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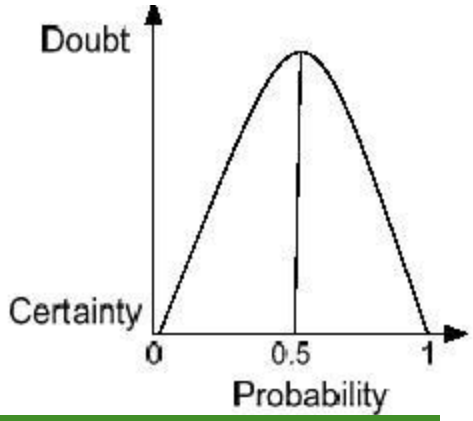
Latent class analysis, Bayesian statistics and the hidden perils of test validation studies

Lesley Stringer



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Zero-inflated random effect Bayesian test evaluation of individual faecal culture and ELISA to detect *Mycobacterium avium* subsp. *paratuberculosis* infection in young farmed deer

L Stringer[1], C Jewell[2], C Heuer[1], P Wilson[1], G Jones[1], A Noble[1], W Johnson[3]

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Background: paratuberculosis

- Caused by *Mycobacterium avium* subsp *paratuberculosis*
- Granulomatous enteritis – malabsorption – diarrhoea – weight loss – death
- Disease seen worldwide in ruminants
- No effective treatment
- In deer, highest clinical incidence is in yearlings

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The problem

- Want to estimate sensitivity and specificity of individual faecal culture (IFC) and a serum ELISA test for paratuberculosis in deer
- There is no ‘gold standard’ test in the live animal

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Traditional gold standard methods

- In theory: error-free diagnostic method which determines whether the target condition (e.g. disease/infection) is present or not
- Results from test under evaluation are compared to panel of “true cases” or “true non-cases” to derive sensitivity and specificity values
- In practice: no perfectly sensitive and specific ante-mortem test exists for any disease

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Latent class analysis

- True disease/infection status of individual is accepted as unknown (latent)

- Test accuracy is derived mathematically from test results using maximum likelihood methods or Bayesian inference

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Advantages of latent class analysis

- New test for evaluation may be more sensitive than the existing gold standard
- Avoids selection of panel of “true positives” biased to those positive to a gold standard
- If Bayesian methods are used to fit the model, smaller sample size needed

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Two test-two population method

- First described by Hui and Walter (1980)*

"If two tests are applied simultaneously to the same individuals from two populations with different disease prevalences, then assuming conditional independence of the errors of the two tests, the error rates of both tests and the true prevalences in both populations can be estimated by a maximum likelihood procedure".

* S.L.Hui, S.D. Walter, Biometrics 36, 167-171

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Two-test, two population model assumptions

- Test sensitivity and specificity are constant across populations

- The disease prevalences of the two populations are distinct

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Solving the equations

- Modelling can be done using maximum likelihood methods: Newton-Raphson or EM (expected maximization) algorithms

On-line tools for analysis: TAGS software

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Solving the equations

www.epi.ucdavis.edu/diagnostictests

TAGS : evaluation of Tests in the Absence of a Gold Standard

1: Enter the number of test(s) to be evaluated -------

2: Enter the number of tested population(s) with an unknown infection status ---------

3: Enter the number and the category of the reference population(s) tested no reference population

Number of parameters to be evaluated 0 **Degree of freedom** 0

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Bayesian latent class analysis

Modelling can also be done using Bayesian inference

Rev Thomas Bayes, 1702-1761

Bayes theorem

$$P(A|B) = P(B|A)\,P(A) \,/\, P(B)$$

$$P(B|A) = P(A|B)\,P(B) \,/\, P(A)$$

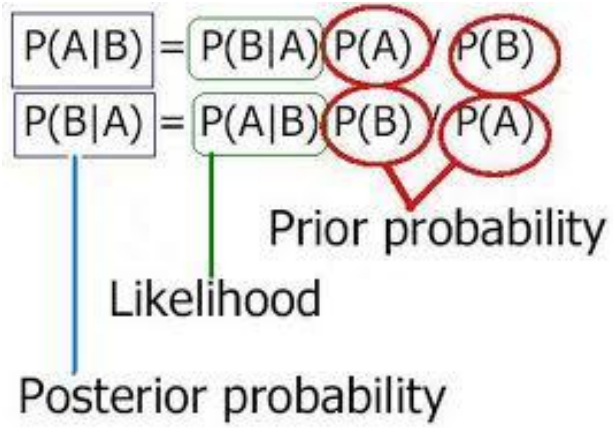
Prior probability

Likelihood

Posterior probability

In words..

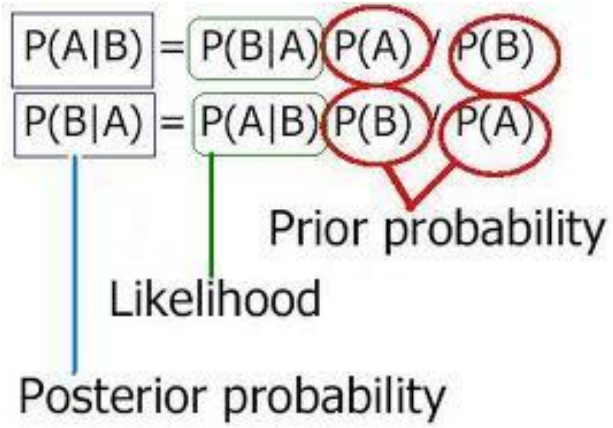
Bayes theorem



To get the posterior probability, multiply the prior probability distribution by the likelihood function and then normalize.

In words..

Bayes theorem



So in a test validation study we use prior information on test performance and prevalence and combine that with the data (the test results) to derive the posterior probability for each parameter

The mean, median or mode of the posterior distribution can be considered the parameter estimate

Probability or **credible** intervals i.e. the interval within which the true value lies with 95% probability are derived by Monte Carlo simulation from the posterior distribution

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Bayesian latent class analysis

The prior probability distribution allows us to incorporate existing scientific information on each parameter. Using estimates from prevalence studies, for example as prior information for the prevalence parameter.

Can also use expert opinion where no data exists.

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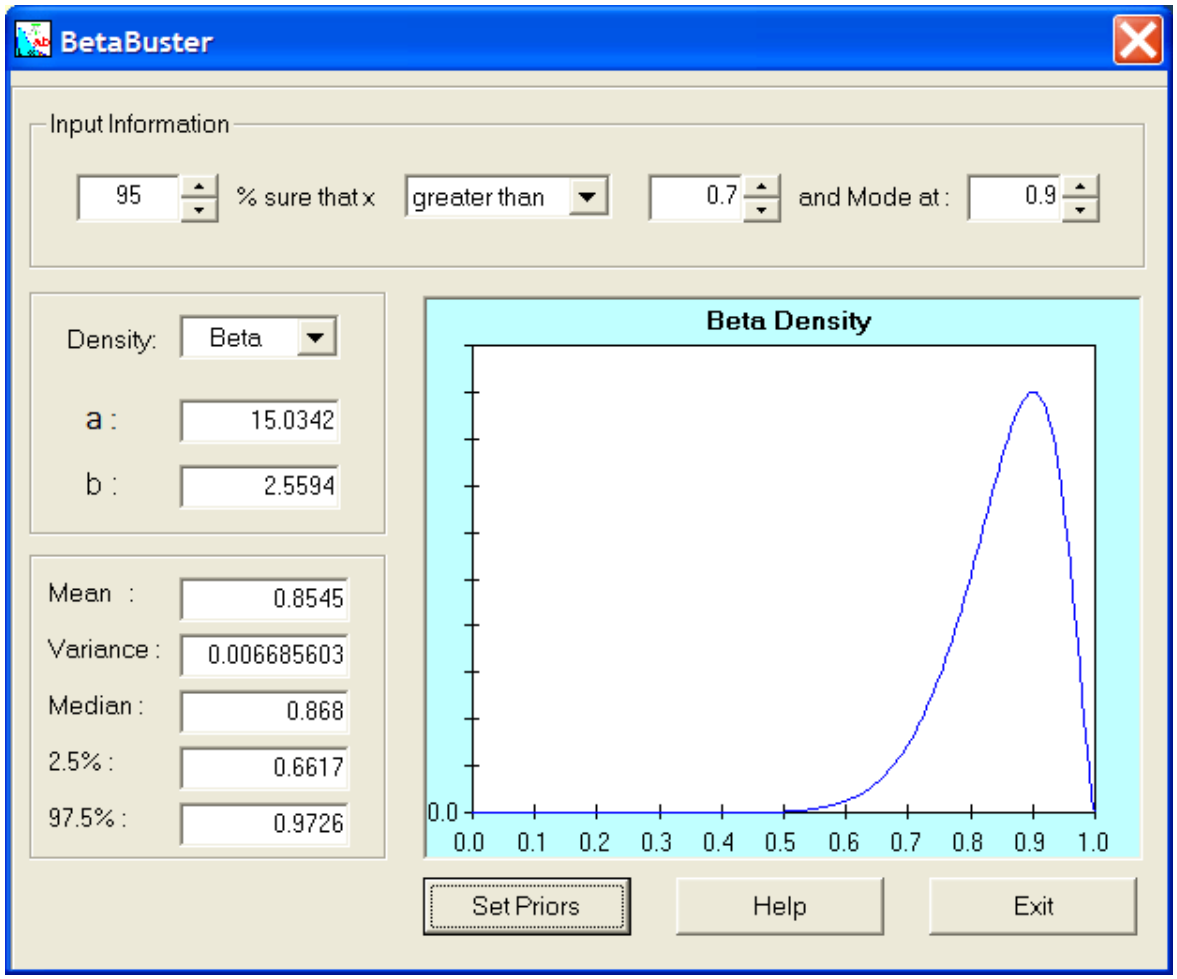
Priors

Expert opinion:

1. elicit most plausible value (e.g specificity of test A is 90%)
2. Elicit value that expert is 95% sure the value exceeds (e.g. 95% certain that specificity is greater than 70%)
3. Translate these into a beta distribution

Betabuster



BetaBuster, University of California, Davis

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Prior values

Parameter	Prior estimate	5th/95th percentile
Herd prevalence North Island	0.50	>0.1
Herd prevalence South Island	0.70	>0.5
Sensitivity faecal culture	0.50	>0.2
Sensitivity ELISA	0.77	>0.1
Specificity faecal culture	0.98	>0.95
Specificity ELISA	0.995	>0.95
Within-herd prevalence	0.30	<0.9

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Two tests two population method

For each population, test results are cross-classified in the appropriate 2 x 2 table

Population 1

	Test 2 +	Test 2-
Test 1 +	a	b
Test 1 -	c	d

n1

Population 2

	Test 2 +	Test 2 -
Test 1 +	e	f
Test 1 -	g	h

n2

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Latent class analysis

For each cell a-h, equations are derived:

e.g. test result in cell "a" (i.e. positive on both tests) could occur if the individual is infected, i.e.

true positive

Pop 1

	Test 2+	Test 2 -
Test 1 +	a	b
Test 1 -	c	d

= pi1*se1*se2

or

false positive = (1-pi1)* (1-sp1)*(1-sp2)

Latent class analysis

So for cell “a” the likelihood equation is based on the sum of these probabilities, i.e. true positive + false positive

a = pi1se1se2 + (1-pi1)(1-sp1)(1-sp2)

Likelihoods for remaining cells are constructed similarly

Latent class analysis

These are the equations to be solved:

Cell (a): pi1se1se2 + (1-pi1)(1-sp1)(1-sp2)
Cell (b): pi1se1(1-se2) + (1-pi1)(1-sp1)sp2
Cell (c): pi1(1-se1)se2 + (1-pi1)sp1(1-sp2)
Cell (d): pi1(1-se1)(1-se2) + (1-pi1)sp1sp2
Cell (e): pi2se1se2 + (1-pi2)(1-sp1)(1-sp2)
Cell (f): pi2se1(1-se2) + (1-pi2)(1-sp1)sp2
Cell (g): pi2(1-se1)se2+ (1-pi2)sp1(1-sp2)
Cell (h): pi2(1-se1)(1-se2) + (1-pi2)sp1sp2

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Latent class analysis

Six parameters can be estimated from the observed data:

- prevalence in pop'n 1 (pi1) and pop'n 2 (pi2)
- sensitivity of ELISA (se1) and culture (se2)
- specificity of ELISA (sp1) and culture (sp2)

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Back to the study question

Estimate the sensitivity and specificity of two diagnostic tests (IFC and ELISA) for paratuberculosis

Test validation: should consider

1. Test purpose (confirmation of diagnosis? Freedom from disease? Test and cull?)
2. Target condition (Infected? Infectious? Clinically diseased?)
3. Target population

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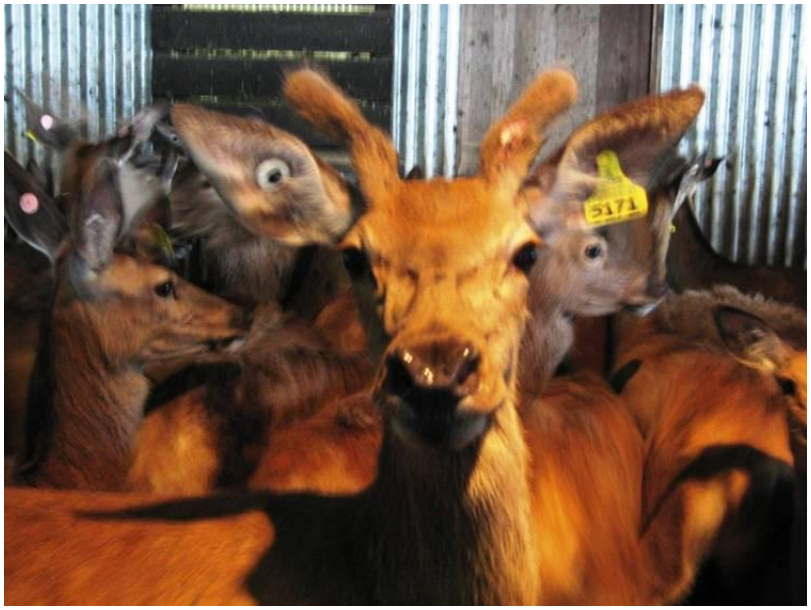
The study question

Estimate the sensitivity and specificity of IFC and ELISA to identify **clinically normal yearling deer** **infected** with MAP for the purpose of **herd classification** (freedom from disease sampling)



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Study design

- Prevalence of MAP infection in farmed deer different in North (29%) and South Island (51%) of New Zealand – two populations
- 20 clinically normal yearling deer sampled (faeces and blood) in 20 herds SI, 18 NI
- Results for each animal of individual faecal culture and a serum IgG1ELISA

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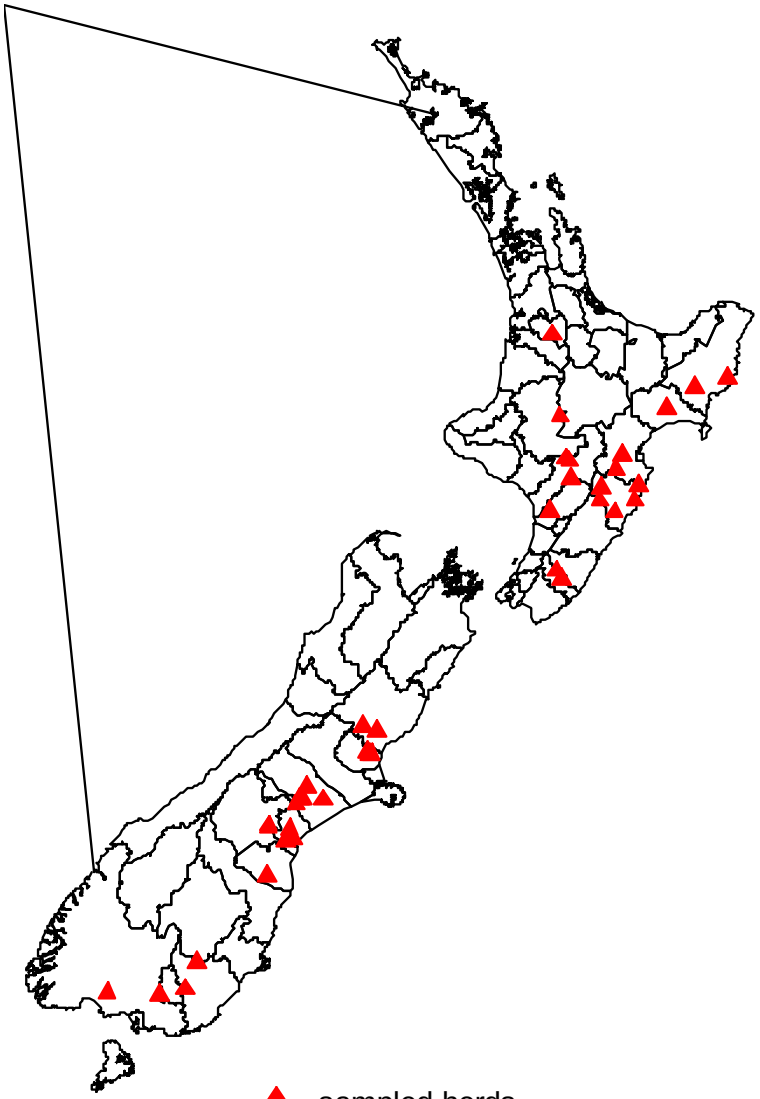

▲ sampled herds

Te Kunenga
ki Pūrehuroa

Statistical problems

- Samples not independent observations, clustered in herds
- Variation in within-herd prevalence expected
- Possibility of non-infected herds

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Solution: extended HW model

- Variation in within-herd prevalence modelled as a random effect

- Zero-inflation effect incorporated, allowing modelling to include probability of herd being non-infected

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Practical problems

- Important that vets collect samples to minimise cross-contamination
- Clear instructions to use fresh gloves each sample
- Test run of questionnaire, instructions and sampling

To be sure, to be sure….contact each vet post-sampling

- "I use a spoon for faeces collection but wash it between bums so it is clean. And gloves cost money too!"

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Results from IFC and ELISA

North Island

	IFC +	IFC-
ELISA +	1	15
ELISA -	1	339

South Island

	IFC +	IFC -
ELISA +	10	27
ELISA -	45	319

757 paired samples:
401 from the South Island
356 from the North island

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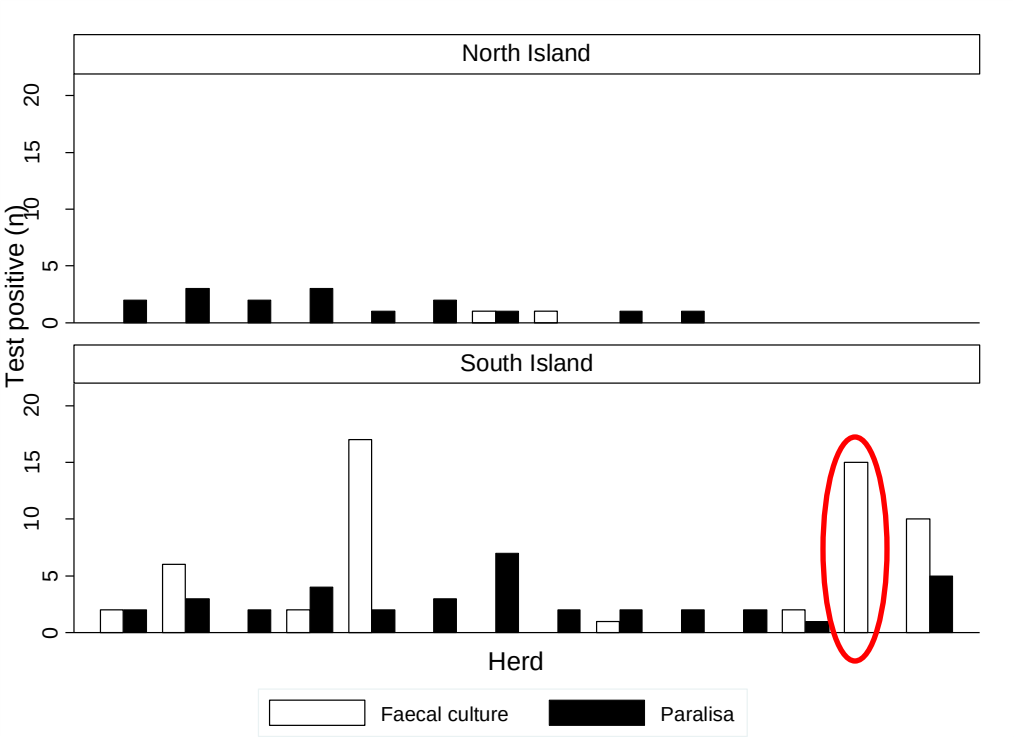
Herd-level results



IFC pos = 2 herds in NI, 8 herds SI

ELISA pos = 8 herds NI, 13 SI

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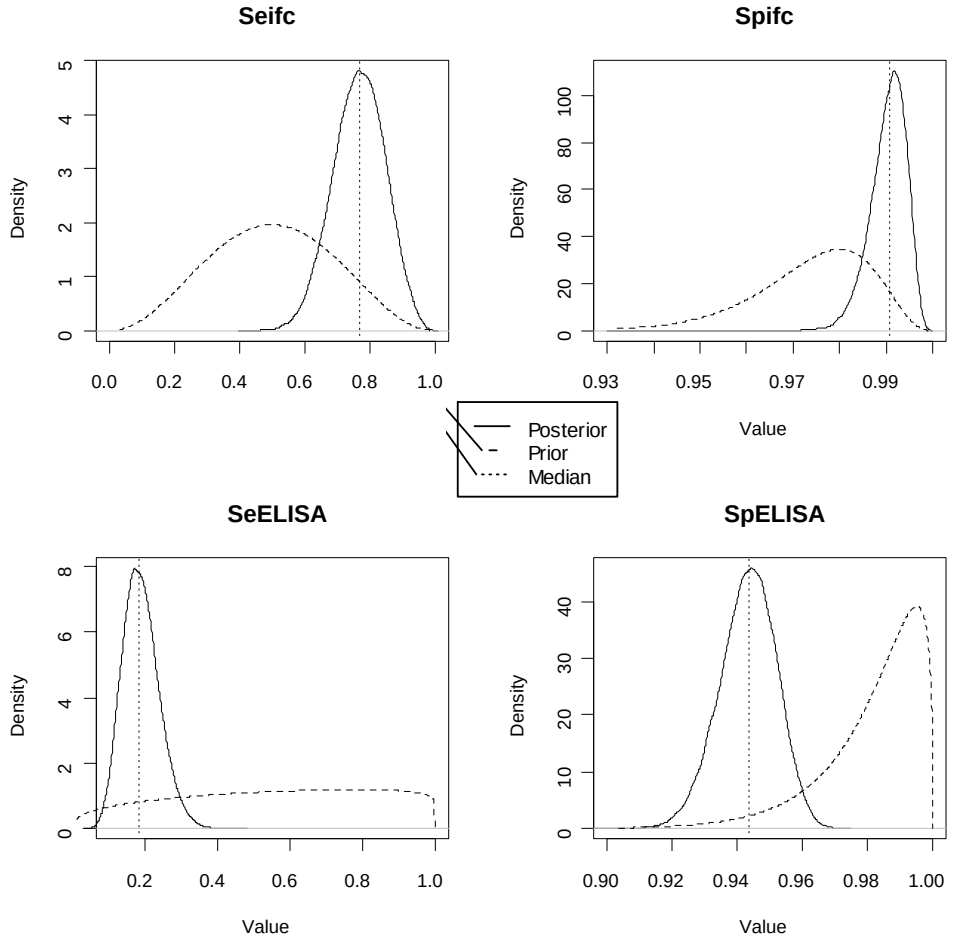
Prior and posterior distributions



Sensitivity estimates (95%CI)
IFC: 77% (61-92)
ELISA: 19% (10-30)

Specificity estimates (95%CI)
IFC: 99% (98-100)
ELISA: 94% (93-96)

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Sensitivity analysis

Model run changing priors for herd prevalence, within-herd prevalence, using pessimistic test sensitivity and specificity priors:

Little effect on posterior distributions

Conclusion: model robust to changes in prior information, i.e. estimates driven by the data rather than the priors

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Example sensitivity analysis (red = changed value)

Original prior for SP ELISA: most likely value = 99.5%
(95% certain that greater than 95%)

Amend prior for SP ELISA to 90%
(95% certain that greater than 20%)

Parameter	Median	SD	2.5% quantile	97.5% quantile
Sensitivity IFC	0.77	0.08	0.61	(0.92)
Sensitivity ELISA	0.18 (0.19)	0.05	0.10	0.30
Specificity IFC	0.99	0.004	0.98	1.00
Specificity ELISA	0.94	0.009	0.92 (0.93)	0.95 (0.96)

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Back to the question

Estimate the sensitivity and specificity IFC and ELISA to identify clinically normal yearling deer infected with MAP for the purpose of herd classification (freedom from infection sampling)

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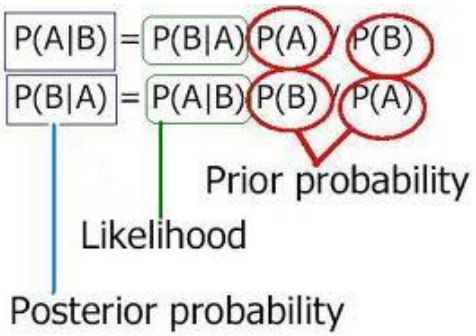
So…

- Applied two tests in two populations of different prevalence
- Used latent class analysis, i.e. non-gold standard method
- Taken into account that herds may not be infected (zero-inflation) and modelled the within-herd prevalence using a random-effects logistic model
- Used Bayesian inference to derive the sensitivity and specificity estimates



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Conclusion

IFC is more sensitive (77% vs 19%) and specific (99% vs 94%) at the individual level than ELISA in yearling deer in New Zealand

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Prior values

- Derived from published literature:

Se IFC: 50% (95% certain that >20%)

Se ELISA: 77% (95% certain that >10%)

Sp IFC: 98% (95% certain that >95%)

Sp ELISA: 99.5% (95% certain that >95%)

- Posterior values of test performance

Se IFC: 77% (95CI 60%-92%)

Se ELISA: 19% (95%CI 10% - 30%)

Sp IFC: 99% (95%CI 98%-99.7%)

Sp ELISA: 94% (95% CI 93%-96%)

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What happened next?

Some comments…

….the analysis is likely to have been greatly biased by just two herds which

(a) are “outliers”
(b) are not biologically plausible; and/or
(c) do not have an explanation with our current knowledge.

…in analyses like this it is not uncommon to drop extreme results that do not appear to be biologically sensible .

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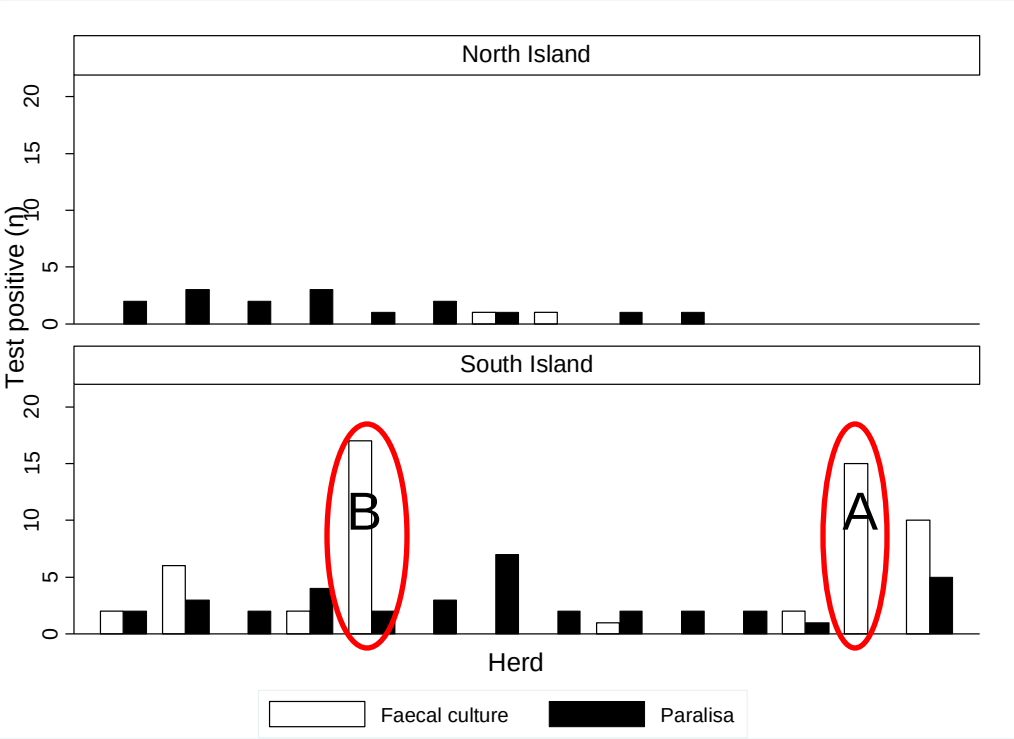
Removing data?



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Removing data?

Results of analysis excluding herd A (15 IFC +, no ELISA +)

Parameter	Median	95% CI
SeIFC	0.76	0.56 - 0.92
Separa	0.27	0.15 - 0.41
SpIFC	0.99	0.98 - 0.997
Sppara	0.94	0.93 - 0.96

Excluding herd B (17 IFC +, 2 ELISA +)

Parameter	Median	95% CI
SeIFC	0.69	0.47- 0.89
Separa	0.22	0.11- 0.36
SpIFC	0.99	0.98- 0.997
Sppara	0.94	0.93- 0.96

Excluding both herds

Parameter	Median	95% CI
SeIFC	0.51	0.27- 0.82
Separa	0.39	0.22- 0.60
SpIFC	0.99	0.98-0.997
Sppara	0.95	0.93- 0.97

Full model estimates

Sensitivity (95%CI)
IFC: 77% (61-92)
ELISA: 19% (10-30)

Specificity (95%CI)
IFC: 99% (98-100)
ELISA: 94% (93-96)


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?

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WINbugs code

```
 # Population 1
for (i in 1:ny1) {
myN1[i] <- y1[i,1] + y1[i,2] + y1[i,3] + y1[i,4]
y1[i,1:4] ~ dmulti(p1[i,1:4], myN1[i])
p1[i,1] <- pi1[i]*SeELISA*Seifc + (1-pi1[i])*(1-SpELISA)*(1-Spifc)
p1[i,2] <- pi1[i]*SeELISA*(1-Seifc) + (1-pi1[i])*(1-SpELISA)*Spifc
p1[i,3] <- pi1[i]*(1-SeELISA)*Seifc + (1-pi1[i])*SpELISA*(1-Spifc)
p1[i,4] <- pi1[i]*(1-SeELISA)*(1-Seifc) + (1-pi1[i])*SpELISA*Spifc
pi1[i] <- z1[i] * pistar1[i]
z1[i] ~ dbern(phi1)
logit(pistar1[i]) <- alpha + U1[i]
U1[i] ~ dnorm(0, tau)
}
# Population 2
for (i in 1:ny2) {
myN2[i] <- y2[i,1] + y2[i,2] + y2[i,3] + y2[i,4]
y2[i,1:4] ~ dmulti(p2[i,1:4], myN2[i])
p2[i,1] <- pi2[i]*SeELISA*Seifc + (1-pi2[i])*(1-SpELISA)*(1-Spifc)
p2[i,2] <- pi2[i]*SeELISA*(1-Seifc) + (1-pi2[i])*(1-SpELISA)*Spifc
p2[i,3] <- pi2[i]*(1-SeELISA)*Seifc + (1-pi2[i])*SpELISA*(1-Spifc)
p2[i,4] <- pi2[i]*(1-SeELISA)*(1-Seifc) + (1-pi2[i])*SpELISA*Spifc
pi2[i] <- z2[i] * pistar2[i]
z2[i] ~ dbern(phi2)
logit(pistar2[i]) <- alpha + U2[i]
U2[i] ~ dnorm(0,tau)
}
```