



Estimation of OJD prevalence from pooled faecal samples

J-A. Toribio & E. Sergeant



The University of Sydney



AusVet



Trial design

- 4-year field trial commenced in 1999
- 3 properties in southern New South Wales with owner estimated mortalities related to ovine Johne's disease (OJD) of 5-15% per year
- 200 vaccinated and 200 control sheep per property

Challenge – Selection of diagnostic test

- Measure disease prevalence
- Cost-effective



Pooled faecal culture

Selected because...

- Higher sensitivity and specificity than serology
- Saving on laboratory work - Cost per culture is the same irrespective of pool size and comparable to the cost of individual culture
 - ❖ Pooled culture A\$110
 - ❖ Individual culture A\$100
- Able to reduce pool size over course of trial as disease prevalence increased



Procedure

- Faecal samples collected from each sheep, pooled and cultured for MAP every 6 months
- Pool size
40 → 20 → 10 **With disease progression**
- Pools further assessed by individual culture to verify PFC prevalence estimates when positive or when among 4 randomly selected negative pools per observation



Pooled faecal culture

Effect of pooling ?

- Dilution impacts on ability to identify the MAP in a pooled sample

| | 20 Multibacillary sheep | | 20 Paucibacillary sheep | |
|------------|-------------------------|-----|-------------------------|----|
| | No. +ve | Se | No. +ve | Se |
| Individual | 20 | 100 | 14 | 70 |
| 1 in 10 | 20 | 100 | 12 | 60 |
| 1 in 50 | 20 | 100 | 10 | 50 |
| 1 in 100 | 18 | 90 | 9 | 45 |

Whittington et al. (2000) *Journal of Clinical Microbiology* 38: 2550-2556.



Pooled faecal culture

Estimating animal prevalence

Control PFC results - 24 months post-vaccination

| Pool size | No of pools tested | No of positive pools |
|-----------|--------------------|----------------------|
| 10 | 15 | 11 |
| 9 | 1 | 0 |
| 14 | 1 | 1 |

Ability to estimate

- Variation in pool size
- Se of PFC at each pool size
- Proportion of multibacillary & paucibacillary cases
- True prevalence in the flock



Pooled Prevalence Calculator



ome

Pilot System

This system is a pilot system only and is still undergoing further development and testing. Results from this system should not be used without cross-checking with other sources. Please send any comments, questions or suggestions

Evan Sergeant



Pooled Prevalence Calculator

This site has been developed with funding from the [Australian Biosecurity Cooperative Research Centre](#). The site is intended for use primarily by participating CRC members and other researchers involved in estimating disease prevalence through structured surveys. The calculators provided here are particularly designed for estimating prevalence where samples are tested individually or in groups (pools) or where one or more tests are used with imperfect sensitivity and/or specificity.

Site Contents

Estimating individual prevalence from pooled samples:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)
- ◆ [Bayesian estimation using a Gibbs sampler](#)

MLE with approx CI (Kline 1989)
MLE with exact CI (Hauck 1991)

Estimating sample sizes required for prevalence estimation from pooled testing:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)

Simulate sampling to assess the validity and precision of pooled testing strategies for estimating individual prevalence:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)

Pooled Prevalence Calculator



ome

Pilot System

This system is a pilot system only and is still undergoing further development and testing. Results from this system should not be used without cross-checking with other sources. Please send any comments, questions or suggestions to [Evan Sergeant](#).



Pooled Prevalence Calculator

This site has been developed with funding from the [Australian Biosecurity Cooperative Research Centre](#). The site is intended for use primarily by participating CRC members and other researchers involved in estimating disease prevalence through structured surveys. The calculators provided here are particularly designed for estimating prevalence where samples are tested individually or in groups (pools) or where one or more tests are used with imperfect sensitivity and/or specificity.

Site Contents

Estimating individual prevalence from pooled samples:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)
- ◆ [Bayesian estimation using a Gibbs sampler](#)

Modified MLE (Tu 1994)

Estimating sample sizes required for prevalence estimation from pooled testing:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)

Simulate sampling to assess the validity and precision of pooled testing strategies for estimating individual prevalence:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)

Pooled Prevalence Calculator



ome

Pilot System

This system is a pilot system only and is still undergoing further development and testing. Results from this system should not be used without cross-checking with other sources. Please send any comments, questions or suggestions to [Evan Sergeant](#).



Pooled Prevalence Calculator

This site has been developed with funding from the [Australian Biosecurity Cooperative Research Centre](#). The site is intended for use primarily by participating CRC members and other researchers involved in estimating disease prevalence through structured surveys. The calculators provided here are particularly designed for estimating prevalence where samples are tested individually or in groups (pools) or where one or more tests are used with imperfect sensitivity and/or specificity.

Site Contents

Estimating individual prevalence from pooled samples:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)
- ◆ [Bayesian estimation using a Gibbs sampler](#)

Extension of modified
MLE (Cowling 1999)

Estimating sample sizes required for prevalence estimation from pooled testing:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)

Simulate sampling to assess the validity and precision of pooled testing strategies for estimating individual prevalence:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)

Pooled prevalence for fixed pool size and tests with uncertain sensitivity and specificity

Input Values

Method 5

Test results:

Size of pools:

Number of pools tested:

Number of positive pools:

Method 5 method assumes a fixed pool size but unknown (or uncertain) test sensitivity and specificity. Uncertainty associated with the point estimates of test sensitivity and specificity is incorporated through the inclusion of additional variance associated with the sample size used to determine the values used for these parameters. The smaller the sample size, the greater the uncertainty about the true values for sensitivity and/or specificity and hence the greater the uncertainty about the resulting prevalence estimate. [Asymptotic confidence limits](#) are based on a normal approximation and may be <0 for low prevalence values. See the [User Guide](#) or [Cowling et al. \(1999\)](#) (Method 6) for more details.

Input values

Test sensitivity & specificity:

Test Sensitivity:

Test Specificity:

Sample size for estimating Sensitivity:

Sample size for estimating Specificity:

Required inputs for these methods are pool size, number of pools tested, number of pools positive, test sensitivity and specificity and desired upper and lower confidence limits for the estimate. The sample size used for estimating test sensitivity and specificity must also be entered. Pool size, number of pools, number of pools positive and sample sizes for sensitivity and specificity estimates must all be positive integers and the number of positive pools must be less than the number of pools tested. Sensitivity, specificity and upper and lower confidence limits must be between zero and one

Outputs

Outputs for these methods are a point estimate, upper and lower confidence limits (asymptotic) and the standard error for the estimated prevalence as specified. A graph and text file listing of estimates and confidence limits for all possible results are also created for downloading if desired, by clicking on the appropriate icon.

Please Note:

Estimates are only valid if the proportion of positive pools is greater than the false positive rate (1 - Specificity) and less than or equal to the true positive rate (Sensitivity). Invalid results are indicated by NA in the results table.

Set confidence limits for output:

Lower CL for output:

Upper CL for output:

Submit

Reset

[[Home](#) | [User guide](#) | [Glossary](#) | [References](#) | [Links](#)]



This site was created by [AusVet Animal Health Services](#)
Under a project of the [Australian Biosecurity Cooperative Research Centre](#)
Project leader: [Euan Scragg](#)

www.ausvet.com.au/pprev

Pooled Prevalence Calculator



ome

Pilot System

This system is a pilot system only and is still undergoing further development and testing. Results from this system should not be used without cross-checking with other sources. Please send any comments, questions or suggestions to [Evan Sergeant](#).



Pooled Prevalence Calculator

This site has been developed with funding from the [Australian Biosecurity Cooperative Research Centre](#). The site is intended for use primarily by participating CRC members and other researchers involved in estimating disease prevalence through structured surveys. The calculators provided here are particularly designed for estimating prevalence where samples are tested individually or in groups (pools) or where one or more tests are used with imperfect sensitivity and/or specificity.

Site Contents

Estimating individual prevalence from pooled samples:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)
- ◆ [Bayesian estimation using a Gibbs sampler](#)

Expanded MLE
(Williams & Moffitt 2001)

Estimating sample sizes required for prevalence estimation from pooled testing:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)

Simulate sampling to assess the validity and precision of pooled testing strategies for estimating individual prevalence:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)

Pooled Prevalence Calculator



[Home](#)

Pilot System

This system is a pilot system only and is still undergoing further development and testing. Results from this system should not be used without cross-checking with other sources. Please send any comments, questions or suggestions to [Evan Sergeant](#).

Pooled prevalence for variable pool size and perfect tests

Input Values

Confidence level:

Testing results
(for up to 10 pool sizes)

| Size of pools: | Number of pools tested: | Number of positive pools: |
|----------------|-------------------------|---------------------------|
|----------------|-------------------------|---------------------------|

| | | |
|--------------------------------|---------------------------------|---------------------------------|
| <input type="text" value="5"/> | <input type="text" value="40"/> | <input type="text" value="20"/> |
|--------------------------------|---------------------------------|---------------------------------|

| | | |
|--------------------------------|---------------------------------|--------------------------------|
| <input type="text" value="1"/> | <input type="text" value="10"/> | <input type="text" value="2"/> |
|--------------------------------|---------------------------------|--------------------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

| | | |
|----------------------|----------------------|----------------------|
| <input type="text"/> | <input type="text"/> | <input type="text"/> |
|----------------------|----------------------|----------------------|

This method estimates prevalence and confidence limits for variable pool sizes and assumed 100% test sensitivity and specificity. See the [User Guide](#) or [Williams & Moffitt \(2001\)](#) for more details.

Input values

Required inputs are the desired level of confidence in the prevalence estimate and the pool size, number of pools tested and number of pools positive for each pool size used. Prevalence estimates can be calculated for sampling strategies with up to 10 different pool sizes used.

The desired confidence level must be a decimal number >0 and <1 (for example, $0.99 = 99\%$ or $0.95 = 95\%$). Pool sizes and numbers of pools must be positive integers (>0) and the numbers of positive pools must be non-negative integers (≥ 0). The number of positive pools must be less than or equal to the corresponding number of pools tested.

Outputs

Outputs from this program are presented as a point estimate of prevalence plus upper and lower confidence limits for the specified level of confidence

Important Note:

It is important to enter pool sizes and associated numbers tested and positive from the top of the table. You must enter at least one row of input data, and any rows entered must be complete. Any row in the input table that includes a blank or invalid value will be ignored.

www.ausvet.com.au/pprev

Submit

Reset

Pooled Prevalence Calculator



ome

Pilot System

This system is a pilot system only and is still undergoing further development and testing. Results from this system should not be used without cross-checking with other sources. Please send any comments, questions or suggestions to [Evan Sergeant](#).



Pooled Prevalence Calculator

This site has been developed with funding from the [Australian Biosecurity Cooperative Research Centre](#). The site is intended for use primarily by participating CRC members and other researchers involved in estimating disease prevalence through structured surveys. The calculators provided here are particularly designed for estimating prevalence where samples are tested individually or in groups (pools) or where one or more tests are used with imperfect sensitivity and/or specificity.

Site Contents

Estimating individual prevalence from pooled samples:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)
- ◆ [Bayesian estimation using a Gibbs sampler](#)

**Bayesian approach
(Cowling 1999)**

Estimating sample sizes required for prevalence estimation from pooled testing:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)

Simulate sampling to assess the validity and precision of pooled testing strategies for estimating individual prevalence:

- ◆ [For fixed pool size and perfect test](#)
- ◆ [For fixed pool size and known test sensitivity and specificity](#)
- ◆ [For fixed pool size and uncertain test sensitivity and specificity](#)
- ◆ [For variable pool sizes and perfect test](#)

Pooled prevalence using a Gibbs sampler

Input Values

Introduction

Test results:



This method uses a Bayesian approach and a Gibbs sampler iterative model to estimate the posterior distribution of the true animal-level prevalence of infection for a given pool size, number of pools tested, number of pools positive and estimated test [sensitivity](#) and [specificity](#). See the [User Guide](#) or [Cowling et al. \(1999\)](#) (Method 7) for more details.

Size of pools:

Input values

Number of pools tested:

Required inputs are pool size, number of pools tested, number positive and alpha and beta parameters for prior Beta distributions for true prevalence and test sensitivity and specificity. *For pooled testing, sensitivity and specificity should be estimated at the pool-level, rather than at the individual-level.* Additional inputs are the number of iterations to be simulated in the Gibbs sampler, the number of iterations to be discarded to allow convergence of the model and lower and upper probability (confidence) limits for summarising the output distributions. The Gibbs sampler is then used to estimate the probability distributions of true prevalence, sensitivity and specificity that best fit the data and prior distributions provided.

Number of positive pools:

Alpha & Beta parameters for prior distributions:

Prior distributions for Prevalence, Se and Sp

Prior prevalence: Alpha Beta

The Gibbs sampler requires prior estimates of the true prevalence and test sensitivity and specificity, based on expert knowledge or previous data. These estimates are specified as Beta probability distributions, with parameters alpha and beta. Beta probability distributions are commonly used to express uncertainty about a proportion based on a random sample of individuals. In this situation, if x individuals are positive for a characteristic out of n examined, then the alpha and beta parameters can be calculated as $\alpha = x + 1$ and $\beta = n - x + 1$. Alternatively, alpha and beta can be calculated using the [Beta distribution utility](#), provided estimates of the mode and 5% or 95% confidence limits are available from expert opinion.

Test Sensitivity: Alpha Beta

If there is no prior information on which to base a prior distribution, $\alpha = \beta = 1$ should be used. This results in a uniform (uninformative) distribution, in which all values between 0 and 1 have equal probability of occurrence.

Test Specificity: Alpha Beta

Outputs

Simulation details:

Outputs for this method are posterior probability distributions for prevalence, sensitivity and specificity. These distributions are described by their minimum, maximum, upper and lower probability limits specified, median, mean and standard deviation. A histogram and density chart and a text file of simulation results can also be downloaded for each parameter.

Number of iterations:

any iterations?

Number to discard:

Because the Gibbs sampler estimates prevalence iteratively, based on the data and the prior distributions, it may take a number of iterations for the model to converge on the true value. It is also important to carry out an adequate number of iterations to support inference from the results. suggested default values for the total number of iterations and the number to be discarded are provided, but can be varied if desired.

Lower CL for output:

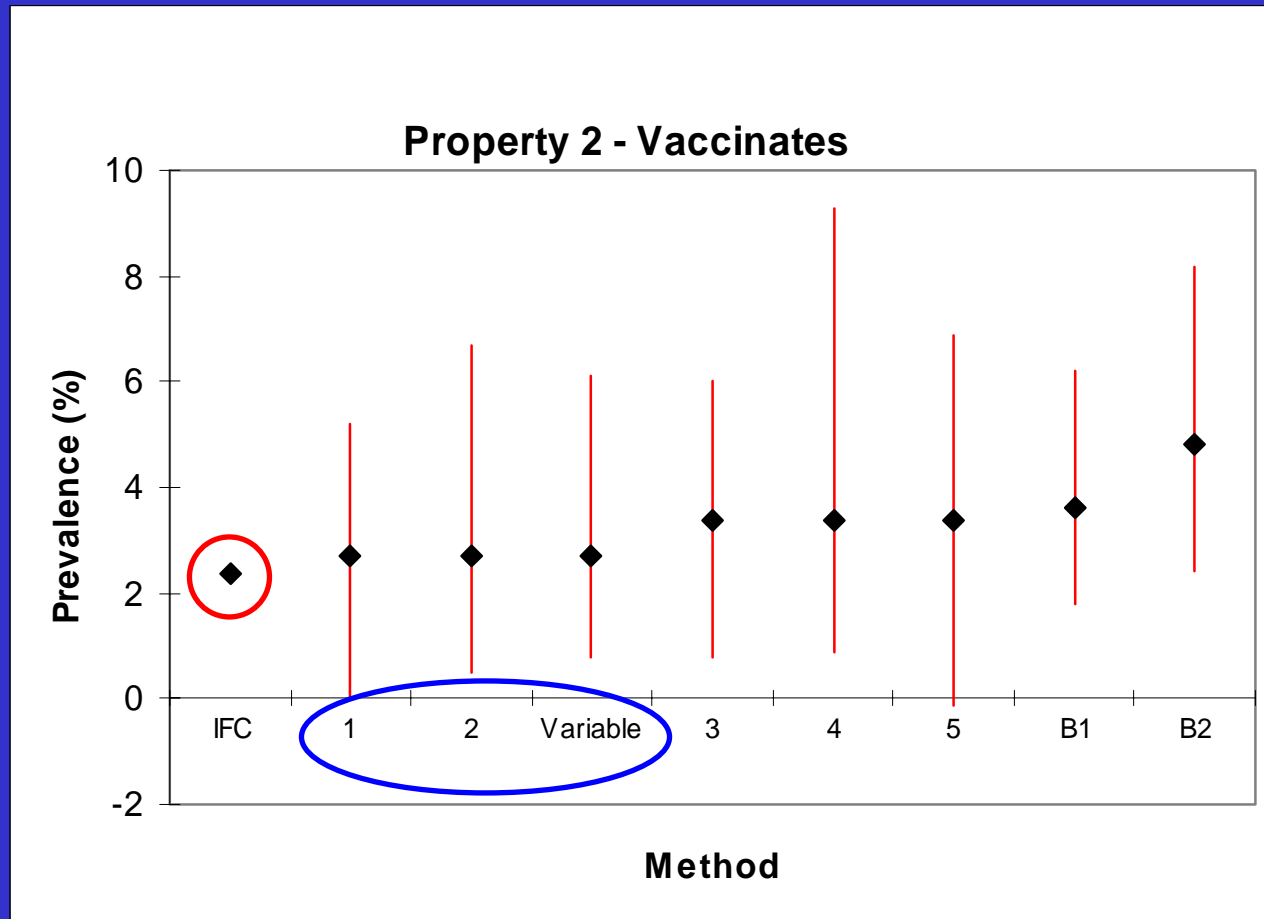
Upper CL for output:

Please note:

PPC includes a Beta distribution utility

OJD Vaccine trial

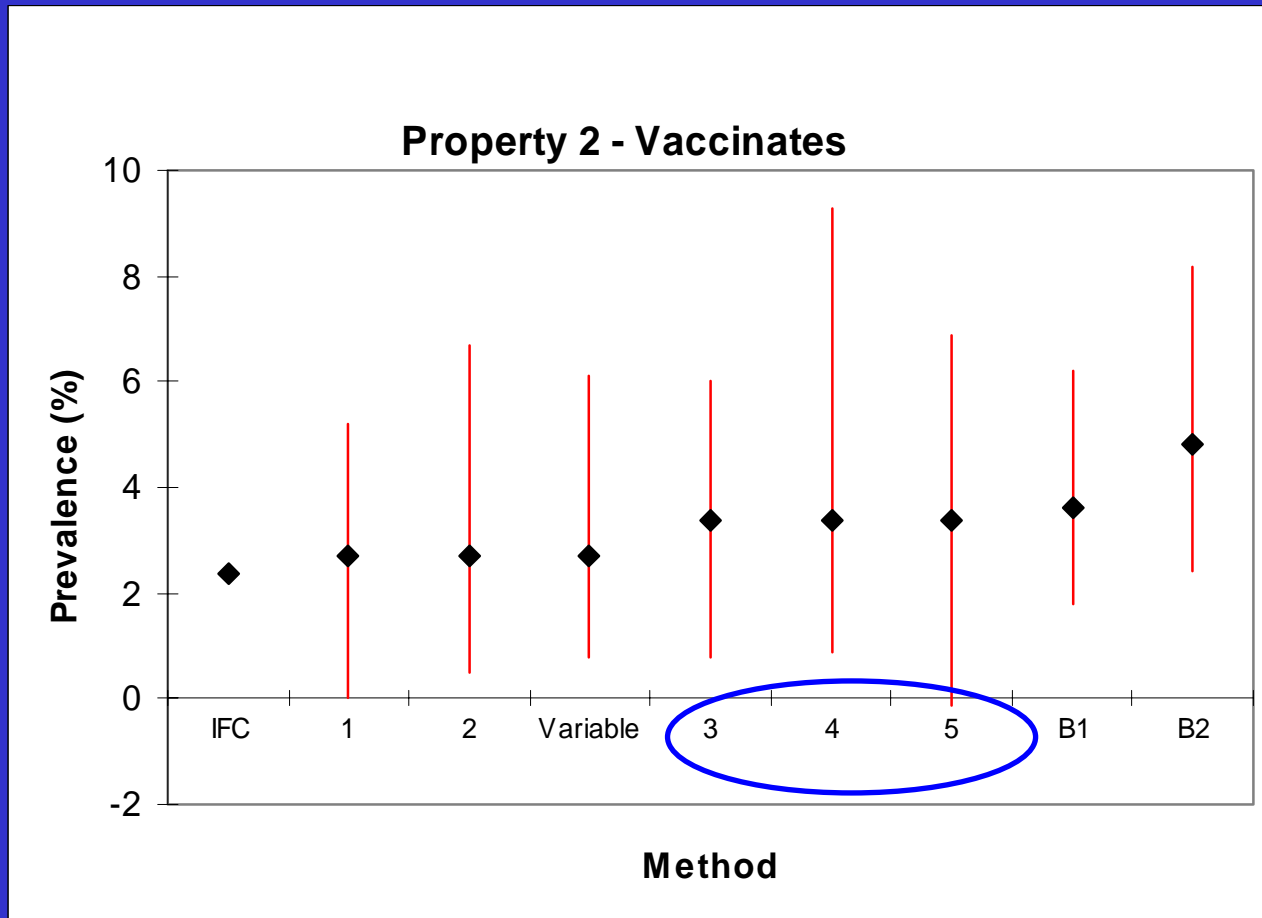
Prevalence - 30 months post-vaccination



Assume perfect test - IFC and Methods 1 / 2 / Variable

OJD Vaccine trial

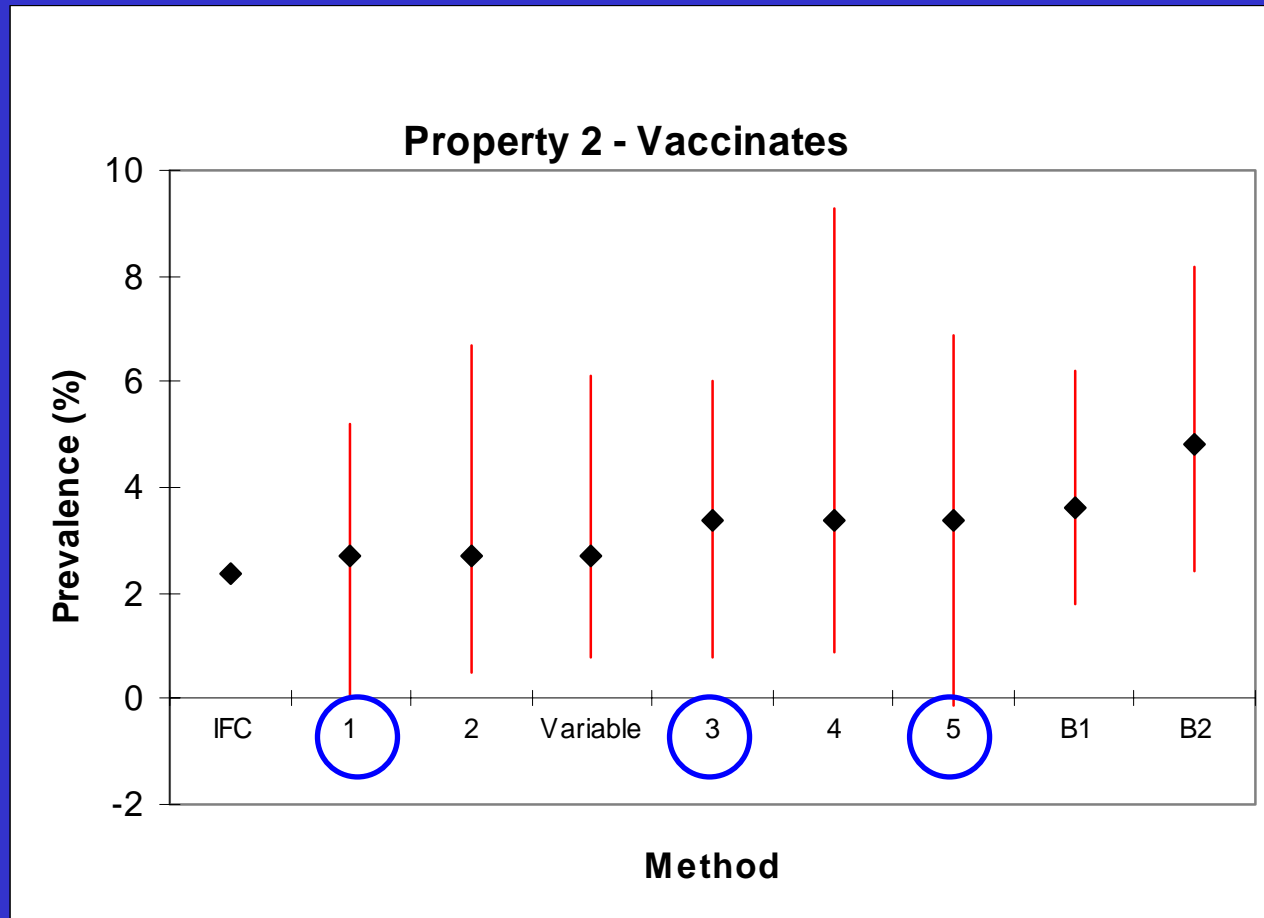
Prevalence - 30 months post-vaccination



Assume fixed pool size but imperfect test –
Methods 3 / 4 / 5

OJD Vaccine trial

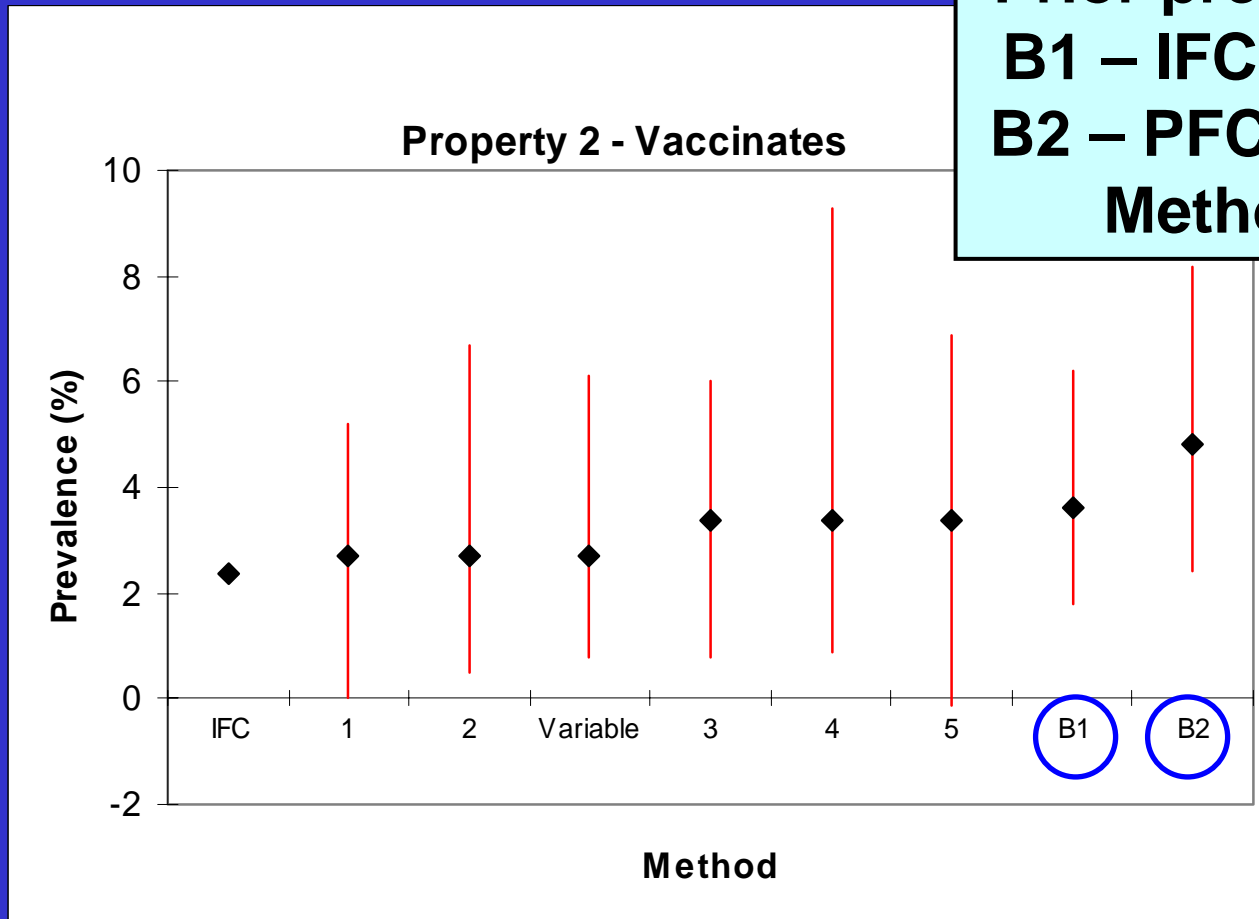
Prevalence - 30 months post-vaccination



Apply large sample theory – negative LCL at low prevalence – Methods 1 / 3 / 5

OJD Vaccine trial

Prevalence - 30 months post-vaccination

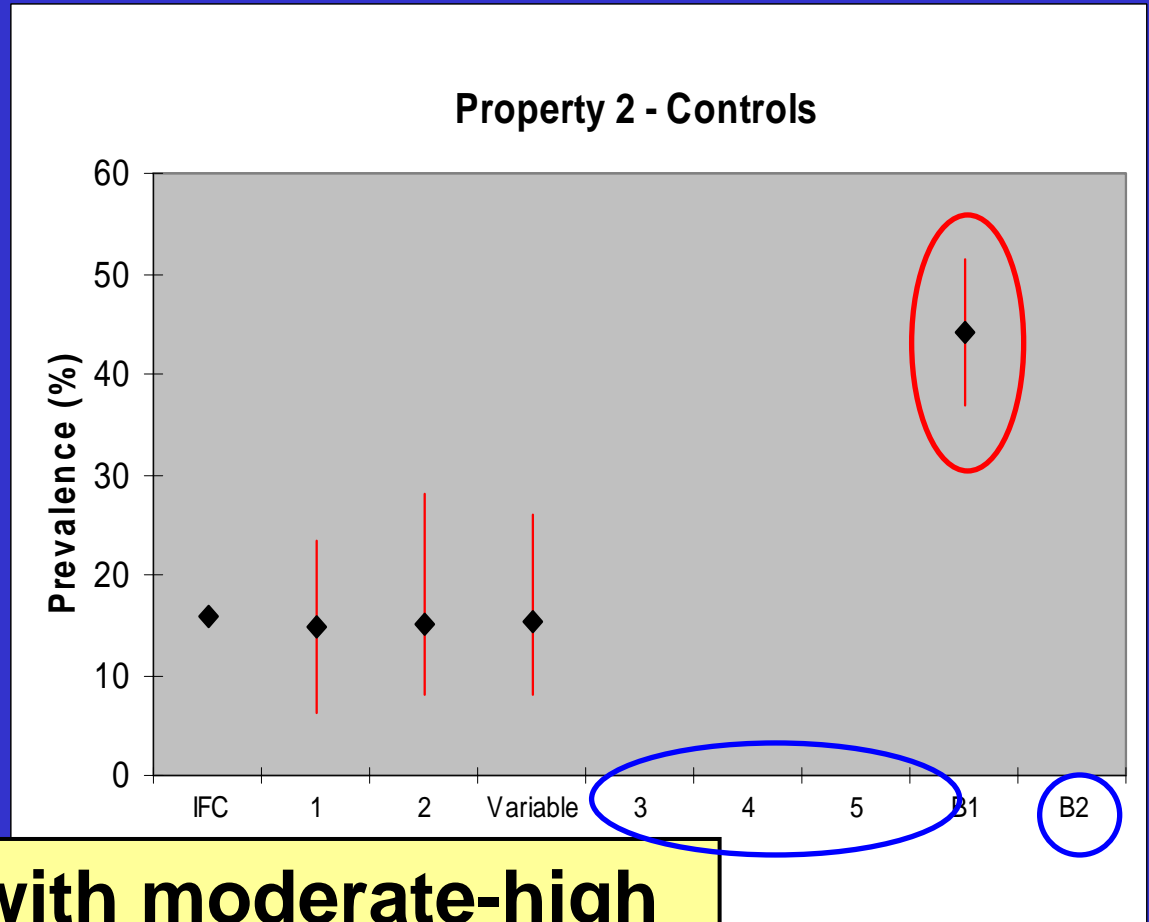
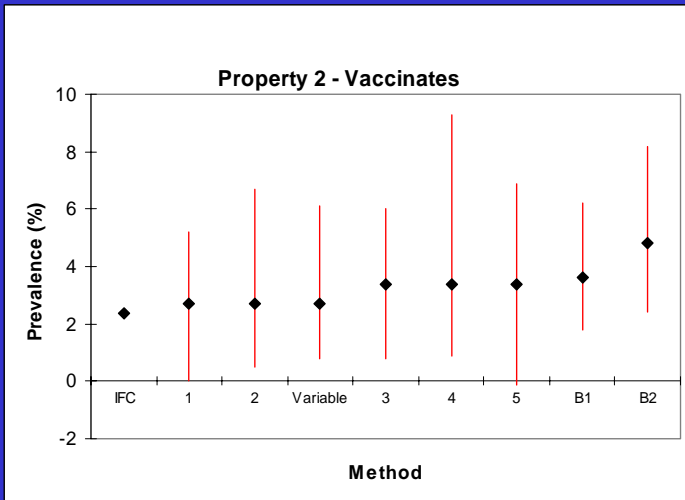


Prior prevalence
B1 – IFC results
B2 – PFC results
Method 5

Higher point estimates and tighter CIs –
Bayesian method

OJD Vaccine trial

Prevalence - 30 months post-vaccination

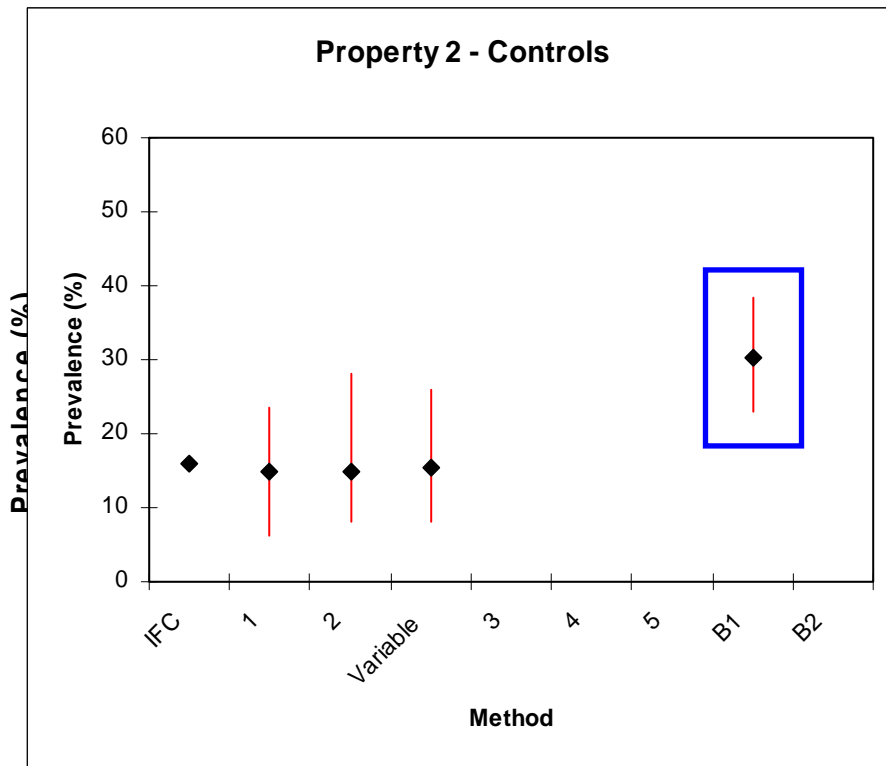


NO RESULTS with moderate-high prevalence inconsistent with PFC Se – Methods 3 / 4 / 5

OJD Vaccine trial

Prevalence - 30 months post-vaccination

Property 2 - Controls



| | Prior prevalence mode | Point prevalence estimate |
|----------|-----------------------|---------------------------|
| Original | 0.44 | 44.1 |
| 1 | 0.40 | 40.2 |
| 2 | 0.35 | 35.2 |
| 3 | 0.30 | 30.3 |

Which method fits best ?

- Method 2 – MLE with exact CIs - Hauck 91
- Variable pool size – Expanded MLE – Williams & Moffitt 2001

Opportunity for...

- Investigation of PFC Se at different pool sizes with different classes of OJD lesions
- Collaboration on Bayesian to:
 - Incorporate variable pool size
 - Identify appropriate prior prevalence



Acknowledgements

- Meat & Livestock Australia
- Australian Biosecurity CRC
- NSW DPI
- Rural Lands Protection Board
- USyd – Farm Animal Health

