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What we work on ? Johne's Disease (Jd)

- Granulomatous enteritis due to *Mycobacterium paratuberculosis* (*Map*)
- We estimate > 60% NZ deer herds are infected by *Map*
- Jd causes clinical losses in a small number of NZ deer herds
- Subclinical *Map* infection causes production/reproductive losses (10%)
- Jd increased by environmental stress and host Susceptible genetics

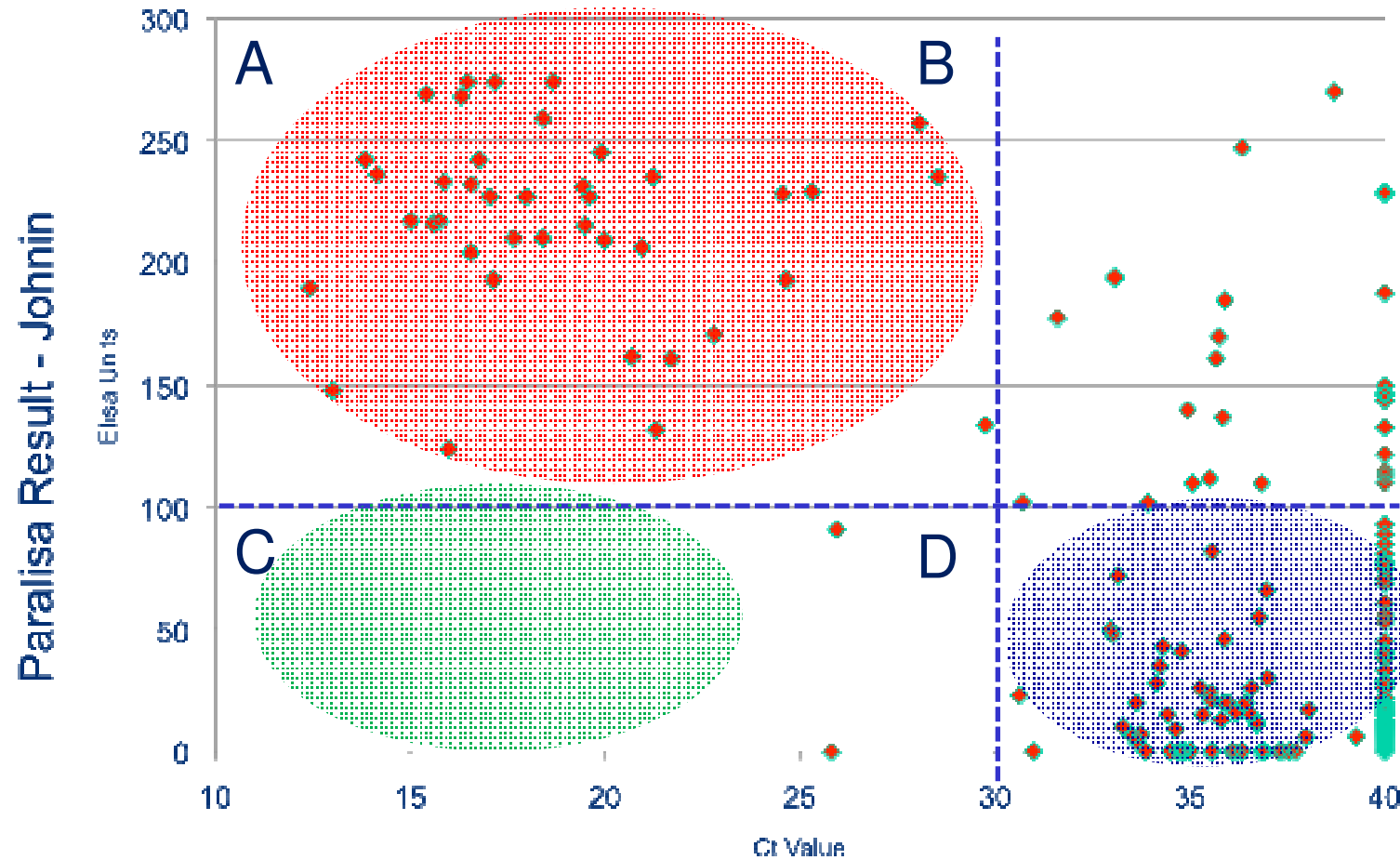
How do we diagnose *Map* ??

A. Immune Diagnosis (IgG₁) – Antibody (Paralisa™)

		Lesion-positive animals		
	Culture (+)			
	Infected	Disease +	Disease ++	Disease+++
ELISA(+)	77/100	39/43	37/40	66/67
ELISA sensitivity	77%	91%	93%	98.5%

Inverse Relationship: [Antibody] & Disease Severity

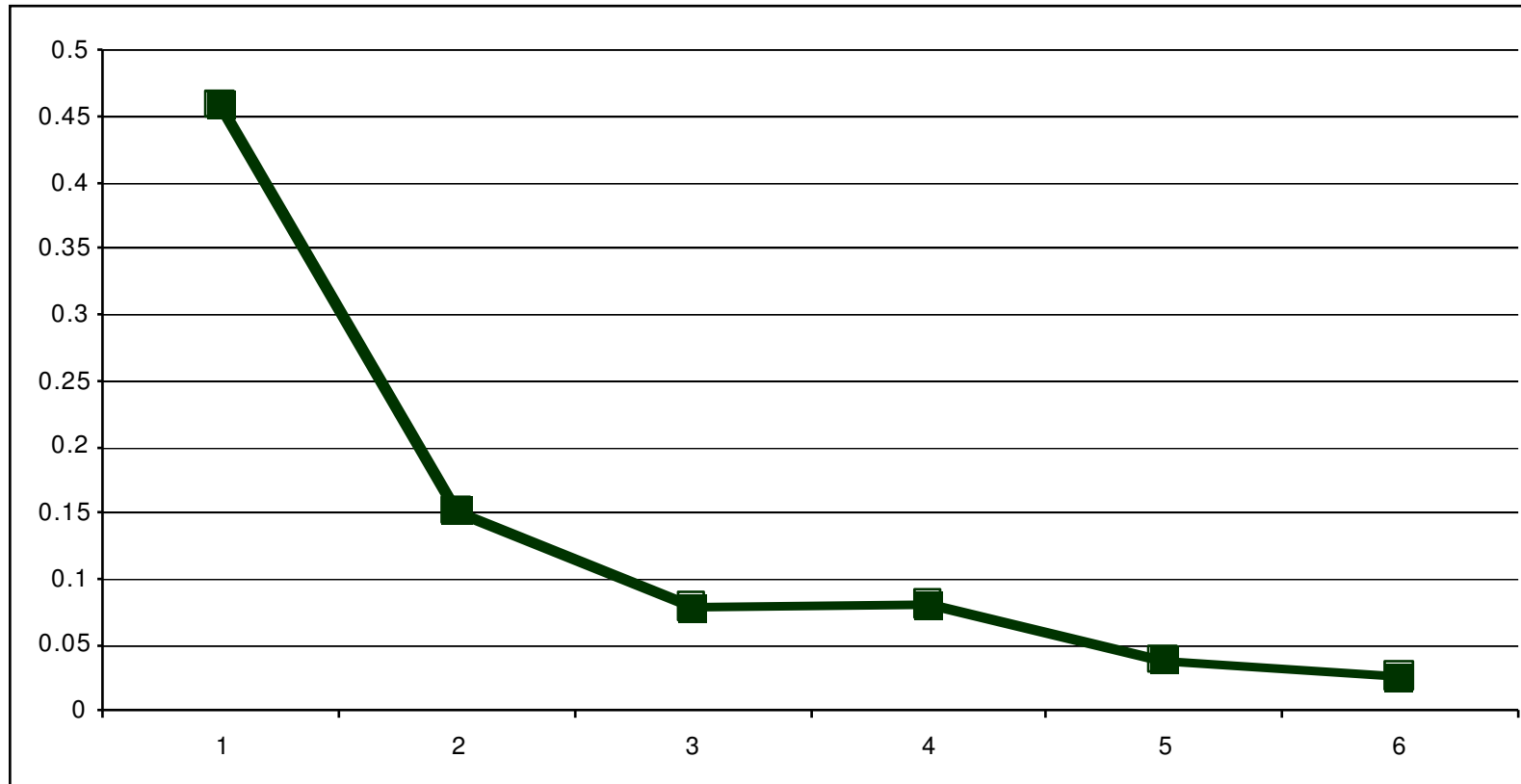
B. Measure Faecal shedding using Molecular techniques (PCR)



qPCR Ct Value – Faecal Shedding (Ct vs ELISA $p < 0.0001$)

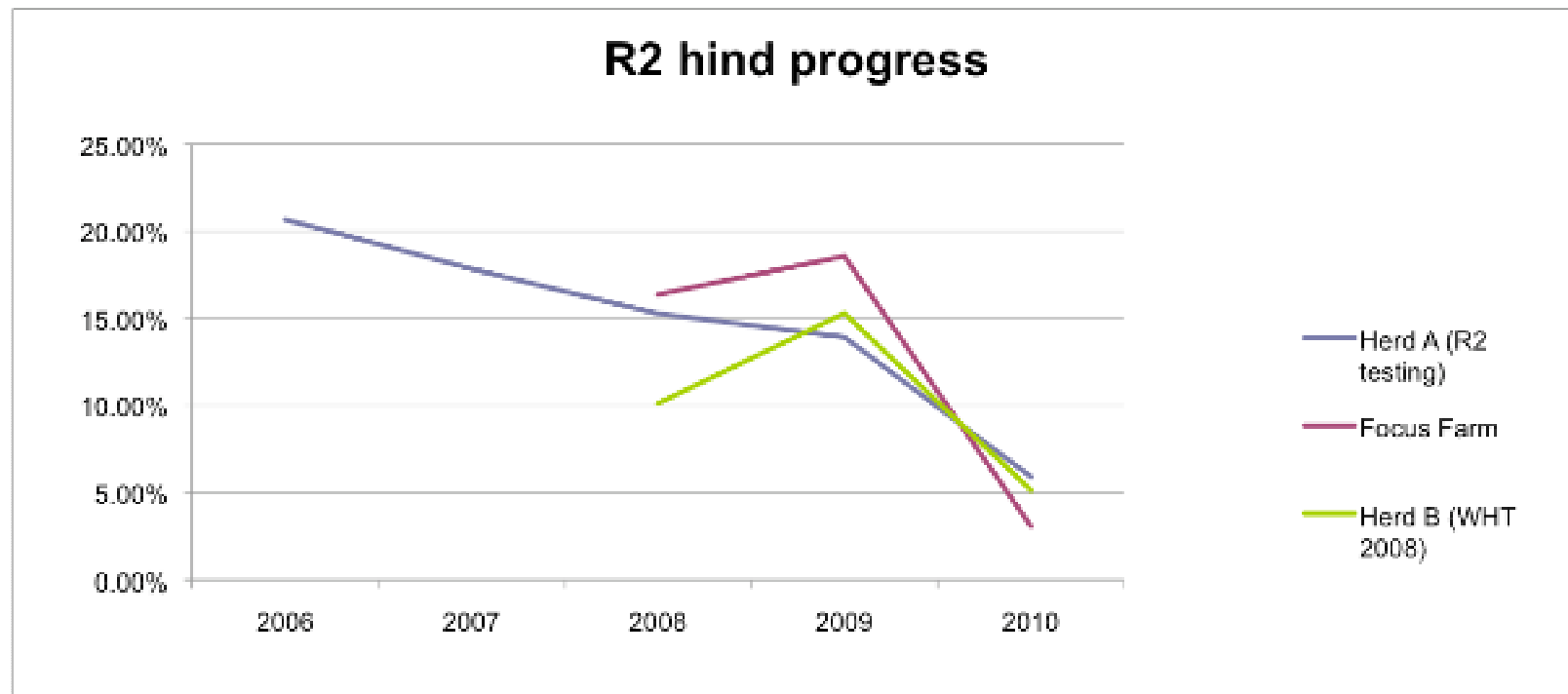
Excellent correlation between high Paralisa titres (>100) and supershedding (Ct <30)

Immunodiagnosis using Whole Herd Testing to Control Infection in 5 deer herds (6,000 animals)

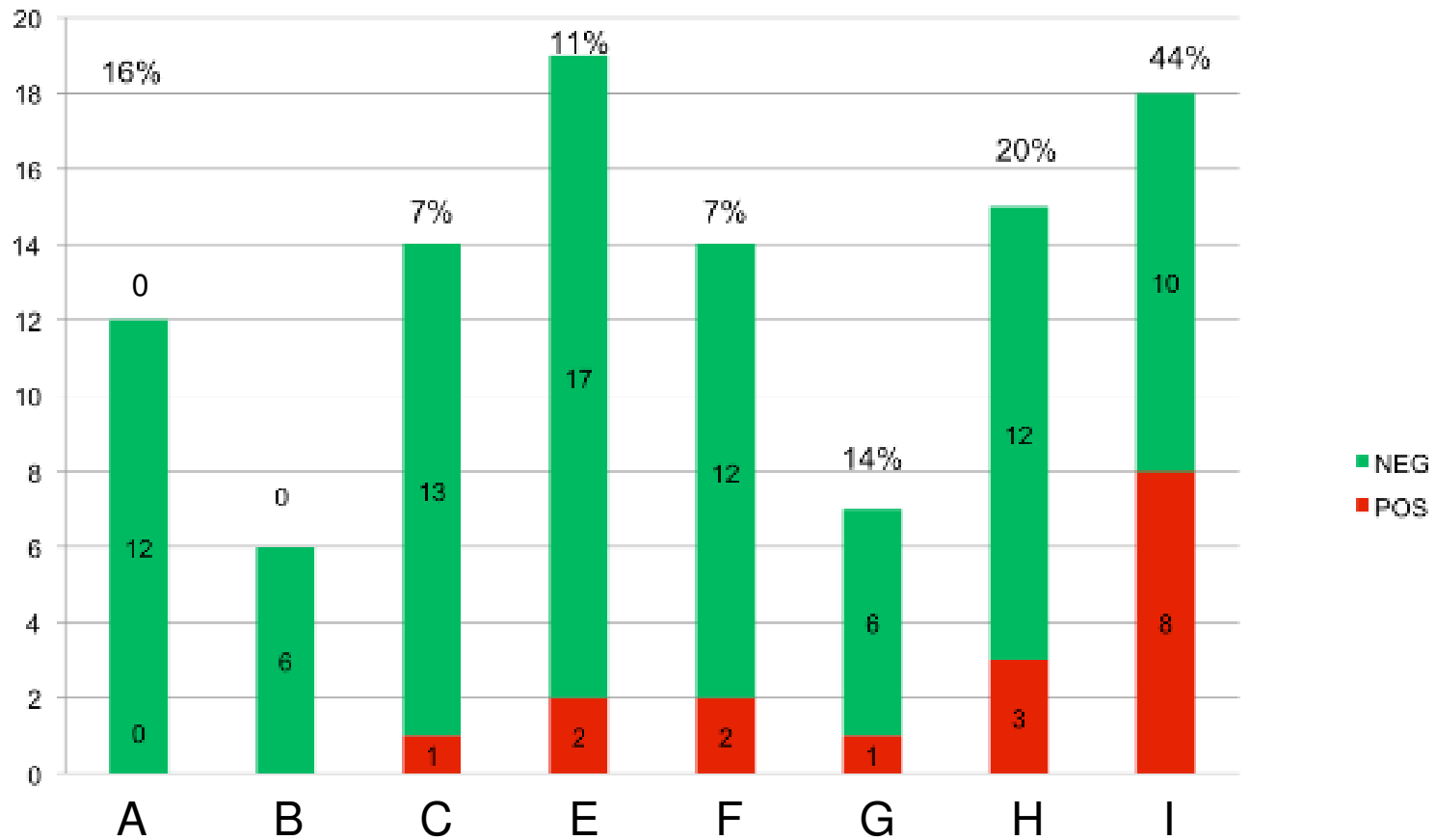


Most disease is removed after the first test

The Story at The Steyning Focus Farm

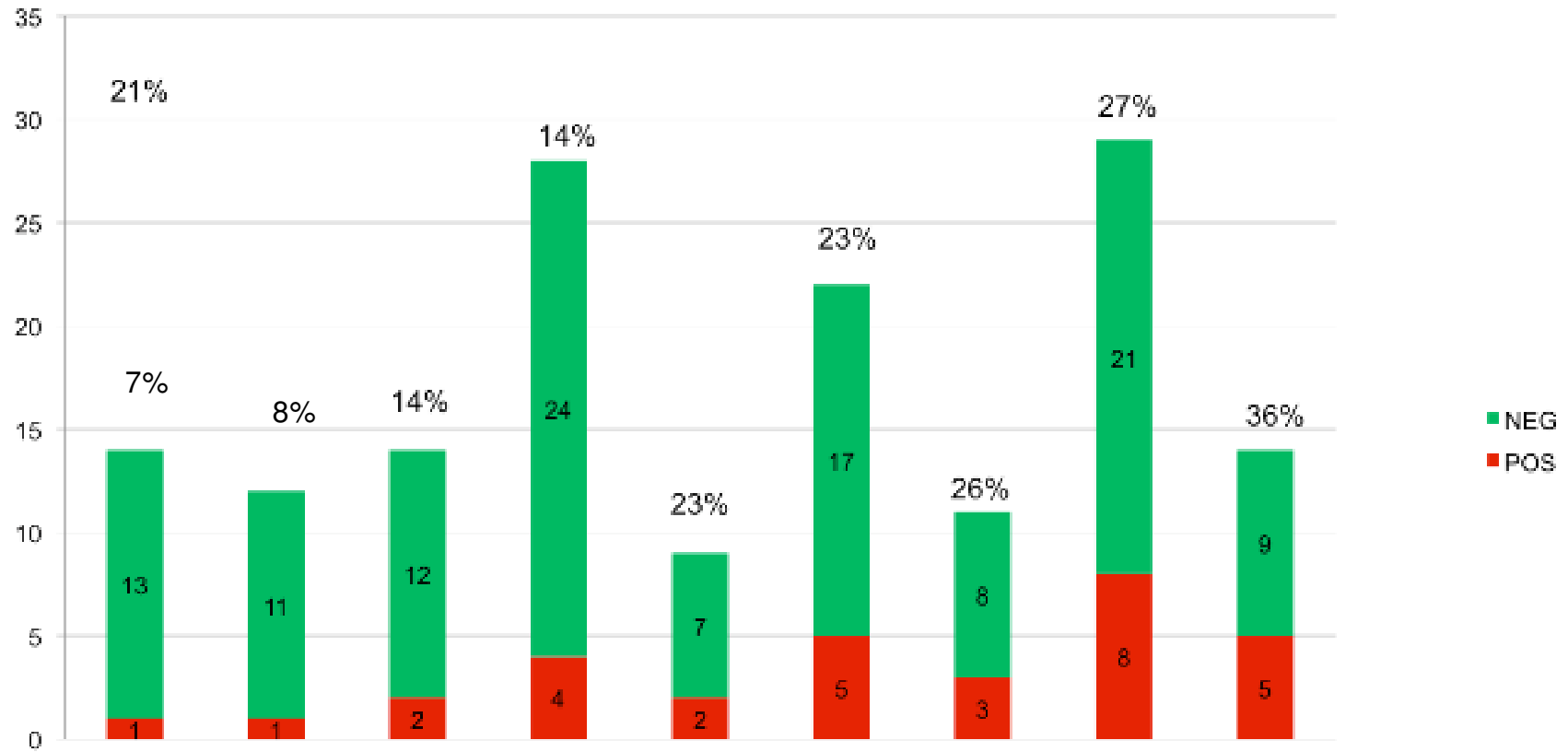


2006 R2 hinds by sire

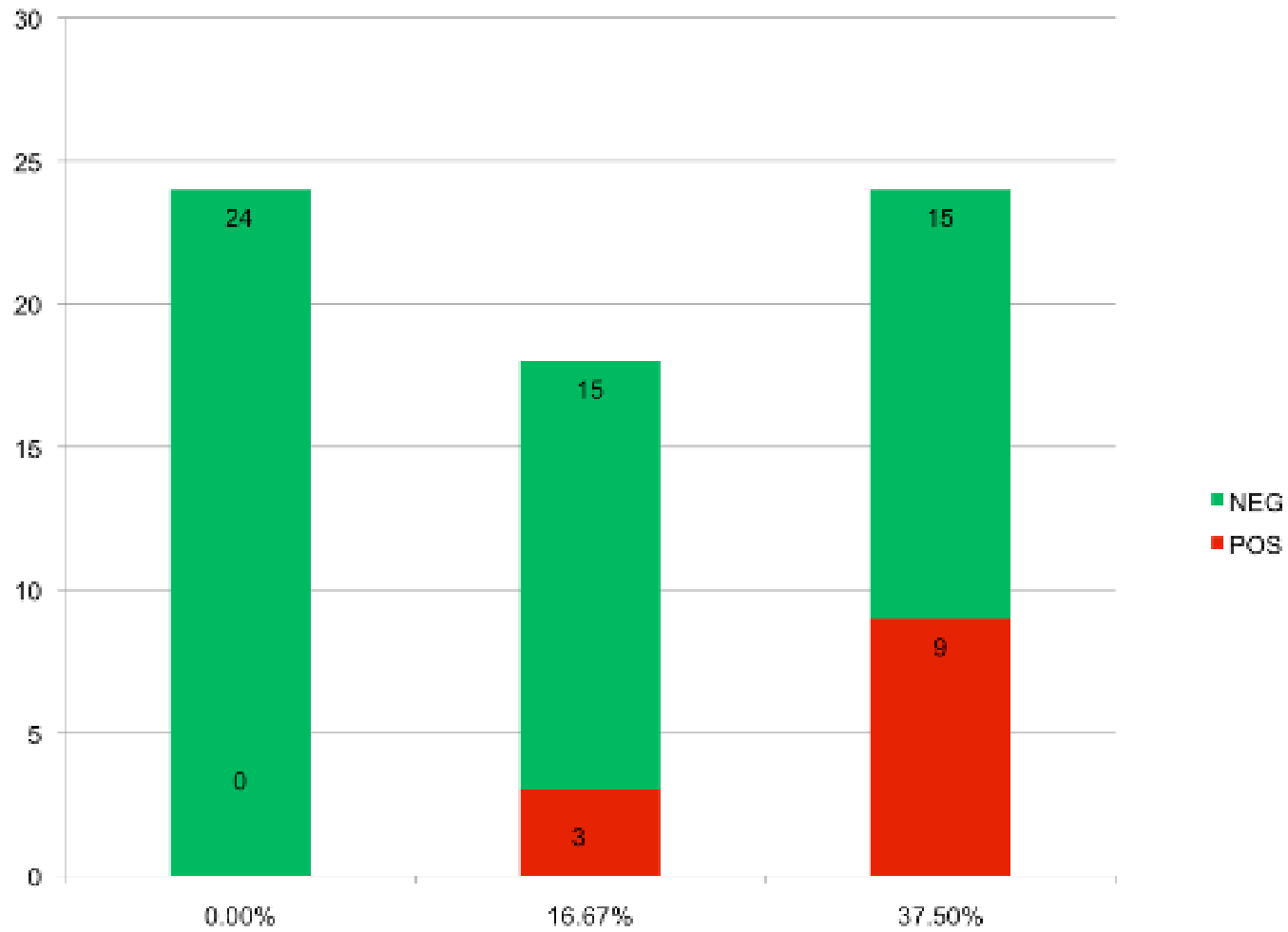


Sire A appears to be Resilient and Sire I is Susceptible

2007 R2 hinds by sire



A South Island Herd with an Outbreak of Clinical Johnes R2 hinds by sire



One sire appears Resilient and another Susceptible

A Diagnostic Curve ball !!!

Year 1 : Stud whole herd test (2000+) 23% Reactors

Future Prediction: Reactors should be <5% in Year 2

Year 2: 122 - 2yo Animals tested: 33 reactors (27%)

A Messup or Serendipity ??

- How could this be explained !!!.

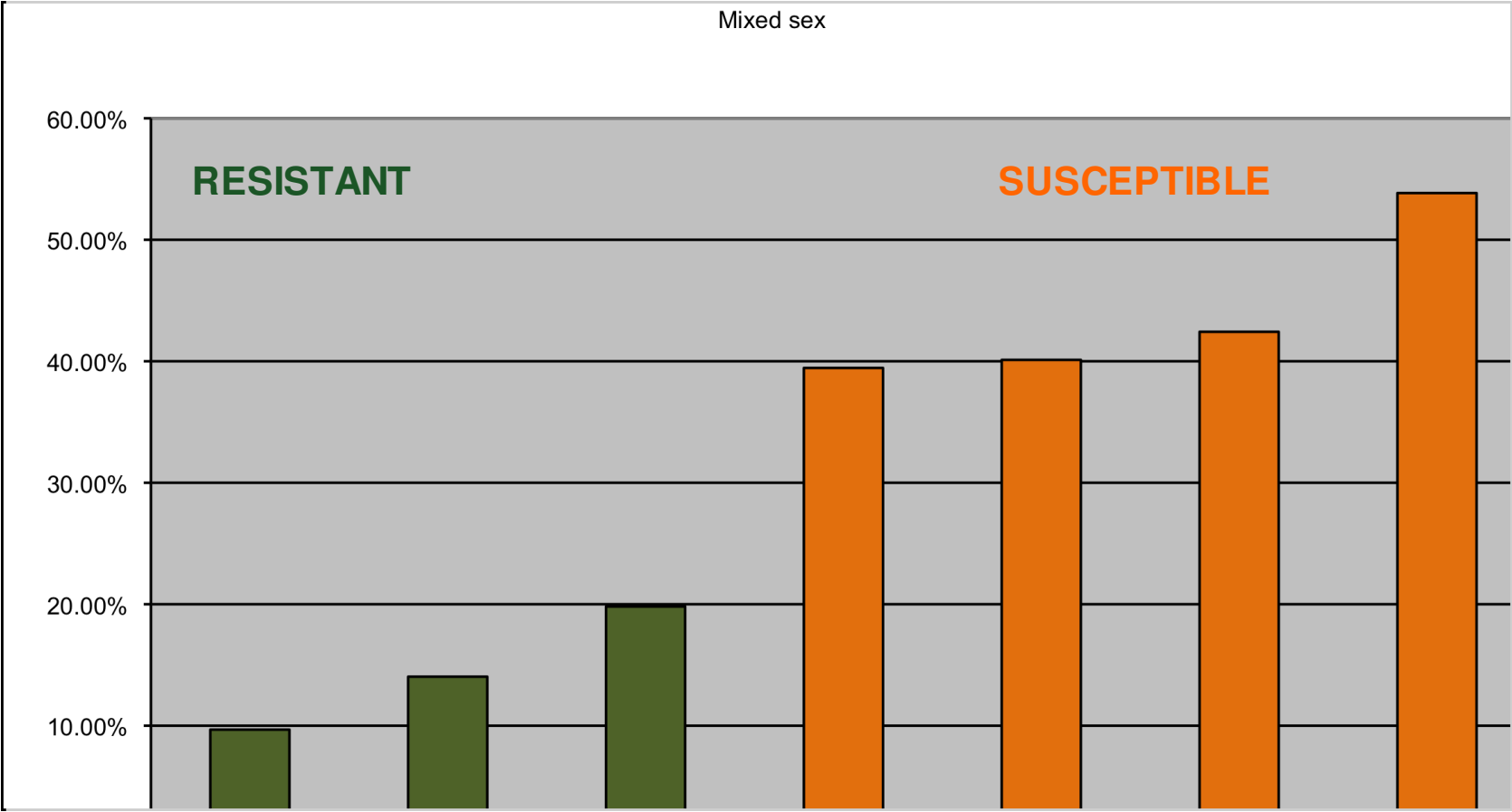
Two Pure Breeds;

Breed 1: 1 / 47 (2%)

Breed 2: 32/75 (43%)

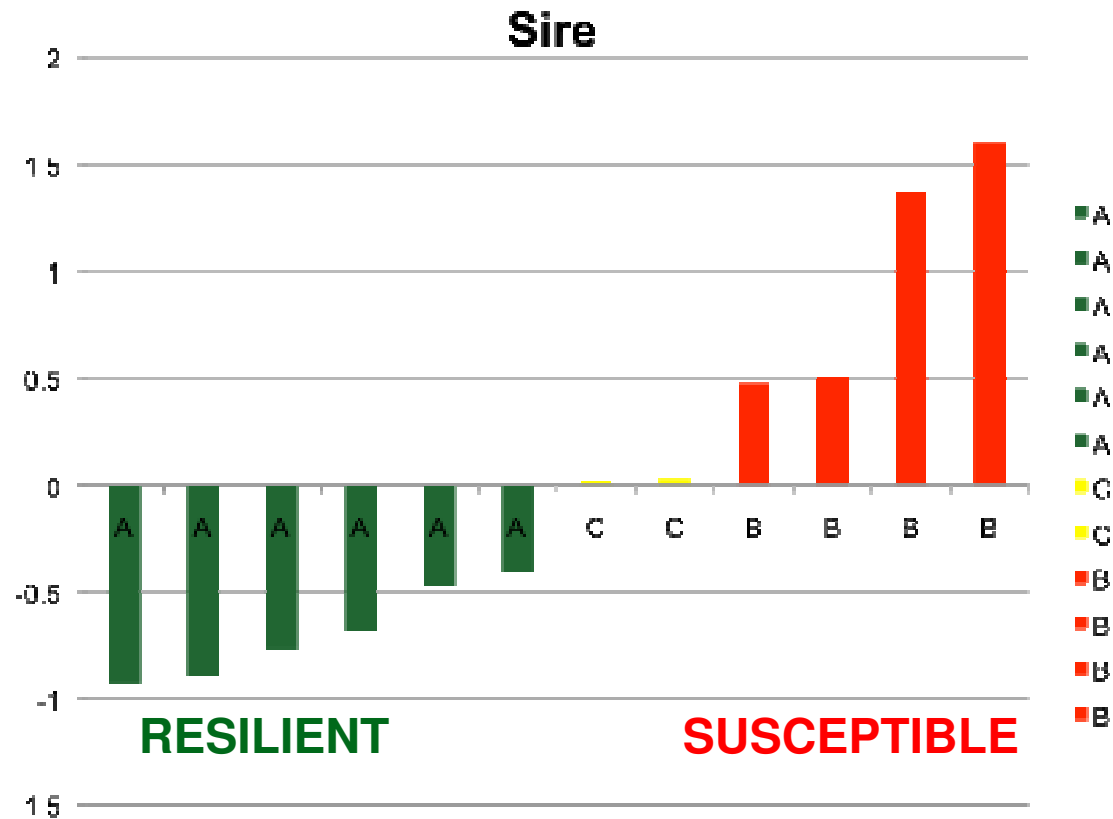
- Serendipity : Genetics must be involved !!!!!

The Answer is in the Genome !
Retrospective analysis of Reactor rates in 7 Different Deer Breeds



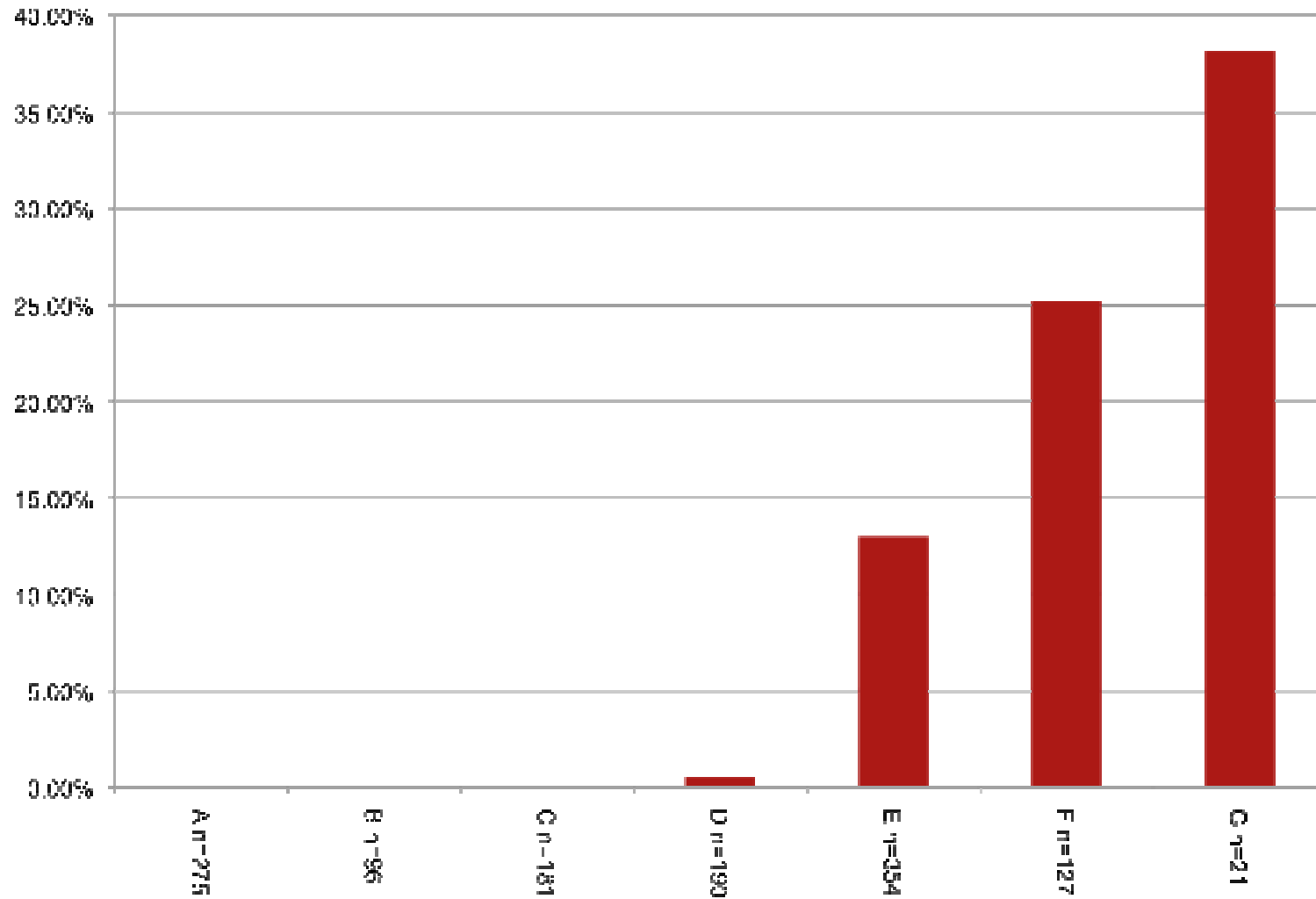
Level of seroreactivity among deer breeds

We now have Individual Breeding Values (BVs) for Resilience (-) and Susceptibility (+) to *Map* in 5257 Deer (12 Examples given)



Overall EBV = 0.27 +/- 0.06

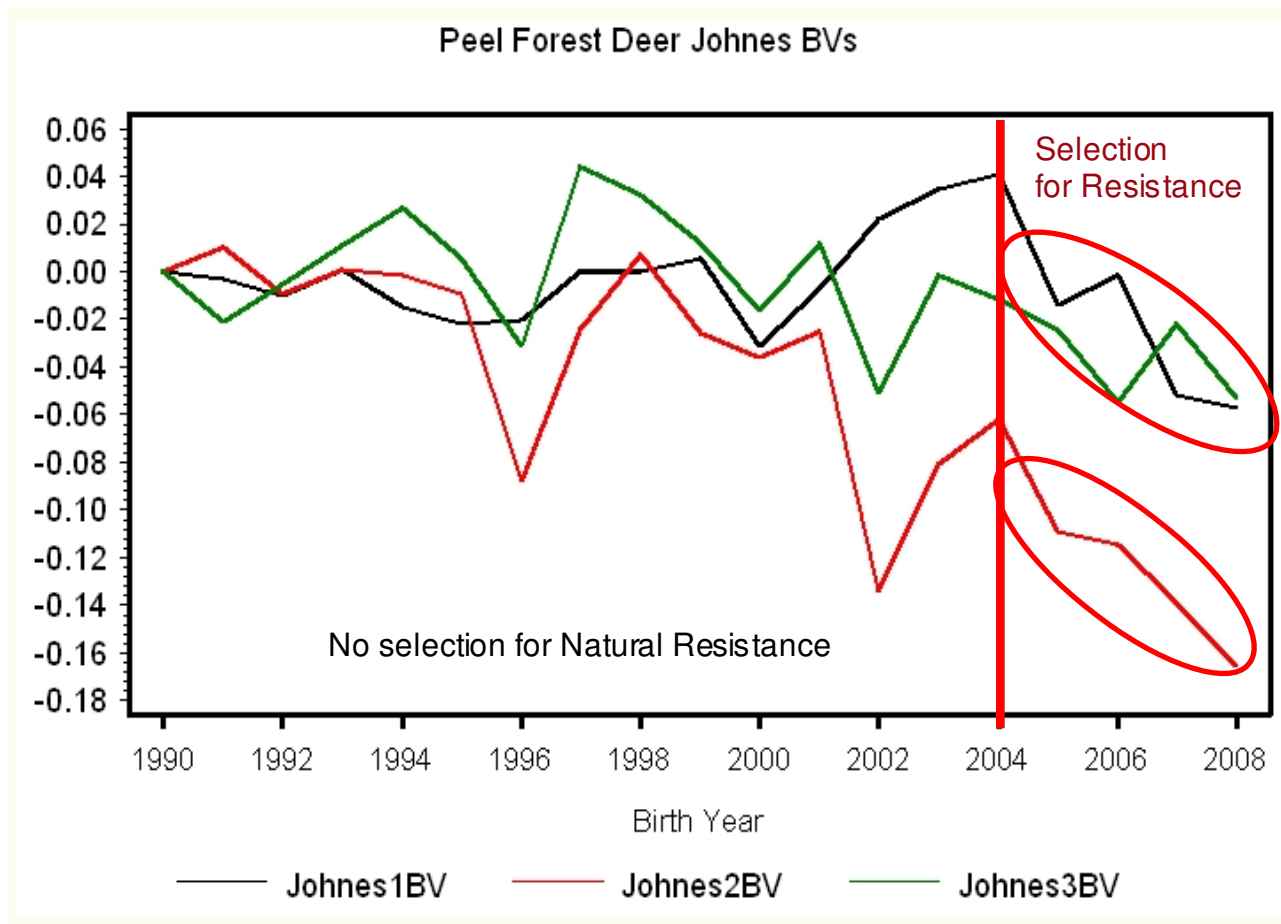
The Good (Resilient) and the Dead Susceptible !!
(Mortality rates per sire)



Genetic Progress towards Resilience using Diagnostic testing to accelerate Natural Selection

No control strategy

Diagnostic Intervention



Values < 0.00 indicate a drive towards Resilience

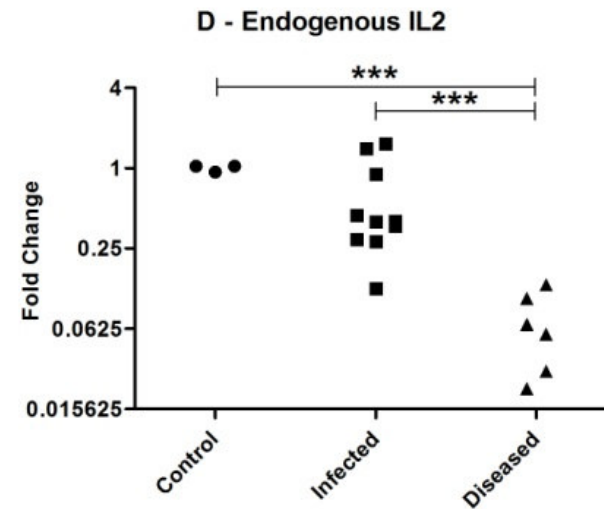
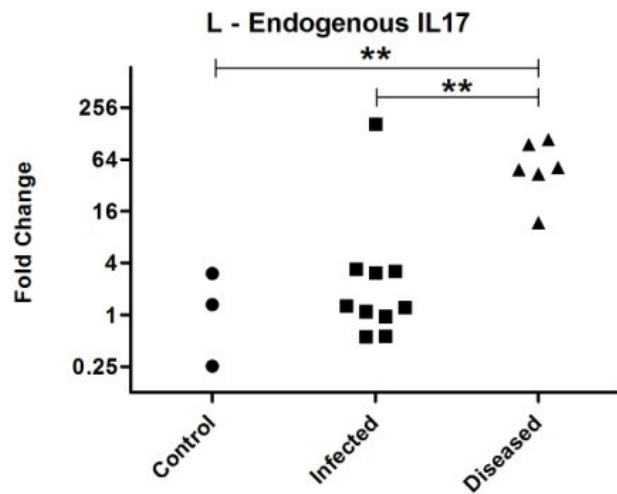
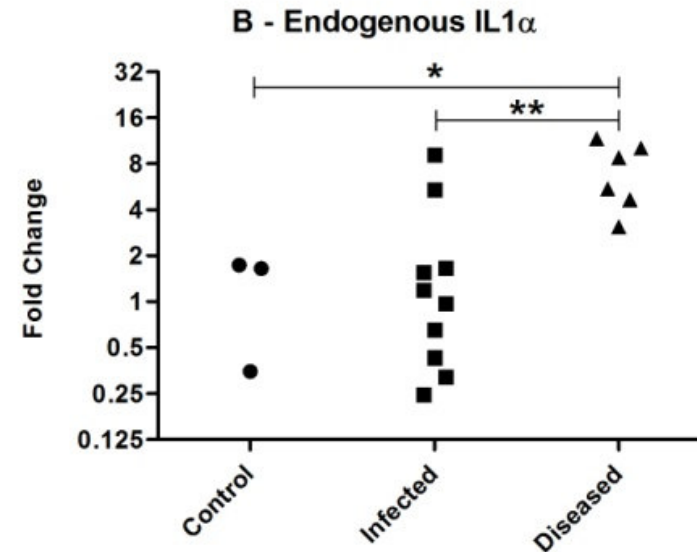
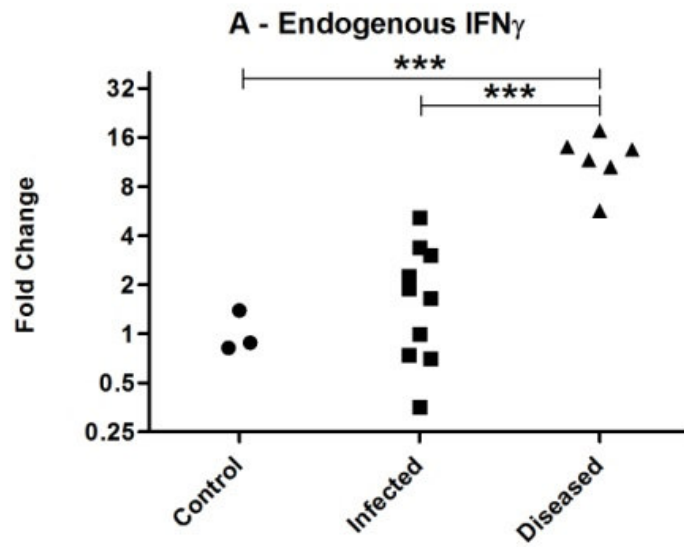
An Otago Approach to chart Immunological markers for Resistance or Susceptibility to *Map*

- **Examine candidate gene expression using rtPCR of deer peripheral blood or Lymph nodes from naturally/experimentally infected animals (Robinson *et al* 2010)**
- **Map all genes from lymph nodes of experimentally infected animals using massive parallel 454 sequencing (Mackintosh *et al* 2010)**
- **Map the transcriptome of deer macrophages by *in vitro* stimulation with *Map* (Brooke Dobson)**

Opportunities to extend *Mptb* research in deer to explore important questions in Disease Biology

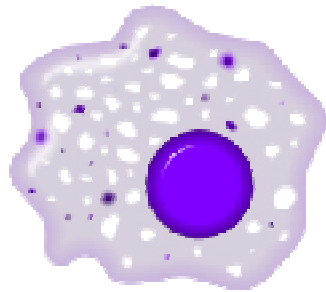
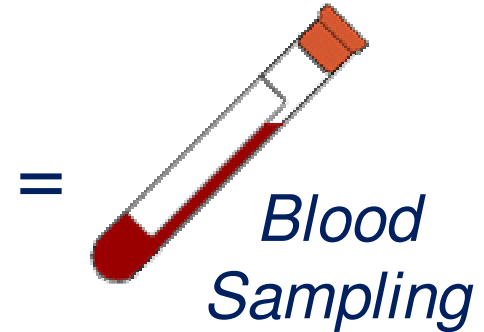
- **Purebred deer provide unique genetics to study disease susceptibility/resistance**
- **Multiple Embryos (ET >10) from deer breeds provide opportunities for classical "Progeny testing"**
- **We Need to understand the biology of disease in at least one host before we can extend our science to other species**
- **In NZ we have an experimental deer model of Johne's disease and a Molecular platform appropriate to study the immunobiology**

Candidate Gene Expression – Lymph Nodes



Gene mapping in resistant and susceptible deer

To be truly useful we need to access tissue in the live animal



Macrophage

= Haematological target of most relevance

Immune markers most easily measured via



Transcriptomics Approach?

Candidate genes can only tell us what we already *think* to look for.

Next Generation Sequencing has made dramatic strides in recent years in terms of cost and amount of RNA necessary.

RNA of quality and quantity suitable for transcriptomics work is now routinely recovered from Macrophage Derived Monocytes.

May help shed some light on differential R/S macrophage responses to Map infection.