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A Bayesian Approach to Estimate OJD Prevalence From Pooled Fecal Samples of Variable Pool Size

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This paper describes a Bayesian approach to prevalence estimation based on pooled samples that accommodates variation in pool size and adjusts for test imperfection. A logistic model was developed for pooled fecal culture (PFC) sensitivity as a function of pool size and a logistic mixed model for ovine Johne's disease (OJD) prevalence as a function of covariates that were found significant in a recent OJD risk factor study conducted in Australia. Available data on these factors and prior information about prevalence and sensitivity were incorporated into a Bayesian model to estimate OJD prevalence from PFC data. Overall, posterior cohort OJD prevalence was estimated to be 0.16 (range of prevalences across cohorts 0.002 to 0.72). The average prevalence was higher in wethers than ewes. PFC sensitivities for pool sizes 10, 30 and 50 were estimated to be 0.91 (95% probability intervals 0.80, 0.96), 0.85 (0.80, 0.90) and 0.77 (0.65, 0.88), respectively. Posterior specificity of PFC was almost perfect though based primarily on the prior. Results suggest the Bayesian model successfully estimated the animal-level prevalence after accounting for variable pool size and imperfect test parameters. The method can be easily adapted for other conditions and diseases where pooled samples are collected. WinBugs code for the article is available online.

Key Words: Diagnostic test; Mycobacterium; Ovine Johne's disease; Paratuberculosis; Prevalence; Sensitivity.

1. INTRODUCTION

Ovine Johne's disease (OJD), a chronic debilitating disease of sheep caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is prevalent in many countries of the

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world, including Australia, and causes significant economic losses to farmers (Bush, Windsor, and Toribio 2006). Pooled fecal culture (PFC), the principal test used for OJD diagnosis in Australia, has better sensitivity and specificity than serological tests and is more cost effective than individual fecal culture (Whittington et al. 2000). It has proved to be very useful for detection of infected flocks, however, pooled results provide only a crude estimate of within-flock prevalence. Statistical methods for estimating animal-level prevalence from pooled results are available, but are in some way limited when applied to OJD field data (Toribio and Sergeant 2007).

Estimation of animal-level prevalence from PFC results is complicated due to variation in PFC sensitivity with pool size and with disease pathology (Whittington et al. 2000). Collection of variable, rather than uniform pool sizes due to the logistics of sample collection in the field, further complicates the estimation of animal-level prevalence. All currently available methods, except that of Williams and Moffitt (2001), assume collection of pools of uniform size. However, the method of Williams and Moffitt (2001) assumes perfect sensitivity and specificity, which is not true for PFC. We are not aware of any method that can be used to estimate animal-level prevalence from PFC results by accounting for both the variable pool size and imperfect or unknown test sensitivity and specificity, highlighting the need for such a method.

This paper describes a Bayesian approach to prevalence estimation based on PFC results that accommodates variation in pool size as well as test imperfection. This new approach was applied to estimate OJD prevalence for sheep cohorts enrolled in a risk factor study and can be easily modified for similar situations or disease conditions.

2. METHODS

2.1. DESCRIPTION OF THE OJD RISK FACTOR STUDY DATA SET

OJD risk factor study was conducted in infected sheep flocks in four states of Australia during 2004–2005. Detailed study design and sampling methodology are available elsewhere (Dhand et al. 2007), and therefore will be discussed here only in brief.

A cohort of sheep, defined as a group of sheep of the same age and sex in a flock, was the unit of interest in this study. Usually one cohort was enrolled per flock, but due to logistics of sampling, two or more cohorts had to be enrolled from some flocks (Figure 1). The data set used for the present analysis consisted of 97 cohorts representing 86 flocks (1 cohort per flock from 75 flocks and 2 cohorts per flock from 11 flocks). We were interested in estimating animal-level OJD prevalence for each cohort.

Most pools were comprised of pellets from 30 or 50 sheep, with each sheep selected using a systematic sampling approach and then one pellet collected per rectum per sheep. Some pools had pellets from less sheep due to insufficient sheep on the farm on the day of sampling. Pooled fecal samples were cultured using a modified BACTEC radiometric method (Whittington et al. 2000). MAP in culture positive samples were further confirmed by polymerase chain reaction (PCR) and restriction endonuclease analysis (REA) by demonstrating the presence of *IS900* (Cousins, Evans, and Francis 1995; Whittington et al. 1998). Only such confirmed positive pools were considered to be PFC positive while

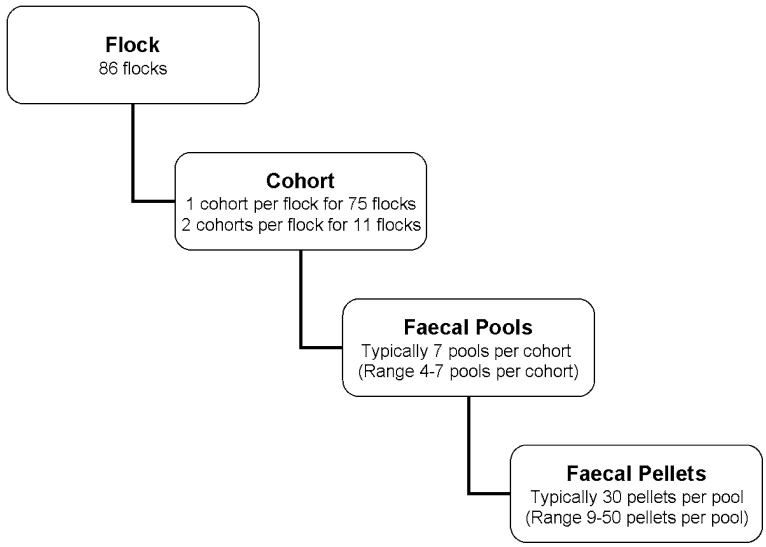

Figure 1. Sampling schema for collecting pooled faecal samples in the OJD risk factor study conducted in 2004–2005 in sheep flocks in Australia. Typically, seven fecal pools were collected from each cohort, with each pool generally constructed by one fecal pellet collected per rectum per sheep from 30 sheep.

all other pools (even if BACTEC positive) were considered to be PFC negative. This composite procedure will be referred to from here on as a single test procedure, the PFC. This procedure will have very high specificity because the three tests were conducted in series but less sensitivity than if the pools were tested by BACTEC method alone. Note that PFC was preferred to individual fecal culture as this is the most commonly used approach for disease diagnosis in Australia.

We now discuss the Bayesian approach developed to estimate animal-level prevalence based on PFC results.

2.2. POOL AND ANIMAL-LEVEL PREVALENCES

We introduce some notation. Consider a cohort from which x pools tested positive out of a total n pools collected, all of the same pool size. We assume x is distributed binomially, that is,

$$x \sim \text{bin}(n, P), \tag{2.1}$$

where P is the apparent pool prevalence of OJD in the cohort calculated as the sum of probabilities for true positive and false positive pools (see (2.2) below). A pool is truly positive if it has fecal pellets from at least one infected sheep and on the other hand a pool is truly negative if it is constituted by pellets from all uninfected sheep.

Let $T+$ denote that a pool has tested positive and let $D+$ denote a pool that has pellets from at least one infected sheep. Then the (population) apparent prevalence can be generically calculated using the law of total probability as:

$$P = \Pr(T+) = \Pr(\text{True Positive Pool}) + \Pr(\text{False Positive Pool})$$
$$= (\text{True Pool Prevalence})(\text{Sensitivity}) + (1 - \text{True Pool Prevalence})(1 - \text{Specificity})$$
$$= \Pr(\geq 1D+) * \Pr(T+ | \geq 1D+) + \Pr(0D+) * \Pr(T+ | 0D+). \quad (2.2)$$

If π is the true animal-level prevalence (proportion of animals shedding MAP in a cohort) and k the number of animals constituting a pool (pool size), the probability that a pool has pellets from any infected sheep is $(1-\pi)^k$. Consequently, the probability that a pool is positive or has pellets from at least one infected sheep is $1-(1-\pi)^k$, which of course is the true pool prevalence. Denote Sp as the PFC specificity, considered to be constant across various pool sizes, and Se_k as PFC sensitivity, allowed to vary according to pool size. Then the (population version of the) apparent prevalence for a pool of size k in the sampled population is

$$P_k = \{1-(1-\pi)^k\} * \text{Se}_k + (1-\pi)^k * (1-\text{Sp}). \quad (2.3)$$

The sampling design considered resulted in multiple binomial counts like the one described above. The animal-level prevalence π was assumed to vary depending on available covariate information (see Section 2.3 below).

As discussed above, the basic sampling unit was flock, and from each flock one or more cohorts were identified (units of interest) from which multiple pools were sampled. Our situation can thus be described by defining x_{ijk} to be the number of positive pools of size k that were observed in cohort i in flock j, and by letting P_{ijk} be the corresponding apparent prevalence. These counts are assumed to be independent and binomially distributed as discussed above. However, since the unit of interest was a cohort, our notation simplifies to x_{ik} as the number of positive pools for the ith cohort with pools of size k and with P_{ik} denoting the corresponding apparent prevalence. In the next subsection, we describe how animal prevalences were modeled as functions of covariate information and how sensitivities were modeled as functions of pool size.

2.3. MODEL FOR ANIMAL-LEVEL PREVALENCE

True prevalence π for animals within a particular cohort was modeled as a function of covariates that were found to be significant in a previous analysis using an ordinal logistic regression model for cohort OJD prevalence level. In addition to covariates, a flock level random effect variable was added to the model to account for clustering within the flock.

Let π_i be the true OJD prevalence of cohort i. Then logit of true prevalence in the ith cohort is modeled as

$$\text{logit}(\pi_i) = g_1 + g_2 s_i + g_3 a_i + g_4 m_{2i} + g_5 m_{3i} + g_6 sr_i + g_7 v_{2i} + g_8 v_{3i} + g_9 f_i + g_{10} w_i$$
$$+ g_{11}(m_2 * \text{sr})_i + g_{12}(m_3 * \text{sr})_i + u(\text{flock}_i) \quad (2.4)$$

where g_1 to g_{12} are unknown regression parameters; s indicates sex of the cohort (0 = females; 1 = males); a indicates the age group (0 = 3-year olds and 1 = 4-year olds); m_2 and m_3 are two indicator variables for the current OJD mortality ($m_2 = 1$ indicates yes for $<2\%$ mortalities and $m_3 = 1$ indicates yes for $\geq 2\%$ mortalities, thus $m_2 = m_3 = 0$

indicates 'no mortalities'); sr is lambing paddock stocking rate (0 corresponds to <14 dry sheep equivalent (dse)/hectare;[1] 1 corresponds to ≥ 14 dse/hectare); v_2 and v_3 are the indicator variables for number of years since commencement of OJD vaccination in the flock ($v_2 = 1$ indicates yes for >2 years and $v_3 = 1$ indicates yes for 'no vaccination,' so $v_2 = v_3 = 0$ implies 1 or 2 years since commencement of vaccination); f indicates the use of fertilizers other than superphosphate and lime on the property ($0 =$ no; $1 =$ yes); w indicates the presence of wildlife other than kangaroos and rabbits on the farm ($0 =$ no; $1 =$ yes); $m_2 * \text{sr}$ and $m_3 * \text{sr}$ represent interactions between stocking rate and current OJD mortality; u's are the random effects for flocks. Refer to Dhand et al. (2007) for further details about the covariates.

Inclusion of random effects for flock was necessary for two reasons. First, there will be variability in overall prevalences among flocks that is due to differences among flocks, perhaps due to differences in management practices, that is unaccounted for by the covariate information that is available. Thus, if we consider a particular combination of covariates, we think of $u_i = 0$ as corresponding to a "typical" flock among those that have that particular combination of risk factors. A well managed flock with those risk factors might have a value of u that is below 0, resulting in a lower prevalence than for a "typical" flock, and similarly for a poorly managed flock the corresponding value of u might be positive and thus result in a larger prevalence. Second, it would be expected that yes/no outcomes of infection might be correlated within flocks, and modeling the random effects accounts for this correlation.

Model selection was performed by considering Posterior Probability of Positive and Negative Association (PPPA and PPNA, respectively) of individual terms in the model and by comparing deviance information criteria (DIC) for models with and without various effects (Spiegelhalter et al. 2002). Smaller values of DIC are associated with better combined goodness of fit and parsimony.

We also compared the selected mixed model with two other variants:

- a model with only three covariates (age, sex and current mortality) and random effects for flocks to evaluate whether this simpler model would suffice rather than a model with all covariates;
- a model with all fixed effects but no random effects.

The uncertainty about regression parameters g_1 to g_{12} and the random effects scale parameter was modeled by eliciting prior information, which is discussed below.

2.3.1. Prior Elicitation for Prevalence Parameters

Since actual prior data for prevalences are unavailable, a partially informative prior was elicited from an expert, Professor R. J. Whittington, with about 12 years of experience in

[1] Stocking density is usually measured in dse per hectare where one dse is the energy requirement of a 50 kg wether just for maintaining weight.

Table 1. Priors for pooled fecal culture (PFC) test sensitivity and specificity, and OJD prevalence elicited in the study.

Priors	Input Values		Prior distributions[a]			
	Mode	Lower/Upper[b]	a	b	Mean	(95% PI)
PFC Sensitivity						
Pool size 10	0.74	0.50	10.20	4.20	0.71	(0.46, 0.90)
Pool size 50	0.60	0.40	10.90	7.60	0.59	(0.36, 0.80)
PFC Specificity	0.995	0.99	1137.50	6.71	0.99	(0.99, 1.00)
Cohort OJD Prevalence						
Nil mortality flocks						
3-year-old ewes	0.05	0.08	8.77	148.70	0.056	(0.03, 0.10)
3-year-old wethers	0.05	0.10	4.57	68.74	0.062	(0.02, 0.13)
4-year-old ewes	0.05	0.10	4.57	68.74	0.062	(0.02, 0.13)
Low mortality flocks (<2%)						
3-year-old ewes	0.07	0.15	3.78	37.95	0.09	(0.02, 0.19)
High mortality flocks (≥2%)						
3-year-old ewes	0.35	0.70	2.68	4.12	0.39	(0.09, 0.75)

[a] a and b are parameters of the respective beta probability distributions.

[b] For sensitivity and specificity, lower 5% limits were obtained from previous literature. For prevalence, upper 90% limits for nil- and low-mortality flocks and an upper 95% limit for high-mortality flocks were elicited from Prof R.J. Whittington. Relatively non-informative priors were used for other parameters including the standard deviation, σ, which was assigned a Unif[0.01,3] prior distribution.

OJD research. Our technique involves specifying prior information about several prevalences where each prevalence corresponds to a particular combination of covariate values. For example, first we asked our expert to give his 'most likely' value for prevalence in a cohort of 3-year-old ewes reared in a flock with nil OJD mortality, say $\tilde{\pi}_1$, and also a value that he is 'virtually certain' that the prevalence would not exceed. We then translated that value to either be the 90th or 95th percentile of a beta(a, b) distribution with mode $([a-1]/[1+b-2])$ equal to his most likely value and solved for the beta distribution that satisfies these characteristics. Our second choice of covariate combination corresponds to a wether cohort, but otherwise has all baseline characteristics. We obtained our expert's most likely value as well as an upper percentile for that prevalence, say $\tilde{\pi}_2$, and solved for a second beta distribution, etc. See Table 1 for inputs and selection of (a, b) values for all five covariate combinations. These prevalence priors were used to induce α and β parameters of beta probability distributions for the regression coefficients (g_1 to g_5) for the prevalence model, adapting the technique described by Hanson et al. (2003) and Bedrick, Christensen, and Johnson (1997) and theoretically justified in Christensen et al. (2010). Note that we only elicited expert opinion for these five coefficients and placed relatively non-informative priors on the rest of the coefficients in the prevalence model.

Briefly, consider g_1 to be the intercept; g_2 and g_3 be the regression parameters for sex (0 = females, 1 = males) and age groups (0 = young, 1 = old); and g_4 and g_5 be the parameters for low (<2%) and high (≥2%) mortality, respectively in five logit beta prior distributions. We assume for the moment that all other variates are set to their reference

values. The logit of prevalences corresponding to five age, sex and mortality combinations (lp_1, lp_2, lp_3, lp_4 and lp_5) are given as:

$$\begin{aligned}
\mathrm{lp}_1 &= \mathrm{logit}(\tilde{\pi}_1) = g_1 + g_2 0 + g_3 0 + g_4 0 + g_5 0,\\
\mathrm{lp}_2 &= \mathrm{logit}(\tilde{\pi}_2) = g_1 + g_2 1 + g_3 0 + g_4 0 + g_5 0,\\
\mathrm{lp}_3 &= \mathrm{logit}(\tilde{\pi}_3) = g_1 + g_2 0 + g_3 1 + g_4 0 + g_5 0,\\
\mathrm{lp}_4 &= \mathrm{logit}(\tilde{\pi}_4) = g_1 + g_2 0 + g_3 0 + g_4 1 + g_5 0,\\
\mathrm{lp}_5 &= \mathrm{logit}(\tilde{\pi}_5) = g_1 + g_2 0 + g_3 0 + g_4 0 + g_5 1.
\end{aligned} \tag{2.5}$$

So, $\tilde{\pi}_1$, $\tilde{\pi}_2$ and $\tilde{\pi}_3$ are the OJD prevalences among 3-year-old ewes, 3-year-old wethers and 4-year-old ewes respectively, in nil mortality flocks and with reference values for other covariates. Similarly, $\tilde{\pi}_4$ and $\tilde{\pi}_5$ are the OJD prevalences among 3-year-old ewes in low ($<2\%$) and high ($\geq 2\%$) mortality flocks, respectively, with reference values for other covariates.

The uncertainty about $\tilde{\pi}_i$'s was modeled with independent beta prior distributions based on expert opinion, as discussed above. It is possible to solve for g's:

$$\begin{aligned}
g_1 &= 1\mathrm{lp}_1 + 0\mathrm{lp}_2 + 0\mathrm{lp}_3 + 0\mathrm{lp}_4 + 0\mathrm{lp}_5,\\
g_2 &= -1\mathrm{lp}_1 + 1\mathrm{lp}_2 + 0\mathrm{lp}_3 + 0\mathrm{lp}_4 + 0\mathrm{lp}_5,\\
g_3 &= -1\mathrm{lp}_1 + 0\mathrm{lp}_2 + 1\mathrm{lp}_3 + 0\mathrm{lp}_4 + 0\mathrm{lp}_5,\\
g_4 &= -1\mathrm{lp}_1 + 0\mathrm{lp}_2 + 0\mathrm{lp}_3 + 1\mathrm{lp}_4 + 0\mathrm{lp}_5,\\
g_5 &= -1\mathrm{lp}_1 + 0\mathrm{lp}_2 + 0\mathrm{lp}_3 + 0\mathrm{lp}_4 + 1\mathrm{lp}_5.
\end{aligned} \tag{2.6}$$

It is clear that information about these g's can be induced from the information about the $\tilde{\pi}_i$'s. This is easily handled in WinBUGS (see Appendix).

Relatively non-informative priors were used for the remaining unknown coefficients in the prevalence model (g_6 to g_{12}), which were set to be normally distributed with mean zero and variance one.

2.3.2. Prior for Random Effects' Scale Parameter

The random effect u_i was assumed to be normally distributed with mean 0 and precision τ, which can be obtained from standard deviation (σ), that is, ($\sigma = 1/\sqrt{\tau}$). Since we have a logistic regression model, values of u_i that are larger than 6 or less than -6 will lead to corresponding probabilities that are near one or zero, respectively. Thus we used a uniform prior on (0.01, 3) in order to be relatively non-informative, subject to the constraint that the largest value that two standard deviations above or below the mean for u_i can be, is about 6.

2.4. MODELING PFC SENSITIVITY

Se_k, the sensitivity of PFC for a pool of size k, was modeled as a function of pool size through a logit transform

$$\text{logit}(Se_k) = b_1 + b_2 * (k - m)/\text{sd}, \tag{2.7}$$

where b_1 and b_2 are unknown regression parameters; m is the mean and sd the standard deviation of k for the values of k in the Whittington et al. (2000) data. Standardizing stabilizes subsequent analysis.

The prior on PFC sensitivity (Se) was derived based on analysis of previous data (Abbott, Whittington, and McGregor 2004; Whittington et al. 2000) and then used to induce priors on the regression parameters (b_1 and b_2) using a similar method to the one used for prevalence parameters. A brief description of the method follows.

2.4.1. Prior Elicitation for Sensitivity

Two major types of pathological entities are associated with OJD, namely, multibacillary and paucibacillary forms of disease. Greater numbers of MAP organisms are shed in the feces in multibacillary sheep resulting in higher sensitivity in culture methods if sheep are manifesting the multibacillary rather than paucibacillary form of disease (Whittington et al. 2000). However, usually sheep with both forms of disease are present in a population and therefore their relative proportion affects the overall sensitivity of PFC, which would be lowest when all the animals are paucibacillary.

Thus, we first estimated multi- to pauci-bacillary ratio in a typical flock based on the results from a recent field trial. In this trial, sheep born to infected and uninfected dams were raised on either an infected or an uninfected pasture, thus mimicking different real field conditions (Abbott, Whittington, and McGregor 2004). Of the sheep in that study that survived up to three years during a trial period, the ratio of multi- to pauci-bacillary sheep was estimated to be 17:83. The multi- to pauci-bacillary ratio depends on a number of factors (Whittington and McGregor 2005); nonetheless, the trial gave a realistic estimate based on a typical situation which is consistent with the current understanding of the biology of OJD. Moreover, it should lead to conservative estimates of PFC sensitivities for different pool sizes. Therefore we assumed multi- to pauci-bacillary ratio of 20:80, an approximation from trial estimates.

Using this ratio, we computed a weighted average of the number of pools likely to be positive in a typical flock based on the data of Whittington et al. (2000) if pools of size 10 and 50 are collected from the flock. This formed the basis of our prior beta distributions for these pool sizes (Table 1). These beta distributions were subsequently used to induce prior distributions on the sensitivity parameters (b_1, b_2) using the method of Hanson et al. (2003), similar to the description for prevalence. This is easily accomplished in WinBUGS by simply defining the b's in terms of the sensitivities (see Appendix for WinBUGS code). Also, see Bedrick, Christensen, and Johnson (1997) for additional details about this method of prior specification.

2.4.2. Elicitation of Specificity Prior

In contrast to sensitivity, PFC specificity was considered to be independent of pool size and other covariates. Uncertainty about specificity was modeled with a beta distribution

$$\mathrm{Sp} \sim \mathrm{beta}(a_{\mathrm{sp}}, b_{\mathrm{sp}}), \tag{2.8}$$

where a_{sp} and b_{sp} are, respectively, the parameters of the beta distribution. Due to the nature of the PFC test, and as the positive pools were further confirmed by PCR and REA, the specificity of PFC was considered to be almost perfect. Therefore lower probability limit and modal values near one were used for the beta distribution for specificity (Table 1) adopting an approach similar to that of Tavornpanich et al. (2004).

2.5. Sensitivity Analysis

A sensitivity analysis was conducted by varying prior specifications for PFC sensitivity and cohort OJD prevalence by $\pm 20\%$ to evaluate their impact on posterior distributions.

2.6. Model Check

We assessed model fit using a method described by Gelman, Meng, and Stern (1996) (GMS). Let $x_{\mathrm{obs}} = (x_1, \ldots, x_n)$ be the observed binomial counts. Analogously to their expression (8), we define Pearson-type chi-square discrepancy

$$D(x_{\mathrm{obs}}; \theta) = \sum_{i=1}^{n} \frac{(x_i - n_i p_i)^2}{n_i p_i},$$

where θ corresponds generically to model parameters and p_i corresponds to (2.3) using (2.4) with (π_i, k_i) substituted for (π, k). If we substituted estimates of π_i's, we would have the classic Pearson chi-square statistic for testing goodness of fit. GMS recommend calculating the "predictive p-value (ppv)"

$$\begin{aligned} \mathrm{ppv} &\equiv \Pr(D(x^{\mathrm{rep}}; \theta) > D(x_{\mathrm{obs}}, \theta) \mid x_{\mathrm{obs}}) \\ &= \int I\big[D(x^{\mathrm{rep}}; \theta) > D(x_{\mathrm{obs}}, \theta)\big] p(x^{\mathrm{rep}} \mid \theta, x_{\mathrm{obs}}) p(\theta \mid x_{\mathrm{obs}})\, dx^{\mathrm{rep}}\, d\theta. \end{aligned} \tag{2.9}$$

Here x^{rep} corresponds to a "repetition" of the sampled data under the presumed model; x^{rep} is sampled from the predictive density of a future vector of data conditional on the observed data. This amounts to thinking of the predictive density $p(x^{\mathrm{rep}} \mid x_{\mathrm{obs}})$ as a reference distribution for simulating future data from the assumed model. Relation (2.9) is numerically approximated by sampling θ^t from the posterior for θ followed by sampling x^t from $p(x^{\mathrm{rep}} \mid \theta^t, x_{\mathrm{obs}})$ and approximating $\mathrm{ppv} \doteq \sum_{t=1}^{r} I[D(x^t; \theta^t) > D(x_{\mathrm{obs}}, \theta^t)]/r$. If ppv is quite small, the inference is that the fit of the observed data to the model is inconsistent with the distribution of fits of data that are sampled from the assumed model.

We also defined the "deviance" measure analogue for goodness of fit and calculated a ppv corresponding to it as well.

2.7. IMPLEMENTATION

All the models were implemented in WinBUGS (Lunn et al. 2000). Brief WinBUGS code is provided in Appendix and a detailed version is available online.

We performed a convergence check by monitoring histories, running quantile plots and the so-called BGR (Brooks and Gelman 1998) plot. All models were run for 30,000 iterations for each of the two chains and the initial 5000 iterations were discarded. Output from WinBUGS was saved in Convergence Diagnostic and Output Analysis (CODA) format for creation of plots in the R language environment (R Development Core Team 2009).

3. RESULTS

3.1. MODEL SELECTION

We first analyzed model (2.4) and found that PPPA or PPNA for coefficients were all at least 0.95, except for 'cohort age,' 'presence of wildlife other than kangaroos and rabbits on the farm' and the two interaction terms '($m_2 * \text{sr}$ and $m_3 * \text{sr}$).' Therefore they were candidates for removal from the model. We did not consider removal of 'cohort age' because it was considered to be a confounder *a priori*. Removal of variable 'presence of wildlife other than kangaroos and rabbits on the farm' and the interaction terms increased DIC values (Table 3) indicating that the model containing these terms was superior to the one without them. The stocking rate by mortality interaction was further investigated by considering estimated odds of OJD with $\text{sr} = 1$, to the odds of OJD with $\text{sr} = 0$, for cohorts in nil, low and high mortality flocks (calculated as: e^{g_6}, $e^{g_6+g_{11}}$ and $e^{g_6+g_{12}}$, respectively). These odds ratios were estimated to be 2.27, 4.53 and 3.85, respectively, for nil, low and high mortality flocks, whereas under the model with no interaction, these three values would be identical. Therefore, we concluded that the interactions were of practical importance and therefore should be retained in the model despite our lack of certainty about their statistical import.

We could have considered model averaging over model (2.4) with and without interactions, and perhaps this would be the preferable model if our goal were prediction, but because we were mainly interested in estimating prevalences, we preferred model (2.4). Parameter estimates and the posterior probabilities for all terms in this model are presented in Table 2.

Comparison of model (2.4) with other variants indicated that this was superior to the model with only fixed effects (Table 3). As expected, model (2.4) was more complex (i.e. had a higher pD), but we preferred it because of its considerably lower DIC. However, interestingly, model (2.4) had a higher DIC value compared to that containing just cohort age, cohort sex and current OJD mortality, even though all the remaining terms in model (2.4) had PPPA or PPNA values > 0.90 (of course, except cohort age and interaction terms, as discussed above). Given that these terms could be biological important, we preferred to choose model (2.4) as our final model.

3.2. ESTIMATES OF PFC SENSITIVITY AND SPECIFICITY

Posterior inferences for PFC sensitivities, Se_k, with pool sizes of $k = 10$, 30 and 50 are 0.91 (0.80, 0.96), 0.85 (0.80, 0.90) and 0.77 (0.65, 0.88), respectively. The estimates

Table 2. Bayesian model for cohort OJD prevalence based on the data of OJD risk factor study conducted in Australia in 2004, with posterior probabilities (PPNA/PPPA) that the estimated coefficients for various covariates in the model were positive or negative (<0 or >0).

Parameters	Categories	b	sd	(95% PI)	Probability	
Constant		−3.15	0.27	(−3.70, −2.63)	1.000	(<0)
Current OJD mortality[a]						
	No mortalities					
	<2% mortalities	0.86	0.40	(0.10, 1.66)	0.986	(>0)
	≥2% mortalities	2.11	0.54	(1.11, 3.25)	1.000	(>0)
Cohort sex						
	Ewes					
	Wethers	0.63	0.33	(0.02, 1.29)	0.972	(>0)
Cohort age						
	3 years					
	4 years	0.43	0.39	(−0.31, 1.23)	0.865	(>0)
Stocking rate in the lambing paddock						
	<14 dse[b]/hectare					
	≥14 dse/hectare	0.82	0.45	(−0.04, 1.73)	0.969	(>0)
Years since commencement of OJD vaccination						
	1 or 2 years					
	> 2 years	−1.00	0.43	(−1.79, −0.08)	0.982	(<0)
	Vaccination not being done	−0.89	0.53	(−1.88, 0.20)	0.950	(<0)
Application of fertilizers other than super and lime						
	No					
	Yes	−1.31	0.52	(−2.32, −0.27)	0.992	(<0)
Presence of wildlife other than kangaroos and rabbits on the farm						
	No					
	Yes	−0.56	0.40	(−1.35, 0.23)	0.924	(<0)
Interaction between stocking rate in the lambing paddock and current OJD mortality						
	<2% mortalities × High stocking rate	0.69	0.63	(−0.49, 1.98)	0.870	(>0)
	≥2% mortalities × High stocking rate	0.53	0.78	(−0.87, 2.20)	0.744	(>0)

[a]Farmer-reported flock-OJD mortality in adult sheep (>2 years old) for previous 12 months.

[b]Lambing ewe = 2.45 dry-sheep equivalent (dse).

Table 3. Deviance Information Criterion (DIC) for all the model variants considered in this paper.

Models	DIC	pD
The final mixed effect model (model (2.4))	297.2	24.7
Model (2.4) without the interaction term	298.0	26.5
Model (2.4) without the variable 'presence of wildlife other than kangaroos and rabbits on the farm'	299.9	24.3
Model (2.4) but with just cohort age, cohort sex and current OJD mortality	289.8	19.2
Model (2.4) without random effects (fixed effects model)	336.5	9.4

of PFC sensitivity displayed in Figure 2a indicate that the PFC sensitivity decreased with increase in pool size. However, the probability intervals were narrower for pools around size 30 than both the lower and higher pool sizes.

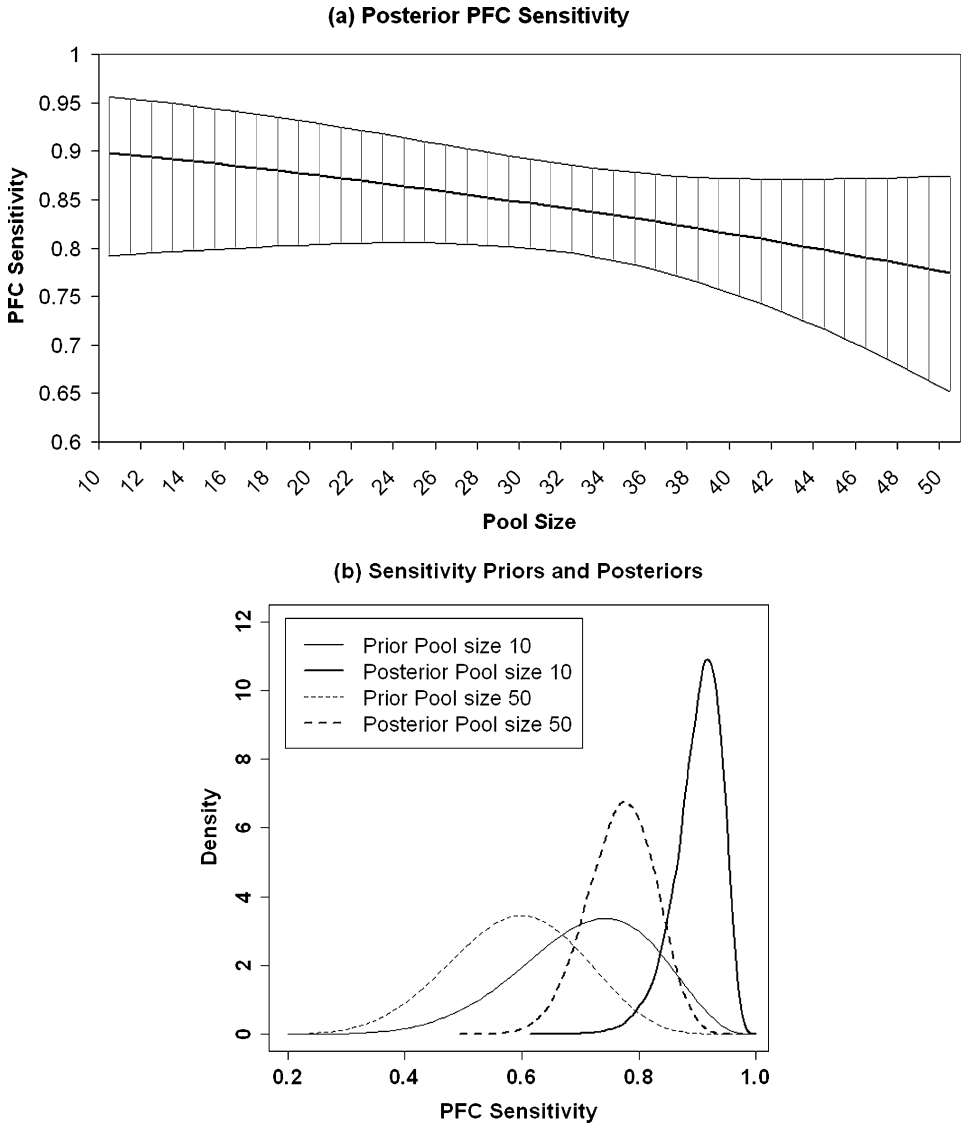

Figure 2. Pooled fecal culture (PFC) test sensitivity based on the analysis of data from the OJD risk factor study conducted in 2004–2005 in sheep flocks in Australia. (a) Posterior median and 95% probability intervals for pools of sizes from 10 to 50; (b) Comparison of priors with the posteriors for PFC sensitivity.

Posterior distributions of PFC sensitivity, Se_k, along with the sensitivity priors presented in Figure 2b indicate that the posteriors were concentrated within the range of priors and the data provided considerable extra information beyond that in the priors. In contrast, posterior specificity of PFC was almost perfect (median 0.994; 95% PI 0.989, 0.998) and the posterior distribution for specificity was aligned almost exactly the same as the prior (figure not shown).

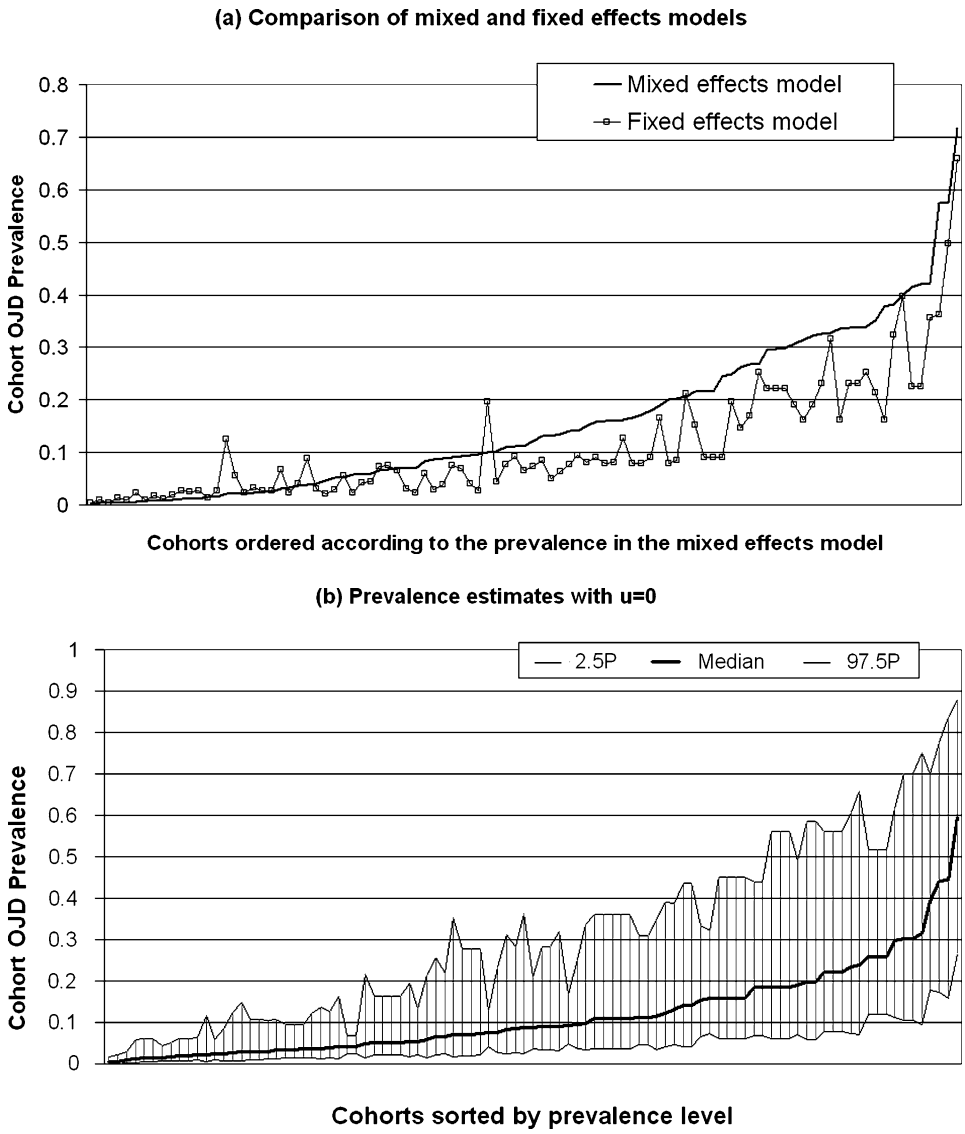

Figure 3. Posterior prevalences for cohorts studied in the OJD risk factor study conducted in 2004–2005 in Australia. (a) Comparison of prevalences based on the random effects model (model (2.4)) and the fixed effects model; (b) Posterior median and 95% probability intervals by keeping $u = 0$.

3.3. ESTIMATES OF OJD PREVALENCE FOR INDIVIDUAL COHORTS

Posterior median prevalence estimates for 97 individual cohorts ranged from 0.002 to 0.72 with an overall average of 0.16. Their comparison with the estimates obtained from the model using only fixed effects is presented in Figure 3a. Both models resulted in estimates that were almost identical for lower prevalence levels, but differences were noticeable at higher levels.

Next, we estimated average OJD prevalences for fixed age by sex combinations, where average corresponds to averaging the prevalences across all data cohorts. Posterior distributions of these average prevalences indicate that the prevalences for wether cohorts were higher than for ewe cohorts, but there were no appreciable differences in estimated prevalences between the age groups: 3-year-old wethers 0.27 (0.15, 0.45); 3-year-old ewes 0.16 (0.09, 0.28); 4-year-old wethers 0.25 (0.11, 0.48); 4-year-old ewes 0.17 (0.08, 0.35).

3.4. INFERENCES FOR OJD PREVALENCE FOR "TYPICAL" FLOCKS WITH GIVEN COVARIATE COMBINATIONS

In order to make inferences beyond the current cohorts/flocks (since flocks outside the data even with the same covariates will necessarily be different due to different u's), we consider prevalences for "typical" flocks, that is, flocks that are just like the ones we have in the data, except with the corresponding u's set to zero. In this way, it is meaningful to obtain probability intervals for such flocks (see Figure 3b). Note that the intervals were wider as the prevalence estimate was increased.

Of even greater interest are inferences for "typical" flocks as we take account of particular covariate combinations. Consider Table 4 where we considered all possible combinations of age, sex and mortality, with other fixed covariates set to zero. Consideration of this table makes it possible to see the practical import of the effect of sex for example, by simply comparing estimated prevalences between sex groups with other variables held fixed (similarly, for age and mortality). Results in Table 4 indicate that the prevalence was higher in flocks with high mortality in all combinations of age and sex. Further, the trends in prevalence observed in ewes and whethers were also evident even after stratifying by current OJD mortality.

3.5. SENSITIVITY ANALYSIS

A sensitivity analysis was conducted by varying prior specifications for PFC sensitivity and cohort OJD prevalence. The modal and the appropriate upper or lower values of priors in Table 1 were increased by 20% of the corresponding values given in Table 1 to construct Prior I (and reduced by 20% to construct Prior II). Results illustrated in Figure 4a, b indicate that the estimated cohort OJD prevalence estimates moderately increased with a decrease in the modal and the lower values of sensitivity priors, and with increase in the modal and the upper values of prevalence priors. There was a greater variability in the estimates at a higher estimated prevalence than at a lower level.

Similarly, the estimates did not change after increasing the number of iterations to 200,000 (and discarding first 20,000 iterations), suggesting that the model and analysis were appropriate.

3.6. MODEL CHECK

The values for ppv for our Pearson and Deviance measures using the GMS predictive model checking method are 0.59 and 0.43 respectively. We are thus not at all tempted to consider revising our model.

Table 4. Inferences for OJD prevalence for "typical" flocks with given covariate combinations obtained by setting $u = 0$. Other covariate values are set to reference values.

Sex	Age	Level of Mortality	Median	(95% PI)
Wethers				
	3-year-old			
		Nil	0.11	(0.05, 0.24)
		Low (<2%)	0.22	(0.08, 0.53)
		High (≥2%)	0.50	(0.19, 0.84)
	4-year-old			
		Nil	0.07	(0.04, 0.13)
		Low (<2%)	0.16	(0.07, 0.32)
		High (≥2%)	0.39	(0.18, 0.70)
Ewes				
	3-year-old			
		Nil	0.06	(0.03, 0.12)
		Low (<2%)	0.13	(0.05, 0.31)
		High (≥2%)	0.35	(0.14, 0.69)
	4-year-old			
		Nil	0.04	(0.02, 0.07)
		Low (<2%)	0.09	(0.05, 0.17)
		High (≥2%)	0.26	(0.12, 0.52)

4. DISCUSSION

Animal-level OJD prevalence was successfully estimated using the Bayesian model. As far as we are aware, this is the first method of its kind that can adjust the prevalences for both variable pool size and imperfect test sensitivity.

Seven methods for prevalence estimation were reviewed by Cowling, Gardner, and Johnson (1999). All these methods assume uniform pool sizes, which is not realistic, at least for OJD studies, due to logistics of sample collection and budgetary constraints. Moreover, some of these methods assume a perfect test or known sensitivity and specificity. In addition, many of them are based on large sample theory and could have negative lower confidence limits for low prevalence flocks, while others cannot compute estimates when prevalence is high. Of the currently available methods, only that of Williams and Moffitt (2001) accounts for variable pool size but it assumes perfect sensitivity and thus will result in biased estimates of true prevalence with intervals that are too narrow. The Bayesian method considered here, on the other hand, assumes an imperfect test, models uncertainty about test characteristics and results in findings that obey probability laws, and does not rely on large sample approximations for its validity.

However, this method requires elicitation of appropriate prior information. We took several steps to obtain priors that reflected available information in the literature so as to reduce the subjectivity inherent in them. First, we carefully developed sensitivity priors based on analysis of previously published data. Second, we increased the width of prevalence priors where our expert was not certain about the estimates. Third, we used prior information for both prevalence and sensitivity to induce priors on model parameters. All

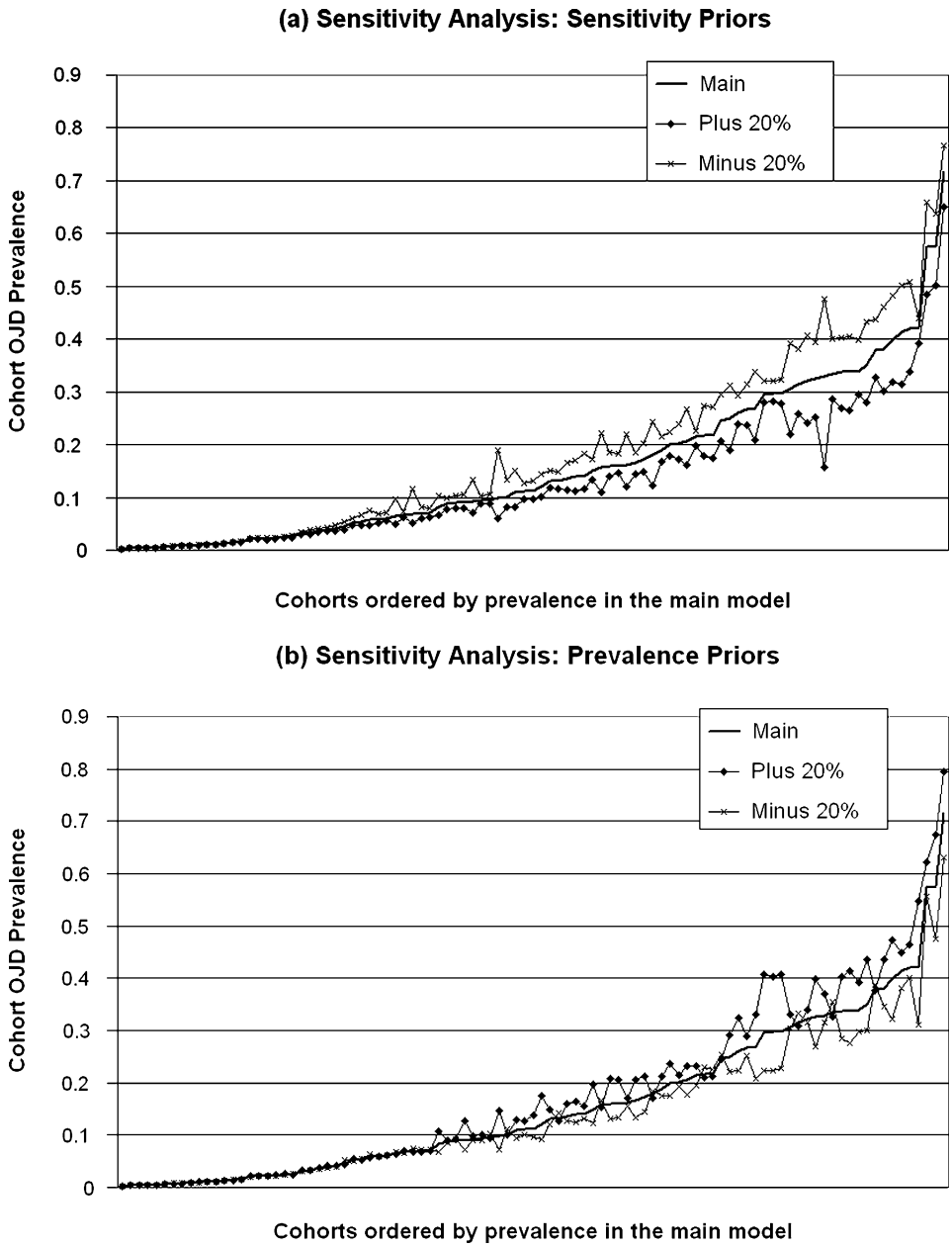

Figure 4. Sensitivity analyses to investigate the effect of changing sensitivity and prevalence priors on posterior cohort OJD prevalence. (a) Influence of changing sensitivity priors; (b) Influence of changing prevalence priors.

this would have improved the appropriateness of our priors. Comparison of the prior and posterior distributions for sensitivity (Figure 2b) indicated that the priors were not obviously unreasonable. Probability intervals of sensitivity posteriors were narrower for pools around size 30 probably due to this corresponding to the "center" of the data.

In contrast, PFC specificity was considered to be nearly perfect because the identity of MAP organisms was confirmed using accepted taxonomic criteria. The test can only be false positive if there is a cross-contamination between samples during pooling or culturing, or misidentification of samples in the field or lab, which although possible, was not likely in the OJD risk factor study. In this study, the sample collector changed gloves after collecting every pool. Further, the pellets from all sheep in a pool were collected directly in a box in the field and thus were not pooled in the lab, reducing the possibility of cross-contamination. Also, due to inclusion of negative controls and other quality control procedures, the possibility of false positive results is negligible. This confidence was reflected in the highly concentrated specificity prior, which, as expected, was very influential in determining the posterior specificity. Other workers have also used concentrated specificity priors for similar tests (Tavornpanich et al. 2004).

We tested several variants of the model to check the importance of random effects and other covariates. Exclusion of the interaction terms did not have a noticeable influence on any inferences except for the relationship between response as a function of stocking rate and mortality, where the effect of the combination of high stocking rate and non-nil mortality is increased over what it would be if the interaction terms were left out. As an alternative to our presentation, we could have analyzed the more parsimonious model (model (2.4) without interactions) and then simply mentioned the possible effect of the interaction. We did not favor deletion of age because it was considered to be a confounder. Based on all evidence, we believe that our final mixed effects model represents the data well.

It was interesting to note that model (2.4) had a noticeably higher DIC value than the "reduced" model with just Current OJD mortality, Cohort age and Cohort sex. To investigate this further, we considered a sequence of nested models by adding terms to the latter model, building up to model (2.4). We first added stocking rate and then the interaction between it and OJD mortality. The DIC values for both of these models were slightly smaller than for the reduced model. Next, we added the vaccination terms, resulting in a DIC that was 5 larger, but where the PPNAs were 0.99 and 0.94, respectively, and where posterior probabilities of negative or positive coefficients for all the other terms remained high. Similarly, after evaluating DIC values and posterior probabilities estimated by adding the remaining terms to the model we conclude that model (2.4) is the most appropriate given our biological and statistical considerations.

Since we included an interaction in the model between stocking rate and mortality, their effects must be discussed together. From Table 2 it is seen that stocking rate and mortality are positively associated with prevalence and that there is a combined effect of high stocking rate and non-nil mortality (since all coefficients including those for interaction are positive), as mentioned in the previous paragraph. OJD prevalence was higher in both low and high mortality flocks, was higher for flocks with a high stocking rate, and was even higher when the two effects were combined (PPPAs of 0.986, 1.000, 0.969 for main effects, and 0.870, 0.744 for interactions). However, interestingly, high stocking rate had a greater influence in increasing prevalence in flocks with $<2\%$ mortalities than flocks with $\geq 2\%$ mortalities.

The effect of lambing paddock stocking could be due to poor nutrition among overstocked dams resulting in nutritional stress in lambs, thereby making them more susceptible to infectious diseases. Poor dam nutrition could also increase MAP fecal shedding, thus increasing MAP pasture and environmental contamination, consequently increasing the availability of MAP to susceptible lambs. Lambs, known to be highly susceptible to infection, are thus exposed to high doses of MAP, increasing the probability of progression to clinal stage.

In contrast to the effect of stocking rate, the flocks that had vaccinated sheep against OJD for more than 2 years (mainly 3–4 years) had lower cohort OJD prevalence compared to flocks that had vaccinated for only 1–2 years (PPNA = 0.982). This may indicate the reduction in MAP contamination across farms that have several age groups of vaccinated sheep who are shedding fewer organisms. In contrast, the OJD level in some flocks not vaccinating at all was also low (PPNA = 0.950). This could be due to the owners/managers of these flocks not observing high OJD mortality and hence deciding not to vaccinate their sheep.

A history of applying less common fertilizers (such as bio-soil, pasture gold, organic manure, reactive phosphorus rock, mono-ammonium phosphate, di-ammonium phosphate, sewage ash, super-potash and pasture special) on the farm was associated with lower cohort OJD (PPNA = 0.992). Most of these fertilizers are associated with cropping and may indicate a spelling effect due to paddocks being cropped or cultivated before stocking sheep. This may reduce the pasture contamination level. The 'presence of wildlife other than kangaroos and rabbits on the farm' was protective but we are not sure about the reasons of such association. The finding of higher OJD prevalence in wethers than in ewes (PPPA = 0.972) could be due to differences in management between wethers and ewes. All these variables had a similar direction of association in the ordinal logistic regression model for three levels of OJD prevalence (<2, 2–10 and >10%) (Dhand et al. 2007), imparting a greater confidence to our previous results as well as to this model.

Robustness of our model was apparent from the sensitivity analyses as there were only minimal changes in the posterior estimates after changing the sensitivity and prevalence priors even by large proportions. Greater variation (and wider prevalence probability intervals) at a higher prevalence level could be due to uncertainty resulting from a majority of the pools testing positive. Further evaluation of this Bayesian approach could in theory be done by comparing the results with culture of all individual samples constituting a pool. However, such results were not available in the OJD risk factor study because individual fecal samples were not collected.

This method can be easily extended to other diseases or conditions where pooled samples are collected. Similar sensitivity and prevalence priors can be constructed based on the previous literature and/or expert opinion.

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SUPPLEMENTAL MATERIALS

WinBUGS code: WinBUGS code to perform the analyses described in the article.

APPENDIX: WINBUGS CODE

```
# Estimation of probability of positive pool:
# nn is the num of distinct cohort/pool size combinations
# x[i] is the number of positive pools out of n[i], of size k[i]

model{
for ( i in 1:nn )
                {x[i] ~ dbin(p[i],n[i])
      temp[i] <- pow(1-pi[i], k[i])
      p[i] <- (1-temp[i])*se[k[i]]+( temp[i])*(1-sp)

# First few rows of data copied below to help you understand the model.
# The first cohort (c) is made up of 1 pool (n) of size 12 (k)
# and 6 pools of size 30. Similarly, the second cohort is made up
# of 1 pool each of size 15 and 20 and 5 pools of size 30.

# c[] k[] n[] x[] a[] s[] m1[] m2[] sr[] v2[] v3[] f[] w[]
#     1       12  1       0         0         0 1 0 1 0 0 1 0
#     1       30  6       3         0         0 1 0 1 0 0 1 0
#     2       15  1       0         0     0   1 0 0 0 0 0 1
#     2       20  1       0         0         0 1 0 0 0 0 0 1
#     2       30  5       3         0         0 1 0 0 0 0 0 1

# a and s are, respectively, the age and sex groups of the cohort
# m1 and m2 are two dummy variables for the current OJD mortality
# v2 and v3 are two dummy variables for the number of years since
# commencement of OJD vaccination in the flock
# f: use of fertilizers other than superphosphate and lime
# w: the presence of wildlife other than kangaroos and rabbits
# Please refer to Section 2.3 for further details.

# Random effects model for animal-level prevalence:
logit(pi[i])<- g[1]+ g[2]*s[i]+ g[3]*a[i] + g[4]*m1[i]
                                          + g[5]*m2[i]+ g[6]*sr[i] + g[7]*v2[i]
                                          + g[8]*v3[i]+ g[9]*f[i] + g[10]*w[i]
                                          + g[11]*m1[i]*sr[i]+ g[12]*m2[i]*sr[i]
                                          + U[flock[i]]

# g1 to g12 are unknown regression parameters. See Section 2.3
# for details about the covariates in the model.

# Prevalences without random effects
logit(prev[i])<- g[1]+ g[2]*s[i]+ g[3]*a[i] + g[4]*m2[i]
                                      + g[5]*m3[i]+ g[6]*sr[i] + g[7]*v2[i]
                                      + g[8]*v3[i]+ g[9]*f[i] + g[10]*w[i]
                                      + g[11]*m1[i]*sr[i]+g[12]*m2[i]*sr[i]
                                          }

# Distribution of the random effects, U;
        for(i in 1: nflocks)
                                       { U[i] ~ dnorm(0,tau)}

# Average Prevalence in cohorts
```

```
for (i in 1:ncohorts){
        for (j in 1:nn){ v[i,j] <- equals(cohort[j],i)
               vv[i,j] <- v[i,j]*pi[j] }
        cprev[i] <- sum(vv[i,])/sum(v[i,])
                }
# Similarly average Prevalence was calculated without
# random effects (code not shown)

# Estimated probabilities of infection for all possible
# combinations of sex, age and mortality with all
# other covariates set to 0
for(i in 1:2){
     for (j in 1:2){
for (r in 1:3){
logit(pprev[i,j,r]) <- g[1] + g[2]*equals(i,1) + g[3]*equals(j,1)
+ g[4]*equals(r,1) + g[5]*equals(r,2)}}}

# Calculation of prevalence for age and sex cohorts:
# Note: Only calculations of prevalence for 3-year-old wethers is shown here
        for(i in 1:nn)
                        { pymtemp[i] <- pi[i]*ym[i]
                                        ym[i] <- s[i]*(1-a[i]) }
                        pym <- sum(pymtemp[])/sum(ym[])

# Priors:
# Priors for sensitivity:
  for(l in 1:50){
                logit(se[l])<- b[1] + b[2]*(l-22.75)/21.84} #standardization of pool size

# Induced priors for beta's: based on analysis of Whittington et al. (2000) data
        se1 ~ dbeta(10.2,4.2) # k= 10; Median = 0.74 and 5p = 0.50
        se2 ~ dbeta(10.9,7.6) # k= 50; Median = 0.60 and 5p = 0.40
        ls1<- logit(se1)
        ls2 <- logit(se2)
        b[1] <- .68125*ls1 + .31875*ls2
        b[2] <- -.546*ls1 + .546*ls2
        prob[1] <- step(b[1])
        prob[2] <- step(b[2])

# Prior for specificity
                        sp ~ dbeta(asp,bsp)

# Prevalence priors (elicited from Prof. Richard Whittington)
# Nil Mortality flocks
        pi1 ~ dbeta(8.77,148.70)# Mode=0.05, 90P =0.08; 3-year-old ewes
        pi2 ~ dbeta(4.57,68.74) # Mode=0.05, 90P =0.10; 3-year-old wethers
        pi3 ~ dbeta(4.57,68.74) # Mode=0.05, 90P =0.10; 4-year-old ewes
# Low Mortality flocks
        pi4 ~ dbeta(3.78,37.95)# Mode=0.07, 90p =0.15; 3-year-old ewes
# High Mortality flocks
        pi5 ~ dbeta(2.68,4.12)# Mode=0.35, 95p =0.7; 3-year-old ewes

# Induced priors for g1 to g5
        lp1 <- logit(pi1)
        lp2 <- logit(pi2)
        lp3 <- logit(pi3)
        lp4 <- logit(pi4)
        lp5 <- logit(pi5)
  g[1] <- 1*lp1 + 0*lp2 +0*lp3
        g[2] <- -1*lp1 + 1*lp2 +0*lp3
        g[3] <- -1*lp1 + 0*lp2 +1*lp3
        g[4] <- -1*lp1 + 0*lp2 +0*lp3 + lp4
        g[5] <- -1*lp1 + 0*lp2 +1*lp3 + lp5
```

```
# Flat priors for g6 to g12 (normally distributed)
        for (i in 6:12) {g[i] ~ dnorm(0,1)}

# Prior for u's (uniformally distributed)
        tau <- 1/pow (sigma, 2)
        sigma ~ dunif (.01, 3)

# Posterior probabilities that g's are positive
   probg[1] <- step(g[1])
   probg[2] <- step(g[2]) # Similar for all g's up to g[9]
}
```

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