for statistical computing). $Se_k$ was defined as the probability of a faecal pool from a flock to test positive, given that it contained pellets from at least one infected animal (true infected pool). This was calculated as a proportion of the true infected pools that tested positive in a given scenario, that is, for a given number of pools of a particular pool size (k) sourced from a flock of a given disease prevalence and with a given proportion of multibacillary among infected sheep. $FSe$ was defined as the ability of the PFC test to detect an infected flock and was calculated as the proportion of infected flocks declared as positive by the PFC test in a given scenario.

The reasons for developing two models instead of just one were firstly, to allow comparison of estimates between the two methods as a form of cross-validation, and secondly to overcome their respective limitations. While Model I did not allow estimation of uncertainty about $FSe$ estimates, Model II could not estimate $Se_k$.

#### 3.1.1. Input parameters

Most input parameters used in probabilistic models are described in Table 2. In addition, beta probability distributions for PFC sensitivity were included in the models based on the experimental results illustrated in Table 1. Numbers of positive and total pools were used to construct $\alpha$ and $\beta$ parameters of beta probability distributions:

$$\alpha = \text{number of positive pools} + 1$$
\[ \beta = \text{total number of pools} - \text{number of positive pools} + 1 \]

For paucibacillary sheep, \( \alpha \) and \( \beta \) parameters were constructed separately for all pool sizes, but as the PFC sensitivity was not significantly different across pool sizes for multibacillary sheep, a single beta probability distribution was used based on the total number of multibacillary pools (60) that tested positive (54).

In contrast to sensitivity, PFC specificity was assumed to be perfect (Table 2) because MAP organisms were detected and their identity confirmed using accepted taxonomic criteria. The possibility of cross-contamination was assumed to be minimal due to inclusion of negative controls and other quality control procedures.

Table 2. Input parameters used to estimate composite pooled sensitivity (CPoolSe) and flock sensitivity in the mathematical models described in the paper.

<table>
<thead>
<tr>
<th>Description</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of iterations</td>
<td>1000</td>
</tr>
<tr>
<td>Number of pools sourced from a flock</td>
<td>7</td>
</tr>
<tr>
<td>Number of pellets constituting a pool (pool size)</td>
<td>5, 10, 20, 30, 40 and 50</td>
</tr>
<tr>
<td>Prevalence of OJD in the flock</td>
<td>0.01, 0.02, 0.05, 0.1</td>
</tr>
<tr>
<td>Proportion of multibacillary among infected sheep</td>
<td>0.2</td>
</tr>
<tr>
<td>PFC specificity</td>
<td>1</td>
</tr>
</tbody>
</table>

The range of pool sizes and use of seven pools for model evaluation (Table 2) reflected real-life situations for most flocks in New South Wales, Australia. However, only low to moderate infection prevalence scenarios (from 1 to 10%) were primarily evaluated because of a greater likelihood of application of this test in such flocks, for example, in the current Australian Johne’s disease market assurance programme.

The proportion of multibacillary sheep was assumed to be 0.2 based on the results of a field trial (Abbott et al., 2004) in which sheep born to infected and uninfected dams were reared for three years under typical field conditions. Although the multi- to pauci-bacillary ratio depends on a number of factors (Whittington and McGregor, 2005), the trial estimates appeared to be consistent with the current understanding of the biology of OJD, and have also been used as an estimate of multi- to pauci-bacillary ratio in our previous study (Dhand et al., in press).

3.1.2. Model i

An OJD-infected sheep flock was simulated with 2000 sheep of a given disease prevalence and with a given proportion of sheep with the multibacillary form of OJD. A random sample of sheep sufficient to create seven pools of a specific pool size was selected, without replacement, from the simulated flock and pools were constructed (Fig. 1). A pool with at least one pellet from either a pauci- or a multi-bacillary sheep was considered true positive. A Bernoulli distribution was used to determine the test status of a true positive pool but all true negative pools were assumed to be test negative due to the assumption of perfect specificity of the test. The model was implemented for 1000 iterations for each combination of model parameters. \( \text{Se}_k \) for an iteration was calculated as a proportion of the true positive pools that tested positive and was then averaged across all iterations for each combination of pool size and prevalence evaluated. \( \text{FSe} \) was calculated as a proportion of the iterations in which at least one pool tested positive.
Subsequent to the main analyses, the models were implemented by changing input values of the ratio of multi- to pauci-bacillary sheep, the number of pools sourced and the number of iterations to evaluate their impact on model outputs.

Besides visual comparison of simulation results, generalised linear mixed model analyses were conducted on simulation results to obtain information about the relative importance of pool size, prevalence and proportion of multibacillary sheep in affecting $S_e$ and $FSe$.

### 3.1.3. Model II

This method involved calculating $FSe$ of PFC based on probability of a pool testing positive. The probability of a pool to be truly contaminated with pellets from multibacillary ($PT_{true_m}$) or paucibacillary sheep ($PT_{true_p}$) was calculated based on the infection prevalence in the flock ($\pi$), the proportion of multibacillary ($Prop_m$) and paucibacillary ($Prop_p$) sheep in the flock, and the pool size ($k$):

- $PT_{true_m} = 1 - (1 - Prop_m \times \pi)^k$
- $PT_{true_p} = 1 - (1 - Prop_p \times \pi)^k$

The probability of a multi- or a pauci-bacillary pool to test positive ($PT_{test_m}$ and $PT_{test_p}$, respectively) was a function of the probability of a pool being truly contaminated and the probability of the pool being detected by PFC. Note that the PFC sensitivity estimates for multi- and pauci-bacillary sheep were based on the experimental results (Table 1).

- $PT_{test_m} = PFC \text{ sensitivity for multibacillary sheep} \times PT_{true_m}$
- $PT_{test_p} = PFC \text{ sensitivity for paucibacillary sheep} \times PT_{true_p}$

The overall probability of a pool to test positive ($PT_{test}$) was then calculated by combining the two probabilities:

$$PT_{test} = PT_{test_m} + PT_{test_p} - PT_{test_m} \times PT_{test_p}$$

$FSe$ was subsequently computed based on this overall probability and the number of pools ($l$) tested, and then averaged over 1000 iterations to enable estimation of uncertainty about $FSe$ estimates.

$$FSe = 1 - (1 - PT_{test})^l$$

### 3.1.4. Sensitivity analyses

Sensitivity analyses were conducted by varying the input values of PFC sensitivity estimates by $\pm$ 20% and by increasing iterations to 10,000 to investigate their impact on $S_e$, and $FSe$ estimates.

### 3.2. Results

Both models produced almost identical $FSe$ results in all scenarios, therefore, only the results based on Model I are presented in all figures except Fig. 7 and 8.

#### 3.2.1. Effect of pool size and prevalence

Estimates of $S_e$, for various pool sizes and prevalence levels are presented in Fig. 2a. In general, $S_e$ decreased with increase in pool size and with decrease in prevalence. However, the relationship of $S_e$ with pool size reversed as prevalence touched a moderate level of 10% (and this trend continued beyond this prevalence level; data not shown). Similar were the results of generalised linear mixed model analyses conducted on simulation results (Table 3).

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1 All figures are located at the end of this document.
In contrast to Se, the FSe increased with both pool size and prevalence (Fig. 2b). At 5% and 10% prevalence, the FSe achieved almost a perfect level for all pool sizes above 20. Odds ratios calculated from generalised linear mixed model analyses based on simulation results indicated a similar association (Table 3).

Table 3. Odds ratios for various combinations of pool sizes and prevalence estimates based on the generalised linear mixed models built to evaluate association of pool size and prevalence with overall pool-sensitivity (Se) and flock sensitivity (FSe) using the datasets created by simulation results from Model I.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Pool size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Overall pool sensitivity achieved in a flock (Sek)</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>1</td>
</tr>
<tr>
<td>2%</td>
<td>1</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
</tr>
<tr>
<td>10%</td>
<td>1</td>
</tr>
<tr>
<td>Flock sensitivity (FSe)</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>1</td>
</tr>
<tr>
<td>2%</td>
<td>1</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
</tr>
<tr>
<td>10%</td>
<td>1</td>
</tr>
</tbody>
</table>

3.2.2. Effect of the proportion of multibacillary sheep
As expected, Se, as well as FSe increased with increase in the proportion of multibacillary sheep in a flock for a given prevalence (Fig. 3 and 4). The proportion of multibacillary cases had the greatest impact on FSe in low prevalence flocks and for large pool sizes.

Generalised linear mixed models constructed based on simulation results also indicated a positive association between proportions of multibacillary sheep and Se and FSe. The interaction terms of proportion of multibacillary sheep with both prevalence and pool size were significant (results not presented).

3.2.3. Effect of the number of pools sourced
FSe increased with an increase in the number of pools sourced for a given prevalence (Fig 5). However, for flocks with 10% prevalence, FSe was >80% even when only three pools of size 30-50 were collected (Fig 5d).

3.2.4. Uncertainty about FSe estimates
Using Model II, we estimated uncertainty about FSe estimates. Fifth and 95th percentiles of FSe for various prevalence and pool sizes shown in Fig. 6 suggest that uncertainty around FSe decreased as the pool size increased, particularly at a higher prevalence. This is an effect of the estimate approaching one, rather than being specifically due to pool size.

3.2.5. Sensitivity analyses
Se, and FSe were re-estimated by varying inputs of PFC sensitivity for multi- and paucibacillary sheep by ± 20%. Only modest changes in the output estimates were observed even after such substantial variation in inputs (Figs. 7 and 8).
Similarly, changing the number of iterations from 1000 to 10000 had a minimal influence on both the estimates.

4. Discussion

Accurate information about PFC sensitivity is crucial for various disease control programmes and OJD research projects. Our estimate of almost perfect PFC sensitivity for multibacillary (3b) sheep up to pool size of 50 are in agreement with those of Whittington et al. (2000), but for paucibacillary sheep, our estimates were lower than those reported earlier. This could be due to animals in the two experiments being at different stages of infection and thus shedding different numbers of organisms. While in the current experiment, faeces were sourced from animals with experimental infection induced with a given dose of MAP, in the previous study samples were obtained from various naturally infected sheep flocks in conjunction with OJD surveillance programmes (Whittington et al., 2000), and thus could have included more clinical animals with advanced disease.

The sensitivity of PFC in sheep with the paucibacillary form of OJD decreased with increases in pool size due to greater dilution of infected faeces, but the impact of dilution was not apparent in the multibacillary pools (Table 1). This is due to the large number of MAP shed in the faeces of sheep with the multibacillary form of the disease, thereby rendering the effect of dilution negligible. Among the multibacillary sheep, although sensitivity was almost perfect in sheep with advanced 3b-type lesions, it was not consistent in sheep with early 3b-type lesions probably due to lower levels of excretion, uneven distribution of MAP or imperfect mixing of faeces. Lack of homogeneity of distribution of MAP in the faeces was also reported previously (Tavornpanich et al., 2004).

In probabilistic models, the observed decrease in $Se_k$ with an increase in pool size was expected but the observed increase in $Se_k$ with pool size at higher prevalence levels was not anticipated (Fig. 2a). This was probably due to a greater likelihood of inclusion of a multibacillary case in a larger pool at a higher prevalence. We tested this proposition by slightly modifying our probabilistic model to count the number of multibacillary pools out of seven sourced and our results were in agreement with the proposition (data not shown). Similar results were obtained by van Schaik et al. (2003) (although for a different range of pool sizes) using a stochastic spreadsheet model to estimate the sensitivity of PFC for paratuberculosis in cattle. It appears that the sensitivity decreases with pool size due to dilution effect and increases with prevalence due to increase in the proportion of multibacillary sheep. With both influences present, however, the effect due to prevalence becomes stronger than the effect due to pool size at prevalence estimates greater than or equal to 10%.

Similarly, an increase in the $FSe$ with pool size (Fig. 2b) was due to the higher likelihood of inclusion of faeces from a multibacillary animal or due to selection of a larger sample from a flock (for example, pellets from 350 sheep are sourced to construct 7 pools of 50 compared to 35 sheep for 7 pools of 5). An increase in the $FSe$ with prevalence is also intuitive due to a higher probability of detection of a greater number of infected animals.

The proportion of multibacillary sheep was assumed to be 0.2 based on the results of a previous investigation (Abbott et al., 2004). As expected, changes in this proportion had a substantial effect on the estimates of both $Sek$ and $FSe$ (Figs. 3 and 4). However, this was more noticeable for larger pool sizes and in low prevalence flocks, because of the increased likelihood of inclusion of a multibacillary case in a pool. Nonetheless, the chances of higher proportions of multibacillary than the assumed 0.2 in low prevalence flocks are not very high, indicating that our model estimates based on this assumption are less likely to vary in real-life.
To delineate the effect of prevalence from the effect of an increase in the proportion of multibacillary sheep, we assumed a constant ratio of multi- to pauci-bacillary sheep (0.2) in our main analyses. However, in practice, this proportion is also likely to increase with increase in prevalence, and therefore the prevalence is expected to have a greater impact than observed in these analyses.

For low to moderate prevalence flocks, collection of >7 pools enhanced FSe. However, 7 pools were shown to be sufficient for flocks with ≥ 5% prevalence because they were able to achieve almost perfect FSe. This suggests that an increase in the number of pools sourced from low prevalence flocks should be considered to improve the probability of detection of infection.

Strengths of this study included estimation of PFC sensitivities for pauci- and multi-bacillary sheep by culturing faeces from infected and non-infected sheep in the laboratory in order to impute input values for probabilistic models. Other input values were based on previous research or past experience and mimicked real-life situations faced routinely in sheep farms in Australia. In addition, sensitivity analyses were conducted to gauge the impact of varying these input values. However, note that the model results may not be applicable to scenarios entirely different to those tested; for example, the results should not be extrapolated to smaller pool sizes used in culturing bovine faeces or to very high prevalence flocks.

The sensitivity of the pooled faecal culture likely to be achieved in a particular scenario is important for both animal health managers and farmers to make appropriate decisions about the number and size of faecal pools to be sourced. Therefore, our study results will help refine guidelines for pooled sampling in disease control programs as well as research projects. The findings will also be relevant to direct faecal PCR tests currently under development (Kawaji et al., 2007) for examination of pooled faeces.

5. Conclusions

The sensitivity of PFC is substantially higher in sheep with multibacillary lesions than those with paucibacillary lesions and varies with different grades of histopathology. Overall pool-sensitivity and flock sensitivity are also influenced by pool size, disease prevalence, and the proportion of multibacillary sheep. The results suggest that in higher overall pool-sensitivity can be achieved lower prevalence flocks by sourcing smaller pools. Higher flock-sensitivity can be achieved by either sourcing a greater number of pools or by increasing pool size.

Acknowledgements

We are thankful to Anna Waldron and other laboratory staff for assisting in microbiology work. This study was funded by Meat and Livestock Australia and the University of Sydney.

References:


Kawaji, S., Taylor, D., Mori, Y., Whittington, R.J., 2007. Detection of Mycobacterium avium subsp. paratuberculosis in ovine faeces by direct quantitative PCR has similar or greater sensitivity compared to radiometric culture. Veterinary Microbiology 125, 36-48.


Figure 1. Schematic representation of the simulated sampling approach (Model I).
Probability of PFC to detect MAP in a pool was a function of PFC sensitivity in pauci- and multi-bacillary pool.

Select a random sample of the required number of sheep (pool size * number of pools) and create pools

Number of sheep in the flock (N)

Number infected (i)  
(Prevalence*N)

Number uninfected  
(N-i)

Number multibacillary sheep (m)  
(Proportion of multibacillary * i)

Number of paucibacillary sheep  
(i-m)

Select a random sample of the required number of sheep (pool size * number of pools) and create pools

Faecal pool  
Faecal pool  
Faecal pool  
Faecal pool  
Faecal pool  
Faecal pool

Does this pool contain pellets from at least one infected sheep?

Yes  
No

True positive pool  
True negative pool

Does it have pellets from a multibacillary pool?

Multibacillary pool

(Paucibacillary pool

(Decision taken similar to the paucibacillary pool except that PFC sensitivity for the multibacillary sheep is used)

Is a randomly selected number from a uniform distribution ≤ the sensitivity of PFC for the paucibacillary sheep?

Yes  
No

Test positive pool  
Test Negative pool

PFC Specificity assumed to be perfect
Figure 2. Effect of pool size and prevalence on overall pool-sensitivity (Sek) and flock-sensitivity (FSe) if 7 pools of a given size were sourced from flocks where 20% of the infected sheep had the multibacillary form of the disease. Detailed input parameters and the priors for PFC sensitivity employed in the analysis are described in Table 2 and Section 3.1.1.
Figure 3. Impact of proportions of multibacillary sheep on overall pool-sensitivity of PFC (Seₖ) when pools of different sizes are collected from flocks with different prevalence levels. Detailed input parameters (except proportion of multibacillary sheep) and the priors for PFC sensitivity employed in the analysis are described in Table 2 and Section 3.1.1.
Figure 4. Impact of proportions of multibacillary sheep on flock-sensitivity of PFC (FSe) when pools of different sizes are collected from flocks with different prevalence levels. Detailed input parameters (except proportion of multibacillary sheep) and the priors for PFC sensitivity employed in the analysis are described in Table 2 and Section 3.1.1.
Figure 5. Impact of the number of pools sourced on PFC flock-sensitivity (FSe) when different sizes of pools are collected from flocks with given prevalence, assuming that 20% of the infected sheep are in the multibacillary form of the disease. Detailed input parameters (except number of pools) and the priors for PFC sensitivity employed in the analysis are described in Tables 2 and Section 3.1.1.
Figure 6. Fifth and 95th percentiles of flock-sensitivity (FSe) estimates calculated using the analytical approach (Model II) assuming that 20% of the infected sheep were in the multibacillary form of the disease. Detailed input parameters (except number of pools) and the priors for PFC sensitivity employed in the analysis are described in Tables 1 and Section 3.1.1.
Figure 7. Sensitivity analysis to evaluate the impact of changing pooled faecal culture (PFC) sensitivity for multi- and pauci-bacillary sheep on overall pool-sensitivity of PFC (Sek) achieved in a flock.

(a) Prevalence = 0.01
(b) Prevalence = 0.02
(c) Prevalence = 0.05
(d) Prevalence = 0.1
Figure 8. Sensitivity analysis to evaluate the impact of changing pooled faecal culture (PFC) sensitivity for multi- and pauci-bacillary sheep on flock-sensitivity.

a) Prevalence = 0.01

b) Prevalence = 0.02

c) Prevalence = 0.05

d) Prevalence = 0.1