Comparison of pre- and post-vaccination ovine Johne's disease prevalence using a Bayesian approach

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Abstract

This study was conducted to evaluate the effectiveness of Gudair™ vaccine in decreasing the prevalence of shedding of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in flocks of varying initial prevalence. Thirty seven self-replacing Merino flocks from New South Wales and Victoria (Australia) that had been vaccinating lambs with Gudair™ for at least five years were enrolled in the study. These flocks had been tested prior to or at commencement of vaccination using pooled faecal culture, agar gel immunodiffusion or both tests. These pre-vaccination test results were used to estimate pre-vaccination prevalence. Post-vaccination prevalence was estimated from culture of usually 7 pools of 50 sheep collected from the enrolled flocks in 2008-2009, approximately five or more years after commencement of vaccination.

A Bayesian model was developed to estimate and compare the pre- and post-vaccination prevalences for the enrolled flocks. Apparent pre- and post-vaccination prevalences for flocks were modelled as functions of the true pre- and post-vaccination prevalences, respectively, and the sensitivities and specificities of the respective diagnostic tests. Logit-normal models were specified on pre- and post-vaccination true prevalences and were then used to make inferences about the median and 90th percentile of the prevalence

distributions and their differences. Priors were mostly specified based on published literature or analysis of abattoir surveillance data for this population of flocks.

The analysis found a significant decline in ovine Johne's disease prevalence from a pre-vaccination median prevalence of 2.72% [95% probability interval (PI): 1.40; 6.86%] to a post-vaccination median prevalence of 0.72% (0.39; 1.27%). However 30 of the 37 flocks still contained sheep that were shedding MAP in their faeces. The results suggest that vaccination with Gudair™ is usually effective in reducing the prevalence of faecal shedding but the response to vaccination is variable among flocks. This approach could be implemented in similar situations to compare prevalences where information from multiple diagnostic tests with varied sensitivities and specificities is available.
Keywords: Ovine Johne's disease; Gudair; Vaccination; Abattoir surveillance; Faecal culture; Agar gel immune-diffusion test.

1. Introduction

Ovine Johne's disease (OJD), a chronic disease of sheep caused by *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*), results in substantial economic losses due to reduced production, increased mortalities and the costs associated with the disease control programmes (Bush *et al*., 2006). The infection usually enters a flock by introduction of infected sheep and then spreads within the flock mainly by the faecal-oral route. Clinically infected sheep can shed up to 10^8 *MAP* per gram of their faeces, thus contaminating the pastures (Whittington et al., 2000b). Susceptible sheep, especially lambs, are infected by ingesting *MAP* organisms whilst grazing contaminated pastures. Although many disease control programmes have been implemented in Australia, vaccination has become the major intervention since the registration of Gudair™ vaccine in April 2002.

There are mixed reports of the effectiveness of Gudair™ vaccination. While it is considered to be very effective in controlling mortalities, several studies in low numbers of flocks have shown that *MAP* shedding continues to occur in vaccinated sheep (Eppleston *et al*., 2005; Reddacliff *et al*., 2006; Windsor, 2006). The persistence of shedding for an extended period following the onset of a vaccination programme presents a risk for spread to other flocks. In addition, there is risk of recrudescence of OJD in vaccinating flocks after cessation of vaccination (Eppleston et al*,* 2011).

This study aimed to assess the long term effect of vaccination on the persistence of shedding by vaccinated sheep, and involved a range of flocks with varying initial prevalence. It was considered timely to estimate the decline in OJD prevalence in commercial flocks that had been vaccinating for five or more years. Although data were available for these flocks on the level of infection at the start of vaccination, these flocks had been tested by either serological or faecal culture tests and in some cases by both tests. This presented difficulties in estimating pre-vaccination prevalences and comparing them to the post-vaccination estimates using frequentist statistical approaches. Therefore, we developed a cohesive Bayesian model to make objective inferences about the differences in prevalence distributions for pre- and post-vaccination flocks.

2. Methods

2.1. Selection of sheep flocks

The reference population for the study was the OJD infected sheep flocks in New South Wales (NSW) and Victoria with a range of prevalence levels. The study population included flocks that met the following selection criteria: (1) high level of willingness to participate in the study and managerial ability of the flock owner; (2) self-replacing Merino flock lambing >500 ewes per year; and (3) OJD positive diagnosis with a continuous vaccination

programme that commenced with lambs in 2002 or earlier, with the inclusion of flocks commencing vaccination in 2003 if required, i.e. five or more years prior to the conduct of this study.

Potential flocks were identified from official data including disease surveillance and laboratory testing information held in the databases at the University of Sydney, the NSW Livestock Health and Pest Authorities (formerly the Rural Land Protection Boards), the NSW and Victorian Departments of Primary Industries. Farm owners or managers were then contacted to determine whether they met the selection criteria and to obtain their consent for participation. Forty-one flocks from NSW and Victoria were enrolled in 2008-2009 to take part in the study. However, four flocks had to be excluded from the analyses reported in this paper because subsequent to sampling it was discovered that they did not meet the selection criteria of being self-replacing Merino flocks or had not vaccinated lambs before 6 months of age.

2.2. Pre-vaccination OJD prevalence data

Majority of flocks had commenced vaccination in 2002 following registration of the vaccine in April of that year. Pre-vaccination prevalence data were obtained from farmers and regulatory agencies by examining property disease records prior to 2002. The diagnostic tests used for estimation of pre-vaccination prevalence levels varied across flocks: (a) Agar gel immune diffusion (AGID) test was used for 7 flocks, (b) pooled faecal culture (PFC) for 20 flocks, and (c) both AGID and PFC for 10 flocks.

2.3. Post-vaccination OJD prevalence data

2.3.1. Faecal sampling

Post-vaccination testing in all of the selected 37 flocks was done using only PFC. Details of the methods have been provided elsewhere (Windsor et al., 2011). Briefly, we aimed to select 7 pools of 50 sheep from each enrolled flock (a cohort of sheep) but the pool numbers and sizes collected from some flocks varied due to logistic issues. Faecal sample collection was performed by the local District Veterinarians and involved collecting one faecal pellet per rectum from each selected sheep. Pellets collected from 50 sheep were pooled in a sterile plastic container and constituted one pool. Note that we tested flocks only post-vaccination but utilised pre-vaccination testing records available with the farmer or animal health authorities. Also note that the sheep sampled post-vaccination were not the same as those tested pre-vaccination.

2.3.2. Pooled faecal culture (PFC)

Pooled faecal samples were cultured using a modified BACTEC radiometric method (Whittington et al., 2000a). Culture positive samples were further confirmed by polymerase chain reaction and restriction endonuclease analysis by demonstrating the presence of IS*900* (Cousins et al., 1995; Whittington et al., 1998). The OJD prevalence of each cohort was calculated from PFC results using a Bayesian model for variable pool size (Dhand et al., 2010).

2.4. Bayesian analyses

We developed a Bayesian approach to obtain true pre-vaccination prevalence estimates after adjusting for sensitivities and specificities of diagnostic tests. These prevalence estimates were then compared to the true post-vaccination prevalence estimates. A schema of the Bayesian approach taken is presented in Fig. 1.

2.4.1. Modelling apparent prevalences

2.4.1.1. Pre-vaccination

The pre-vaccination prevalences were estimated based on three types of test data since flocks were tested by AGID alone, PFC alone or both AGID and PFC. In each case, we modelled either a single apparent prevalence or two apparent prevalences, as functions of

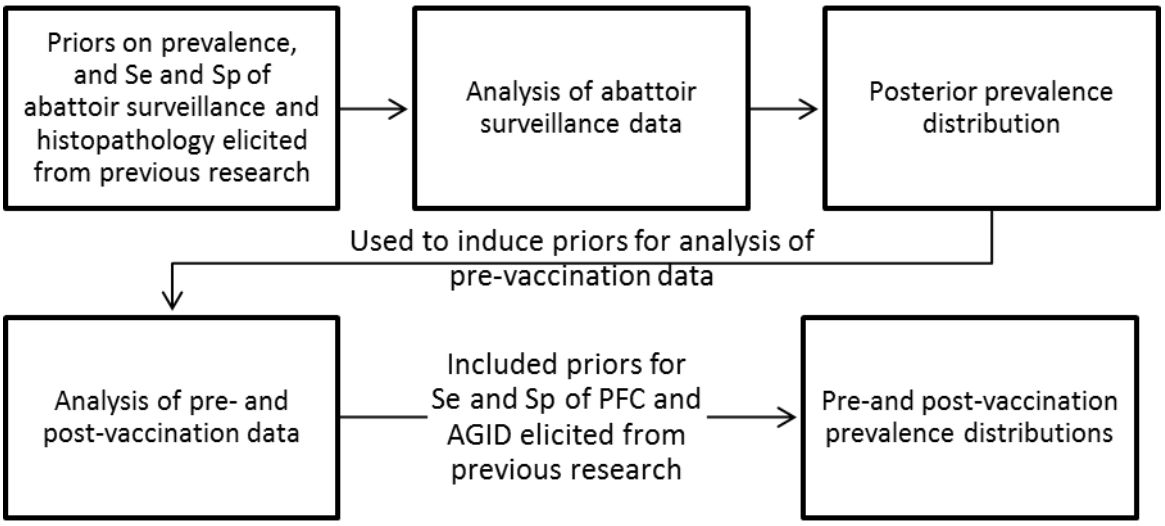

Figure 1. A schema of the models developed for analysis of pre- and post-vaccination data. Se: sensitivity; Sp: specificity; PFC: pooled faecal culture; AGID: agar gel immunodiffusion test

the true prevalences, the sensitivities and specificities of the particular tests, all using a Bayesian adaption of the Rogan and Gladen (1978) approach. Note that for flocks tested using both tests the model determined one true prevalence estimate per flock, although different apparent prevalences were estimated based on AGID and PFC results.

The number of positive animals (or pools) for a flock i (Y_i) was assumed to be binomially distributed (Eq. (1)) as a function of apparent pre-vaccination OJD prevalence of the flock (P_i) and the total number of samples (or pools) tested (N_i). The apparent prevalence was related to the true animal-level pre-vaccination OJD prevalence for that flock (π_i) through test sensitivity and specificity as described by Rogan and Gladen (1978) for individual samples (Eq. (2)) and by Dhand et al (2010) for pooled samples (Eq. (3)). The approach is diagrammatically presented in Figure 2 using flocks tested with AGID as an example.

$$Y_i \sim dbin(P_i, N_i) \quad \text{... (1)}$$

$$P_i = \pi_i * Se_g + (1 - \pi_i) * (1 - Sp_g) \quad \text{... (2)}$$

$$P_i = \{1 - (1 - \pi_i)^k\} * Se_f + (1 - \pi_i)^k * (1 - Sp_f) \quad \text{... (3)}$$

where k is the pool size, i.e. number of sheep constituting a pool; Se_g and Sp_g are the AGID sensitivity and specificity; and Se_f and Sp_f are the PFC sensitivity and specificity, respectively.

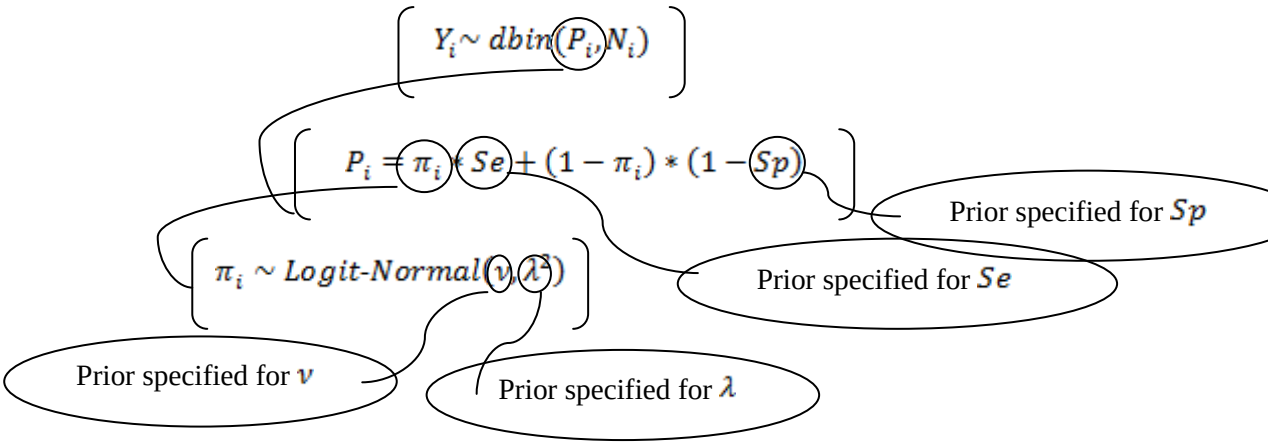

Figure 2. A diagrammatic representation of the code for modelling pre-vaccination prevalence for flocks tested with Agar gel immunodiffusion test (AGID). The number of AGID positive animals for a flock i (Y_i) was assumed to be binomially distributed as a function of apparent pre-vaccination OJD prevalence of the flock (P_i) and the total number of samples tested (N_i). Apparent prevalence for the flock was related to its true prevalence (π_i) through AGID test sensitivity (Se) and specificity (Sp). A logit normal distribution was specified on true prevalences. Priors were specified on the mean (v) and the standard deviation (λ) of the logit normal prevalence distribution and on sensitivity and specificity of AGID test.

2.4.1.2. Post-vaccination

In contrast to pre-vaccination data based on two types of tests, only PFC data were available for post-vaccination animal-level prevalence estimation. The modelling of post-vaccination prevalence data follows a similar procedure, except that the pool sizes were variable rather than fixed at k for the post-vaccination PFC data. Therefore, k was replaced by the actual pool size for each of the individual pools as in Dhand et al. (2010). Thus, if pool j in flock i is of size k_{ij}, then the post-vaccination apparent pool prevalence (PP_{ij}) is:

$$PP_{ij} = \left\{1 - (1 - p\pi_i)^{k_{ij}}\right\} * Se_f + (1 - p\pi_i)^{k_{ij}} * \left(1 - Sp_f\right) \quad \text{... (4)}$$

where $p\pi_i$ is the true post-vaccination prevalence in flock i.

Pre-vaccination and post-vaccination true prevalences (π_i and $p\pi_i$, respectively) were modelled using logit-normal (LN) distributions (see Section 2.4.2) while priors were later specified on test sensitivities and specificities (see Section 2.4.4.1).

2.4.2. Modelling true prevalences and obtaining inferences

2.4.2.1. Pre-vaccination

Pre-vaccination true prevalences (π_i) were modelled as independent and sampled from a LN distribution:

$$logit(\pi_i) = v + v_i, where\ v_i \sim N(0, \lambda^2), \quad \text{... (5)}$$

which implies that:

$$\pi_i = \frac{exp(v + v_i)}{1 + exp(v + v_i)} \quad \text{... (6)}$$

Here ν is the mean and v_i a random effect corresponding to flock *i*; λ is the standard deviation of the prevalence distribution on the logit scale in the selected flocks. Priors were later induced for ν and λ based on analysis of abattoir surveillance data (see Section 2.4.4.2).

The median of the prevalences among flocks of the same type (μ_{pre}) is simply the formula in Eq. (6) with the random effect set to be zero. The 90th percentile of pre-vaccination prevalences ($\mu_{pre,0.90}$) is the above expression (Eq. (6)) with the random effect replaced by $1.28\,\lambda$. Note that the 90th percentiles are the values for which 90% of all prevalences will be smaller, and 10% larger.

$$\mu_{pre} = \frac{e^{\nu}}{1+e^{\nu}} \quad \text{... (7)}$$

$$\mu_{pre,0.90} = \frac{e^{\nu+1.28\lambda}}{1+e^{\nu+1.28\lambda}} \quad \text{... (8)}$$

2.4.2.2. Post-vaccination

A similar approach as pre-vaccination prevalence was used to model post-vaccination prevalences (Eqs. (9) and (10)).

$$logit(p\pi_i) = \nu + \Delta + w_i \quad \text{... (9)}$$

$$p\pi_i = \frac{\exp(\nu+\Delta+w_i)}{1+\exp(\nu+\Delta+w_i)} \quad \text{... (10)}$$

where w_i is a random effect for flock i and is modelled with a $N(0,\sigma^2)$ distribution. Inferences for the median prevalence and the 90th percentile of post vaccination prevalences were similarly obtained as shown below in Eq. (11) and (12):

$$\mu_{post} = \frac{e^{\nu+\Delta}}{1+e^{\nu+\Delta}} \quad \text{... (11)}$$

$$\mu_{post,0.90} = \frac{e^{\nu+\Delta+1.28*\sigma}}{1+e^{\nu+\Delta+1.28*\sigma}} \quad \text{... (12)}$$

Priors were later specified on σ and Δ (see Section 2.4.4.3).

2.4.3. Model variations

The model discussed above (called Model I from here onwards) assumes statistical independence of pre- and post-vaccination prevalences, and allows for both different medians and different levels of spread. We considered two variants of this model: (a) where $w_i = v_i$, meaning that the median prevalence was allowed to change, but that variability in prevalences was assumed to be similar for pre- and post-vaccination (labelled Model II) and (b) where (w_i, v_i) was modelled with a bivariate normal distribution, which would allow for non-zero correlation between pre and post-vaccination prevalences (labelled Model III). Model II is a simplification of Model I and is analogous to having a random effect for each flock since v_i is shared by flock i for pre and post-vaccination. Model III is a generalisation of our assumed model. We compared all three models by calculating the Deviance Information Criterion (DIC) as suggested by Spiegelhalter et al. (2002).

2.4.4. Elicitation of priors

We required priors for sensitivity and specificity for both the tests; for ν and λ for the pre-vaccination true-prevalence model; and for σ and Δ for the post-vaccination prevalence model.

2.4.4.1. Sensitivity and specificity priors

Beta priors for sensitivity and specificity of AGID and PFC tests were elicited based on previous research (Sergeant et al., 2003; Dhand et al., 2010). Sergeant et al. (2003) estimated average AGID sensitivity to be 24.6% using data from six known infected and 12 assumed uninfected sheep flocks (Table 1). Our concentrated specificity prior reflected a very high specificity for AGID reported in that study.

We used the same priors for sensitivity and specificity of PFC as used in Dhand et al. (2010) based on analysis of Whittington et al. (2000a) data (Table 1). See Dhand et al. (2010) for further details.

2.4.4.2. Priors on ν and λ for the pre-vaccination true-prevalence model

Abattoir surveillance data from the same population of flocks as our data were available. We used these data to obtain prior information for the pre-vaccination prevalence distribution (See Appendix A for details about analysis of abattoir surveillance data). Results of surveillance data indicated that our best guess of the median of the Logit prevalence distribution of the flocks was 0.08 (μ_0) and that we were 95% sure that the median prevalence was less than 0.3 (u). We consider a Normal prior for ν:

$$\nu \sim N(a, b^2),$$

where a is the mean and b is the standard deviation.

The mean, *a,* was calculated to be -2.46 [=$logit(0.08)$] as our best guess for the median prevalence based on analysis of abattoir surveillance data.

The standard deviation, *b*, was calculated to be 0.97, assuming the 95th percentile of the prior be 0.30:

$$b = \frac{[logit(u) - logit(\mu_0)]}{1.645} = \frac{[logit(0.30) - logit(0.08)]}{1.645} = 0.97$$

Similarly, we used information about the 90th percentile of prevalences, namely, the prevalence for which 90% of the prevalences were smaller and 10% larger, in order to induce a uniform prior (0, 3) for λ. It was determined that this value could not be larger than 0.8, with virtual certainty. The value of 3 was obtained as:

$$\frac{[logit(max) - logit\ (\mu_0)]}{1.28}$$

where max is the value such that we are virtually 100% certain that this value cannot exceed (assumed to be 0.80), given our prior guess for μ_0. The ideas behind this specification can be found in Christenson et al (2010); see Appendix B for the details.

2.4.4.3. Priors for σ and Δ for the post-vaccination true-prevalence model

A diffuse uniform distribution prior (–6, 6) was specified for Δ in the absence of any prior information. This prior allows for a broad range of differences in pre and post vaccination prevalence distributions, without attaching too much mass to the possibility that they are radically distinct.

Table 1. Priors for sensitivities and specificities for agar gel immunodiffusion test and pooled faecal culture test used in the study.

Priors	**Input values**		**Prior distributions**[2]				**Prior distributions**[2] **for sensitivity analyses**				Source of priors
	Mode	Lower/ Upper[1]	*a*	*b*	Mean	(95% PI)	Mode	Lower/ Upper[1]	*a*	*b*	
Agar gel immuno-diffusion test (AGID)											
Sensitivity[3] (Se_g)	0.25	0.60	2.43	5.30	0.31	(0.06, 0.65)	0.30	0.72	1.98	3.30	Sergeant et al. (2003)
Specificity (Sp_g)	0.995	0.99	1137.5	6.71	0.99	(0.99, 1.00)	-	-	-	-	Sergeant et al. (2003)
Pooled faecal culture test (PFC)											
Sensitivity[4] (Se_f)	0.60	0.40	10.9	7.6	0.59	(0.36, 0.80)	0.48	0.32	11.89	12.80	Dhand et al (2010)
Specificity (Sp_f)	0.995	0.99	1137.5	6.71	0.99	(0.99, 1.00)	-	-	-	-	Dhand et al (2010)

[1]Lower 5% limits were elicited for sensitivity and specificity estimates; [2]*a* and *b* are parameters of the respective beta probability distributions. [3] AGID sensitivity estimates (Se_g) were increased by 20% and [4]PFC sensitivity estimates (Se_f) was reduced by 20% for sensitivity analyses. AGID and PFC specificity estimates (Sp_g and Sp_f respectively) were not changed.

Similarly, a relatively diffuse uniform distribution prior (0, 3) was specified for σ. This prior places the mild restriction that we are virtually certain that the 90th percentile is less than 0.84, which is quite conservative.

2.4.5. Implementation

The models were implemented in WinBUGS (Lunn et al., 2000). The WinBUGS code is available for download as a Supplementary material (Supplementary 1). Convergence was checked by monitoring histories and running quintiles based on distinct initial values. All models were run for 50,000 iterations for each of the two chains with distinct starting values; the initial 5000 iterations were discarded

2.4.6. Sensitivity analyses

A standard sensitivity analysis involves modification of the prior distributions and re-analysis to determine the effect of the prior on inferences. We thus modified our priors on the sensitivities and specificities of the AGID and PFC tests to determine their effect on prevalence estimates. PFC sensitivity estimates were reduced by 20% while the AGID sensitivity estimates were increased by 20% (Table 1).

Another aspect to our sensitivity analysis involves the model for the data itself. Some of the flocks had moderate to large counts of positive post vaccination pools, indicating that some flocks may not have a reduced prevalence. We investigated this by using a modification to the model that allowed for the post vaccination prevalence distribution to be a mixture of two logit-normal distributions rather than a single unimodal distribution (labelled as Model IV) and compared models using the DIC criteria.

3. Results

Data were analysed for 37 flocks after excluding four of the 41 flocks that did not meet the selection criteria. Pre-vaccination prevalence was estimated based on PFC for seven flocks, AGID for 20 flocks and both PFC and AGID for 10 flocks while post-vaccination prevalence was estimated using PFC test for all 37 flocks. Details of the samples collected pre- and post-vaccination are presented in Appendix C.

3.1. Pre- and post-vaccination prevalences

Model I had a lower DIC (335.43) than the two other variants tested (DIC for Model II = 369.94, and DIC for Model III = 335.76). Moreover, Model I was simpler so it was preferred over Model III although their DICs were similar. Hence Model I was selected for all results presented in this paper.

Inferences for pre- and post-vaccination prevalences are presented in Table 2 and Figure 3a. The median pre-vaccination prevalence was 2.72% (1.40, 6.86%) which declined to 0.72% (0.39, 1.27%) post-vaccination. The percent decrease in prevalence was estimated to be 73.4% (95% PI: 42.0, 90.2%). The posterior probability that the median pre-vaccination prevalence exceeded the median post-vaccination prevalence was almost one, indicating that we could be virtually certain that the median post-vaccination prevalence was lower than the median pre-vaccination prevalence.

To examine the spread of the prevalence distribution, we estimated 90^{th} percentiles of the pre- and post-vaccination prevalences. The results suggest that 90% of the flocks had prevalences lower than 3.16% after vaccination, whereas the same proportion of flocks had prevalence lower than 16.04% before vaccination. However, 30 of the 37 flocks were still shedding MAP post vaccination.

We also calculated the proportion of flocks with prevalence greater than 2% and 5% pre- and post-vaccination of the 37 flocks. Pre-vaccination, 16 flocks (43.2%) had prevalence greater than 2% while 12 (32.4%) had prevalence greater than 5%. Post-vaccination, however, four flocks (10.8%) had prevalence greater than 2% and one flock (2.7%) had prevalence greater than 5%.

Table 2. Posterior estimates for pre- and post-vaccination prevalences from the primary analyses (Model I) as well as from the sensitivity analyses.

Parameters	Results from the primary analyses (Model I)			Results of sensitivity analysis			
				After increasing prior AGID sensitivity estimates by 20%		After reducing prior PFC sensitivity estimates by 20%	
	Median (%)	95% PI	Probability[1]	Median (%)	95% PI	Median (%)	95% PI
Median Prevalence							
Pre-vaccination	2.72	(1.40, 6.86%)		2.76	(1.40, 7.48%)	3.42	(1.64, 9.61%)
Post-vaccination	0.72	(0.39, 1.27%)		0.73	(0.39, 1.29%)	0.81	(0.42, 1.52%)
Difference	1.99	(0.64, 6.00%)	0.999	2.01	(0.65, 6.64%)	2.59	(0.79, 8.68%)
90th Percentile of prevalence							
Pre-vaccination	16.04	(5.79, 58.33%)		16.54	(5.78, 62.40%)	22.09	(7.19, 71.04%)
Post-vaccination	3.16	(1.59, 10.29%)		3.14	(1.58, 10.49%)	3.85	(1.82, 15.3%)
Difference	12.40	(1.53, 54.01%)	0.987	12.85	(1.66%, 57.81%)	17.44	(1.63, 65.49%)

[1]Posterior probability that the difference is positive; 95% PI: 95% probability interval; AGID: agar gel immunodiffusion test; PFC: pooled faecal culture.

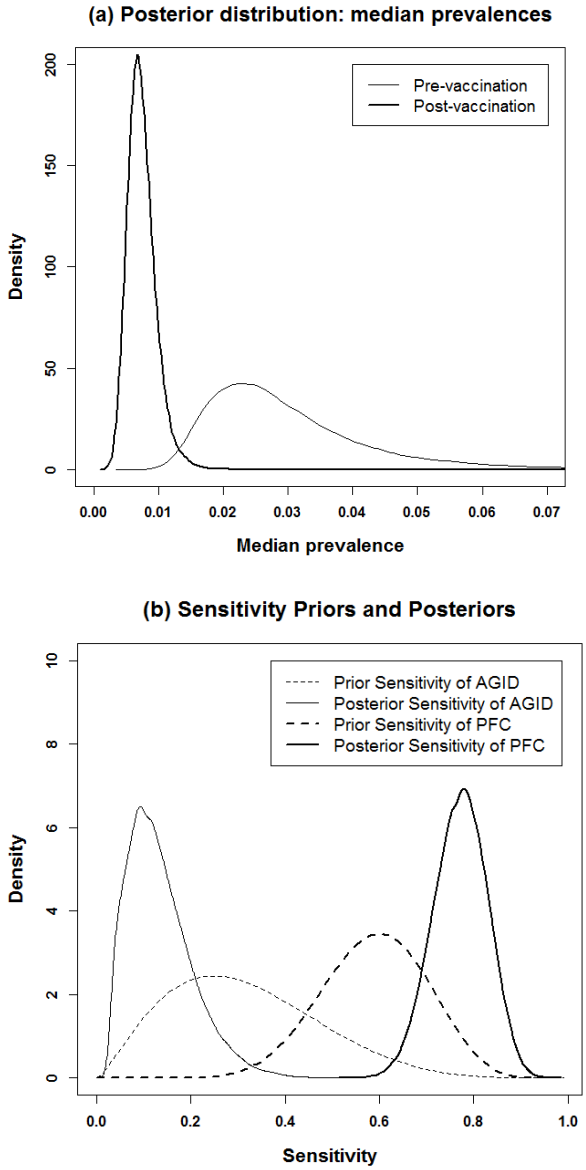

Figure 3. (a) Pre-and post-vaccination median prevalences. (b) Priors and posteriors for agar gel immunodiffusion test (AGID) and pooled faecal culture (PFC) sensitivities used for analysis of pre- and post-vaccination data.

3.2. Sensitivity and specificity estimates

Priors and posteriors for sensitivity of AGID and PFC are shown in Figure 3b. The results suggest that posterior estimate of PFC sensitivity was higher than the inputted prior PFC sensitivity, but posterior estimate of AGID sensitivity was lower than the prior estimate. Posterior estimates of sensitivities and specificities are summarised in Table 3.

3.3. Sensitivity analyses

Sensitivity analyses produced minor to moderate changes in prevalence estimates after modification of prior estimates (Table 2). When AGID sensitivity estimates was increased by 20%, the pre-vaccination and post-vaccination prevalence estimates remained

almost stable but the reduction in prior PFC sensitivity estimates resulted in a greater change.

Table 3. Posterior estimates for sensitivities and specificities of agar gel immunodiffusion test and pooled faecal culture based on Bayesian analyses.

Parameters	Median	95% PI
Agar gel immuno-diffusion test (AGID)		
Sensitivity	0.12	(0.04, 0.30)
Specificity	0.997	(0.995, 0.999)
Pooled faecal culture test (PFC)		
Sensitivity	0.77	(0.66, 0.88)
Specificity	0.994	(0.989, 0.998)

Allowing for the post vaccination prevalence distribution to be a mixture of two logit normal distributions (Model IV) did not improve model fit as the DIC increased to 338.25.

4. Discussion

Following the registration of Gudair™ vaccine in April 2002, OJD control in Australia has largely relied on voluntary vaccination and biosecurity, the latter facilitated by producer awareness of disease risk at trading (Windsor, 2006; Eppleston et al., 2011). This risk-based trading approach is a farmer self-declaration system for OJD risk and involves vendor declaration in a national Sheep Health Statement when sheep are sold, with allocation of Assurance Based Credit (ABC) points for the key risk factors in the spread of OJD (AHA, 2010). Each credit represents an approximate 4-fold decrease in the risk that the sheep are infected, with up to 4 of the 12 ABC points available being allocated for approved vaccination. This programme aims to encourage flock owners to commence vaccination programmes as a precaution to improve their ability to sell re-stocked sheep through the risk based trading ABC scheme. Given the emphasis on vaccination in control of OJD, it is obvious that the industry needs credible evidence for effectiveness of vaccination (Windsor, 2006), particularly over time when losses due to OJD are less apparent in vaccinating infected flocks (Eppleston et al., 2011).

Our study aims to provide information on the decline in OJD prevalence following the introduction of a vaccination strategy in flocks with varying disease prevalence. It is based on a convenient sample of flocks as it was considered sensible to utilise commercial flocks that had been vaccinating for five or more years. A randomised controlled clinical trial – as conducted in the initial evaluation of the vaccine in Australia when mortalities were high (Reddacliff et al., 2006) – would have been an ideal for evaluating effectiveness of a vaccine, but it is difficult to implement in current real-life on-farm situations. It would be almost impossible to ask farmers to agree on not vaccinating sheep for 4-5 years for OJD, particularly, if they have high disease prevalence.

However, this convenience sampling did introduce a number of sources of variation in the study. Firstly, selection of farmers who had high willingness to participate in the study may have caused some selection bias towards flocks that have better management practices. Therefore, the effectiveness of vaccine could be different (lower or higher) in farmers with poorer biosecurity and management. Secondly, variation in prevalence estimates within and between flocks could in part be due to other biosecurity and management factors. These have been investigated based on crude classification of

prevalences and reported elsewhere (Windsor et al., 2011). Further analyses are required to investigate the effect of these factors based on objective prevalence estimates determined in this study. Thirdly, different tests or combination of tests were used pre- and post-vaccination and the tests used different reference units (individual animal for AGID and a pool of faeces for PFC). The differences in use of diagnostic tests partly reflected the emergence of PFC as a test of choice in Australia as it replaced the previously preferred test, the AGID (Reddacliff et al., 2006). This necessitated the use of Bayesian approach which accounted for these uncertainties and allowed us to calculate and objectively compare true prevalence after adjusting for these differences in diagnostic tests. Fourthly, different animals tested pre- and post-vaccination and passage of variable time interval since pre-vaccination testing could also have biased our results. Both of these sources of variation could not be avoided due to selection of convenience sample. It is acknowledged that these could have biased our estimates of median prevalences, despite the estimates being adjusted for variation in diagnostic test performances. However, the estimated differences in median prevalences pre- and post-vaccination should be reflective of what they would have been, if random samples of flocks had been taken.

One of the other perceived limitations of the Bayesian approach is the potential use of subjective prior information which could impact the final results. However, all of our priors were based on previously published research or analysis of available data. We made substantial efforts in eliciting the prevalence prior distribution for the enrolled flocks by analysing abattoir surveillance data for many of the flocks in the study. This enabled improved estimates of the pre- and post-vaccination prevalences in various flocks, contributing to a superior evaluation of the effectiveness of vaccination in reducing prevalence.

We further conducted sensitivity analyses to evaluate the effect of priors and determined that change in PFC prior estimates had a greater influence on the model than changing AGID prior estimates. It suggests that our prevalence estimates could be slightly biased if the PFC sensitivity information is incorrect.

It is expected that there might be correlation between pre and post-vaccination prevalences for the same flocks. Therefore, we considered two models that allowed for this possibility, and one that assumed independence. However, one of these models was found to be considerably inferior to the independence model, and the second was slightly inferior to it according to the DIC (Spiegelhalter et al., 2002). We thus presented the results of only the independent model in this paper (Model I). Although statistically speaking, there was a clear preference for the independence model, yet the inferences were quite similar regardless of whether we used dependence or independence model. A possible explanation for the lack of dependence is the period of time between pre and post vaccination was somewhat large (more than 5 years). It is well known that correlation diminishes with time. For example, milk yield observations of a cow would be highly correlated if taken in a particular week than if taken six months apart. Secondly, completely different animals tested at each farm pre- and post-vaccination could have influenced correlation.

Interestingly, some of the flocks had moderate to large counts of positive pools post-vaccination, indicating that vaccination may not have been effective for those flocks. It suggests that the post-vaccination prevalence distribution may be a mixture of two distributions. Further investigation into this aspect of the data was done by allowing for the post vaccination prevalence distribution to be a mixture of two logit-normal distributions rather than a single unimodal distribution (Model IV). However, this model was not found to be better than the simpler model based on the DIC criterion.

Based on the results of Model I presented here, the median prevalence reduced from a pre-vaccination level of 2.72% to a post-vaccination level of 0.72%, indicating the

effectiveness of the vaccination in reducing MAP shedding. The Bayesian 95% credible interval was (0.64%, 6.0%) meaning that we are 95% sure (after seeing the observed data) that the median prevalence among flocks at pre-vaccination would be between 0.64% and 6.0% larger than the median prevalence in flocks post-vaccination. Although a non-random sample of sheep flocks was obtained, we have no reasons to believe that these sheep flocks are substantially different from other fine wool Merino flocks in Australia. Therefore, we believe that the reduction in prevalence would be similar in the broader sheep population to what was observed in this study. However, please note that despite the median prevalences being different, there could be some flocks having lower or similar prevalence pre-vaccination than at post-vaccination.

However, sheep in 30 of the 37 flocks were still shedding *MAP* organisms in their faeces. This suggests that despite a rapid decrease in OJD mortality in flocks following the commencement of a vaccination programme, shedding of *MAP* persisted for at least five years in a majority of flocks and that many of the flocks have shedding at rates that would be of concern if sheep were being traded from these flocks or vaccination ceased. Similar were the findings of a longitudinal observational study, which found that vaccinates had significantly lower prevalence of shedding than unvaccinated sheep but 10 of the 11 flocks had sheep with detectable shedding (Windsor, 2006). Further, in 6 of 7 flocks where vaccination ceased in wethers but not ewes, shedding rates significantly increased in the wether cohort (Eppleston et al., 2011). These studies provide evidence that long term vaccination is required to suppress the risk of OJD infection spreading or recrudescing in infected Australian sheep flocks.

It must be noted that many of our flocks in this study had a low pre-vaccination prevalence. Effectiveness of the vaccine is likely to be better in flocks with higher prevalence, as found previously. For example, a study conducted in three heavily infected flocks found that vaccination delayed the onset of faecal shedding of *MAP* by 12 months, and reduced the prevalence of shedders by 90% compared to unvaccinated lambs (Reddacliff et al., 2006). Another investigation conducted on seven heavily infected farms demonstrated persistence of shedding but with a significant decline in the mean flock prevalence (Eppleston et al., 2005).

Conclusions

The results confirms that a Bayesian approach can be successfully used to calculate and objectively compare true prevalence when apparent prevalence estimates from inconsistently used diagnostic tests are available. Moreover, information from multiple sources can be incorporated in a cohesive Bayesian model as illustrated by the use of data from abattoir surveillance to elicit prevalence priors. The results also suggest that vaccination with Gudair™ is usually effective in reducing prevalence of shedding but the response to vaccination is variable between flocks and that long-term vaccination is required to reduce the risks of disease spread or recrudescence. It appears that in the study area annual vaccination of lambs with Gudair™ for as long as five years is unlikely to prevent the spread of OJD associated with sale of sheep.

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Appendix A: Analysis of abattoir surveillance data to elicit priors for prevalences

A1. Model for analysis of abattoir surveillance data

The abattoir surveillance involves determination of lesion status of sheep at slaughter for samples of animals from given flocks. Data for abattoir surveillance were available from 25 of the flocks in the current study. Thus for each flock, there is a binomial count of the number of animals with detected lesions. These counts have a probability of being positive that is formulaically just like our previous ones:

$$Y_{la} \sim dbin(P_{la}, N_{la})$$

$$P_{il} = \pi_i * Se_l + (1 - \pi_i) * (1 - Sp_l)$$

where Y_{la} are number of lesion positive animals of the total number of samples tested (N_{la}) for a flock i; P_{il} is the apparent animal-level OJD prevalence of lesions in flock i; π_i is again the true animal-level OJD prevalence, now for the i^{th} flock in this sample; and Se_l is the sensitivity and Sp_l the specificity of lesion detection.

In addition, for each flock, a certain proportion, *prop*, of the observed lesions among those that were lesion positive (L+) were re-tested using histopathology (N_{ha}). The counts of histopathology positive (Y_{ha}) results are again binomially distributed with probability (P_{ha}):

$$N_{ha} = Y_{la} * prop$$

$$Y_{ha} \sim dbin(P_{ha}, N_{ha})$$

$$P_{ha} = \pi_i * Se_l * \frac{Se_h}{P_{il}}$$

where Se_h is the sensitivity of a sample being histo-positive given that it lesion-positive. The (augmented data) likelihood function for this analysis combines binomial contributions from the lesion surveillance and binomial contributions from the histopathology results.

The true animal-level prevalences (π_i) across flocks were modelled as independent and sampled from a $Beta(\alpha, \beta)$ distribution, as was done in (Hanson et al., 2003). Briefly, the mean μ, and variance σ^2 for this distribution are related to α and β as:

$$\alpha = \mu\psi$$

$$\beta = (1 - \mu)\psi$$

where $\psi = \alpha + \beta$. So the mean, μ, is the average prevalence among the super population of flocks, and the standard deviation, $\sigma = \sqrt{\frac{\mu(1-\mu)}{\psi(\psi+1)}}$ is large if ψ is small, and is small if ψ is large. For example, if $\mu = 0.2$ and $\psi = 10$, then $\alpha = 2$ and $\beta = 8$, we obtain a distribution that has 95% of the prevalences between 3% and 48% and a standard deviation of 0.12, whereas if we leave the mean alone and let $\psi = 400$, we obtain a prevalence distribution with 95% of the prevalences between 16% and 24% and a standard deviation of 0.02.

A2. Priors for analysis of abattoir surveillance data

Uncertainty about sensitivity and specificity for abattoir surveillance and for histopathology was modelled with independent Beta distributions which were elicited from previous published research (Bradley and Cannon, 2005; Dennis et al., 2011). Bradley and

Cannon (2005) estimated sensitivity of abattoir surveillance to be 52.5%, 74.1%, and 87.3%, for three inspectors. We calculated an average value of sensitivity and adopted it as a mode for the Beta prior distribution. Our estimate for the lowest value of sensitivity (5th percentile = 0.4) was based on the lowest 95% confidence interval for sensitivity reported in the paper (0.44). Similarly, our prior for surveillance specificity was based on the reported specificity of 97 to 100% in the paper (Table A.1).

Table A.1. Priors for sensitivities and specificities for abattoir surveillance and histopathology used for analysis of abattoir surveillance data.

Priors	**Input values**		**Prior distributions**[2]				Source of priors
	Mode	Lower/ Upper[1]	a	b	Mean	(95% PI)	
Abattoir surveillance							
Sensitivity (Se_i)	0.70	0.40	6.33	3.28	0.66	(0.35, 0.90)	Bradley and
Specificity (Sp_i)	0.98	0.95	151.7	4.08	0.97	(0.94, 0.99)	Cannon (2005)
Histopathology							
Sensitivity (Se_h)	0.65	0.40	7.98	4.76	0.63	(0.36, 0.86)	Dennis et al (2011)
Specificity (Sp_h)	0.995	0.99	1137.5	6.71	0.99	(0.99, 1.00)	(Same as PFC)

[1]Lower 5% limits were elicited for sensitivity and specificity estimates except for abattoir surveillance sensitivity for which the upper 95% limit was incorporated because the mode was less than 0.5); [2]*a* and *b* are parameters of the respective beta probability distributions.

Dennis et al. (2011) reported results of an investigation conducted to describe changes in infection status and enteric lesions of sheep naturally exposed to *MAP*. In this study, histopathological lesions could be detected only from 30 of the 46 infected sheep indicating a sensitivity of about 65%, which was used as a mode in forming a prior Beta distribution prior for histopathology sensitivity (Table A.1). We assumed the lower 5% value of sensitivity to be the same as for abattoir surveillance (Table 1).

Analysis of the abattoir data was also performed in WinBUGS using priors for the mean of the prevalence distribution, μ, from previous work (Dhand et al., 2010), and using a non-informative prior for ψ (Table A.2).

Table A.2. Priors for prevalence distributions for analysis of abattoir surveillance model: $Beta(\mu\psi, (1-\mu)\psi)$.

Parameters	**Input values**		**Priors**	**Source of priors**
	Mode	Upper[1]		
μ	0.16	0.70	$Beta(2.89, 0.32)$	Dhand et al (2010)
ψ	-	-	$Uniform(1, 1000)$[2]	Vague

[1]Upper 95% limit for prevalence; [2]Vague prior on dispersion parameter of prevalence distribution.

We note that these priors were used for analysis of abattoir surveillance data, where the output, in particular the mean and 95th percentile of the estimated prevalence distribution, was then used to form priors for the pre-vaccination OJD prevalence (Figure 1).

A3. Results of analysis of abattoir surveillance data:

The mean for the posterior distribution for μ based on abattoir surveillance was estimated to be 0.08, and the corresponding 95% probability interval (PI) was (0.044, 0.14), meaning that we are 95% sure, after seeing these data, that the mean prevalence would be in this interval. However, we wanted to be conservative, so we selected 0.3 instead of 0.14 to be the 95th percentile for our prior on the median prevalence for conducting principle analyses reported in the manuscript.

Prior and posterior distributions for μ, shown in Figure 3a, indicate that the posterior μ was well supported by the prior. Priors and posteriors for sensitivity of abattoir surveillance and histopathology shown in Figure 4 suggest that posterior sensitivity of abattoir surveillance was similar to the prior sensitivity but posterior sensitivity of histopathology was inferred to be higher than under the posterior than under the prior. Posterior estimates of sensitivities and specificities are summarised in Table A.3.

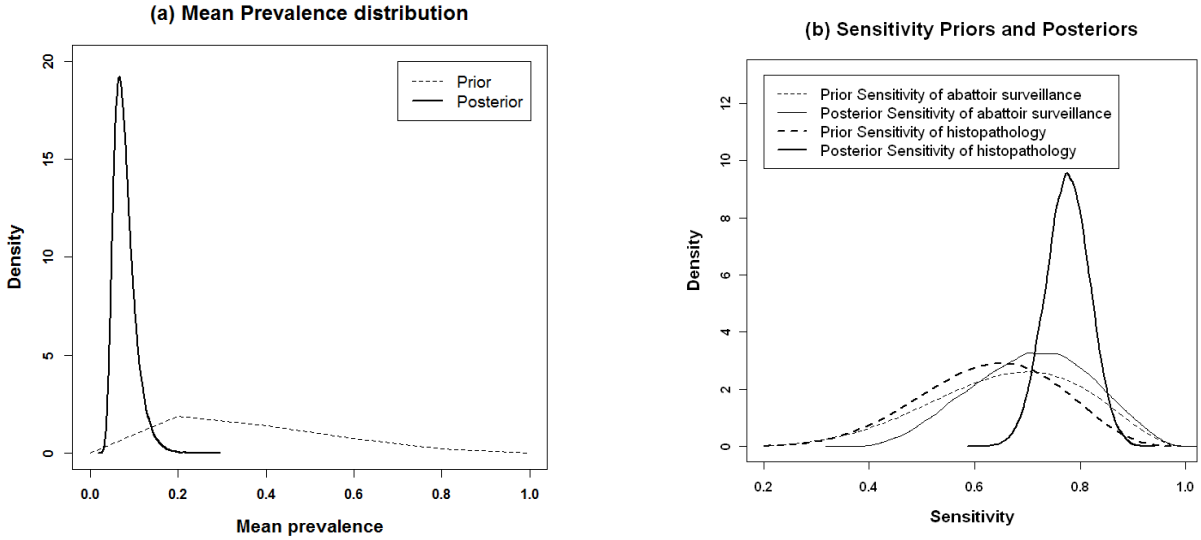

Figure 4. Comparison of prior and posterior distributions for analysis of abattoir surveillance data. (a) Mean prevalence (b) Sensitivity and specificity of abattoir surveillance and histopathology.

Table A.3. Posterior estimates for sensitivities and specificities of abattoir surveillance and histopathology based on analysis of abattoir surveillance data.

Test	Median	95% PI
Abattoir surveillance		
Sensitivity (Se_l)	0.71	(0.49, 0.91)
Specificity (Sp_l)	0.998	(0.997, 0.999)
Histopathology		
Sensitivity (Se_h)	0.78	(0.69, 0.86)
Specificity (Sp_h)	0.998	(0.997, 0.999)

Table A.4. Number of samples tested (and positive) for agar gel immunodiffusion test (AGID) and pooled faecal culture (PFC) test, pre- and post-vaccination.

Farm	Pre-vaccination				Post-vaccination	
	# of positive AGID tests	Total # of AGID tests	# of positive pools	Total # of pools tested	# of positive pools	Total # of pools tested
A. Flocks tested with AGID pre-vaccination and PFC post-vaccination						
1	1	467	-	-	0	7 [a]
2	8	449	-	-	1	7
3	4	450	-	-	4	8
4	29	900	-	-	3	7
5	2	522	-	-	2	7[a]
6	2	848	-	-	3	7
7	2	454	-	-	2	7
B. *Flocks tested with PFC both pre- and post-vaccination*						
8	-	-	6	7	1	7
9	-	-	2	7	1	7
10	-	-	3	7	3	7
11	-	-	3	11	2	7
12	-	-	5	7	0	7
13	-	-	1	7	0	7
14	-	-	7	7	1	7
15	-	-	3	7	1	8
16	-	-	5	7	1	7
17	-	-	5	10	1	7 [a]
18	-	-	4	10	1	7
19	-	-	19	19	6	7
20	-	-	9	10	1	7 [a]
21	-	-	2	7	2	7
22	-	-	3	14	5	7
23	-	-	1	7	5	7
24	-	-	6	7	3	7
25	-	-	0	7	0	7
26	-	-	3	7	1	7
27	-	-	3	7	4	8 [a]
C. *Flocks tested with both AGID and PFC pre-vaccination but PFC post-vaccination*						
28	0	450	1	1	0	7
29	4	217	3	3	2	7
30	1	957	6	20	4	7
31	3	678	8	10	1	8
32	0	50	4	7	7	7
33	1	1	4	7	0	7
34	0	34	1	4	3	7
35	1	130	2	7	1	7
36	0	50	1	7	0	7
37	11	644	4	7	3	7

[a] At least one of the pools was of a size different than 50, i.e. contained pellets from less than 50 sheep.

Appendix B: Estimation of values of a, b and c for prevalence priors

The logit-normal (LN) model for prevalences is specified as: $\pi_i \sim Logit\text{-}Normal(v, \lambda^2)$ or $logit(\pi_i) \sim N(v, \lambda^2)$. We show here how we elicited priors for v and λ. The ideas behind this specification can be found in Christenson et al. (2010).

Let c_α be the $1-\alpha$ percentile of the LN distribution and z_α the $1-\alpha$ percentile of the standard normal distribution, and v_α the $1-\alpha$ percentile of the $N(v, \lambda^2)$ distribution. So if $\alpha = 0.05$, $z_\alpha = 1.645$, then $v_\alpha = v + z_\alpha \lambda = v + 1.645\,\lambda$. Note that $v_{0.5} = v$ since $z_{0.5} = 0$. Then we can derive c_α by noting that:

$$1-\alpha = Pr(logit(\pi_i) < v_\alpha)$$

$$= \Pr\left(\pi_i < \frac{e^{v_\alpha}}{1+e^{v_\alpha}}\right)$$

$$= \Pr\left(\pi_i < c_\alpha\right)$$

Thus,

$$c_\alpha = \frac{e^{v_\alpha}}{1+e^{v_\alpha}}$$

Then the median of the LN distribution is

$$\mu \equiv c_{0.5} = \frac{e^{v}}{1+e^{v}}$$

and the 95th percentile of the LN distribution is

$$c_{0.95} = \frac{e^{v+1.645\,\lambda}}{1+e^{v+1.645\,\lambda}}$$

So half of the prevalences in the population of prevalences are less than μ and 95% of them are less than $c_{0.95}$.

We place independent priors on v and λ. We first focus on specifying a prior for v. We specify $v \sim N(a, b^2)$ so we need to induce real prior information to determine a and b. It is easiest to think about the median prevalence . Since $\mu = \frac{e^v}{[1+e^v]}$, it is clear that if our best guess for μ is say μ_0, then our best guess for v is simply:

$$v_0 = logit(\mu_0),$$

where v_0 is the same as a.

In addition, if we are 95% sure that $\mu < u$, then we are equivalently 95% sure that $v < logit(u)$. We thus equate:

$$logit(u) = v_0 + 1.645b = logit(\mu_0) + 1.645b.$$

Solving for b, we obtain:

$$b = \frac{[logit(u) - logit(\mu_0)]}{1.645}$$

So we have completely specified our prior for v.

Now we need a prior on λ. We will place a Uniform (0, c) prior on λ. We need some idea about how to pick c. We do this by thinking about $c_{0.9} = \frac{e^{v+1.28\,\lambda}}{[1+e^{v+1.28\,\lambda}]}$, which is the 90th

percentile of the prevalence distribution. Thus 90% of all prevalences are less than this number. We want to pick a value, say *max*, such that we are virtually 100% certain that this value cannot exceed, given our prior guess v_0. So we write

$$Pr\left(\frac{e^{v_0+1.28\,\lambda}}{[1+\,e^{v_0+1.28\,\lambda}]} <\; max\right) =\; 1.0$$

But this is equivalent to:

$$1.0\; =\; Pr\left(v_0\; +\; 1.28\,\lambda < \left(logit(max)\right) = \frac{Pr(\,\lambda < logit(max) - v_0]}{1.28}\right)$$

Thus we are 100% sure that $\lambda\; < \frac{[logit\,(u)\,-\,v_0]}{1.28} \equiv\; c$.

Appendix C.

See Table A4.

Appendix D. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2013.03.003.

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