



Johne's Disease: Diagnosis and Management systems

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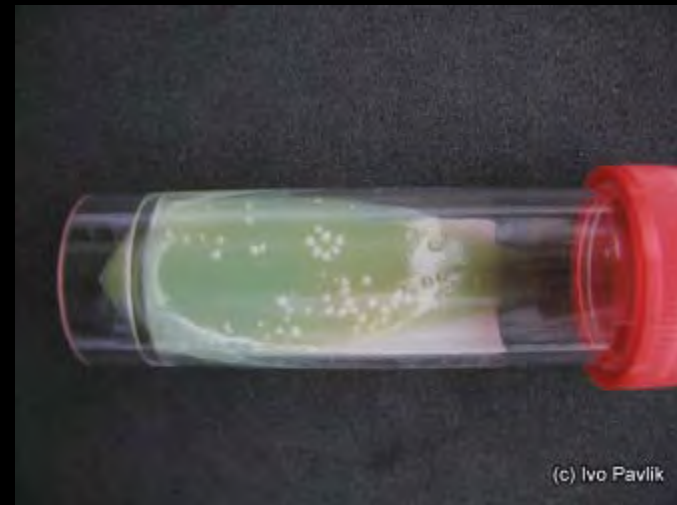
Mycobacterium avium spp *paratuberculosis* (*Map*)
causes Johne's disease



Map looks harmless

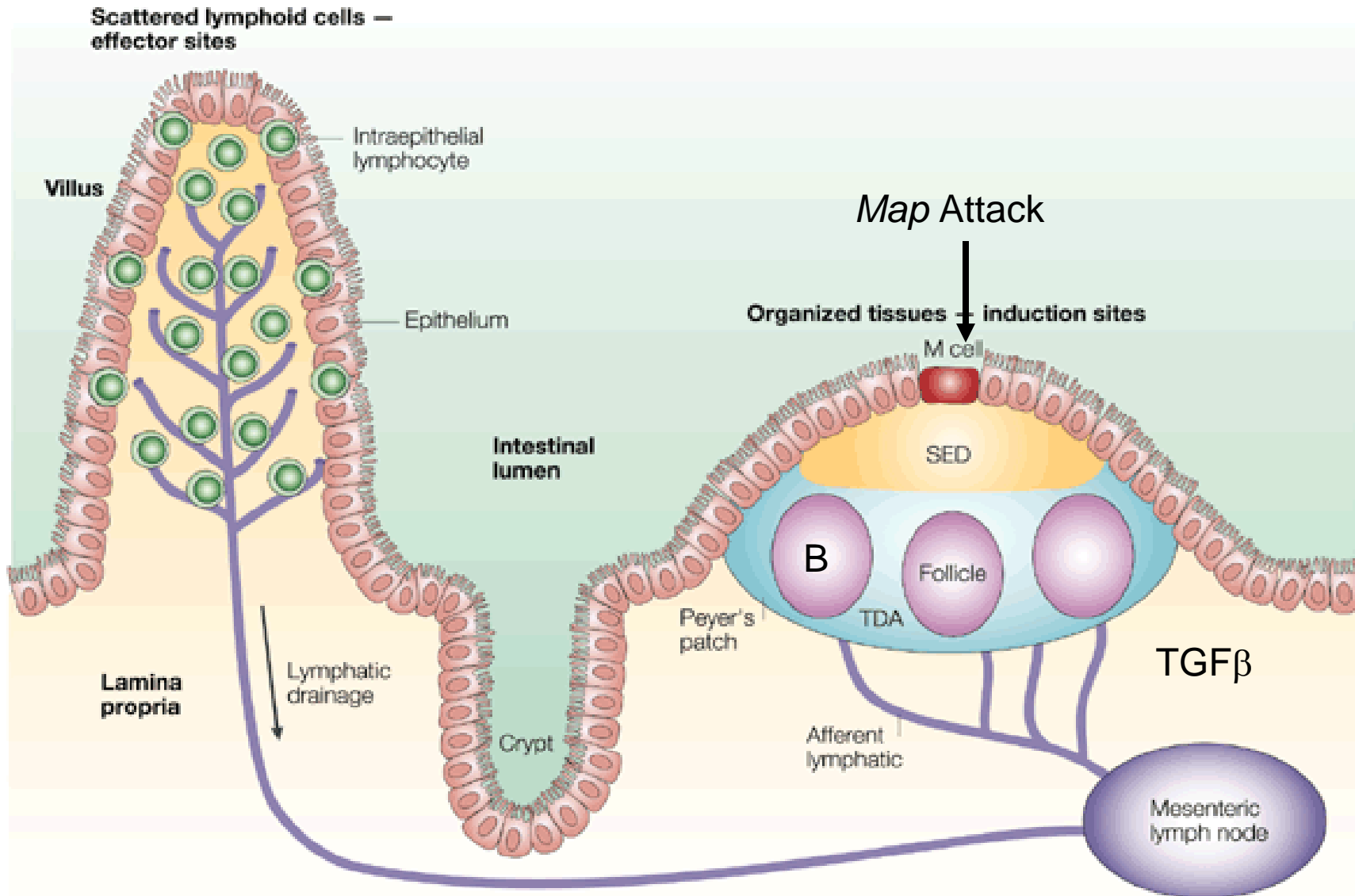


Map has waxy cell wall



Map grows very slowly

A *Map* Attack in the gut tissues of animals

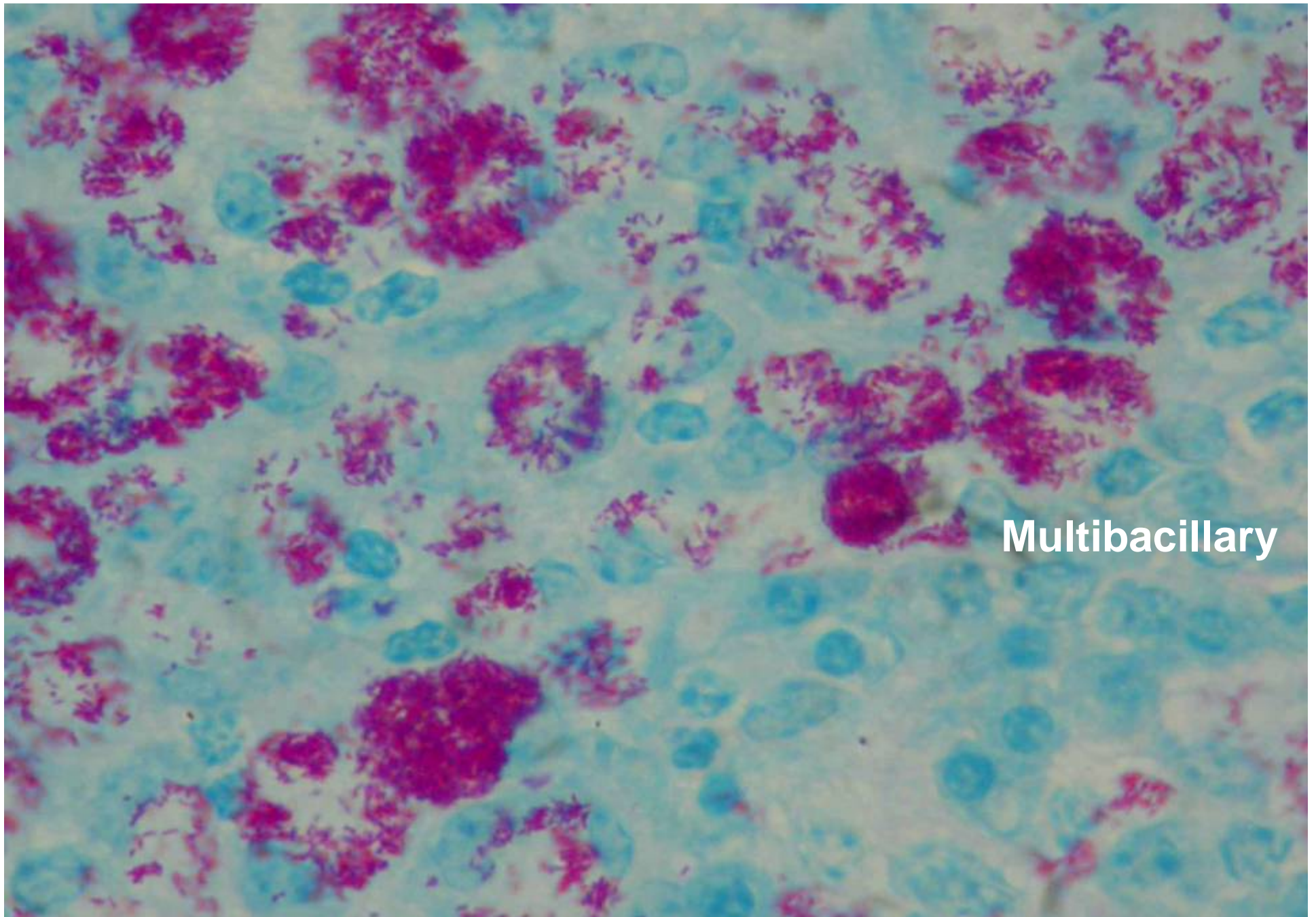


What is Johne's Disease (JD)

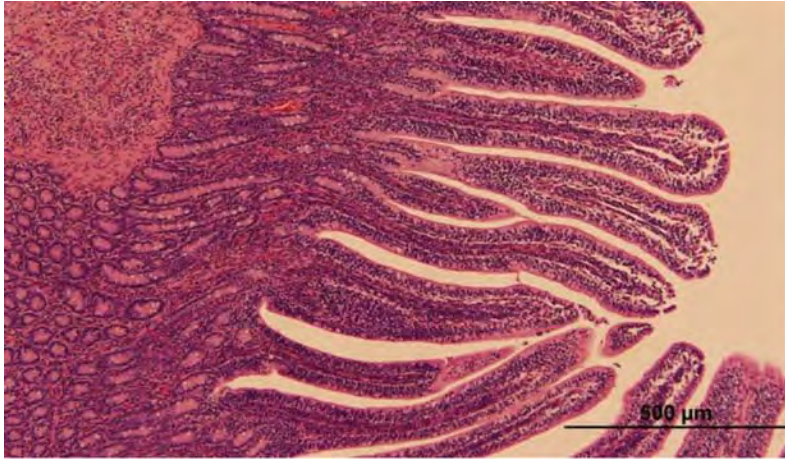
- Granulomatous enteritis caused by *Map*
- Estimated >60% NZ deer herds are infected
- No idea how many dairy herds are affected



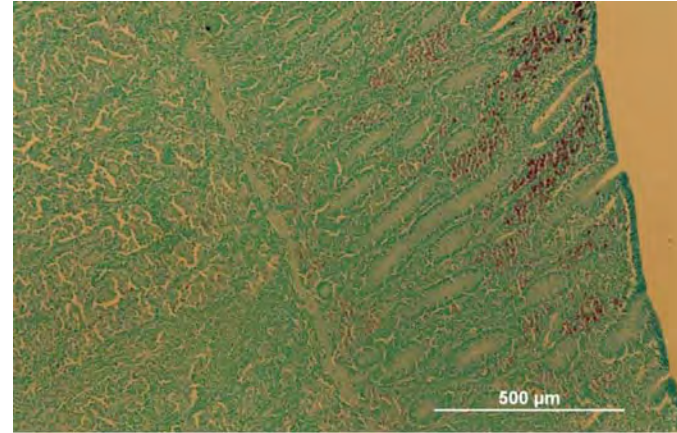
Extreme form of *Map* attack in deer gut tissues



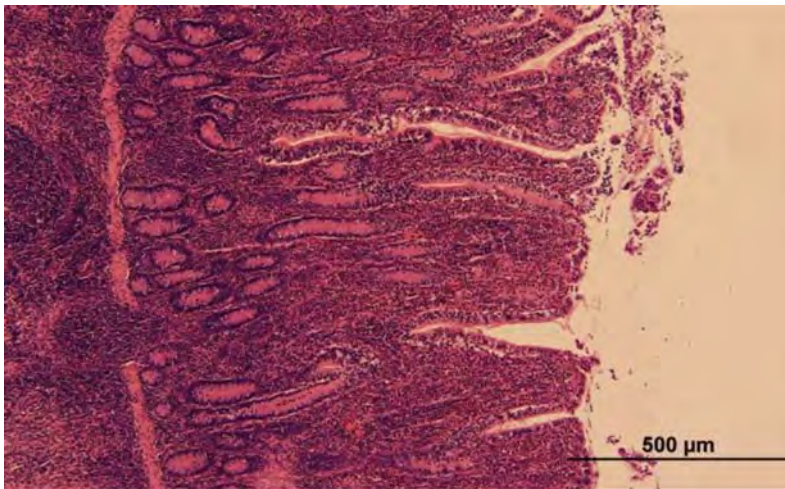
Multibacillary



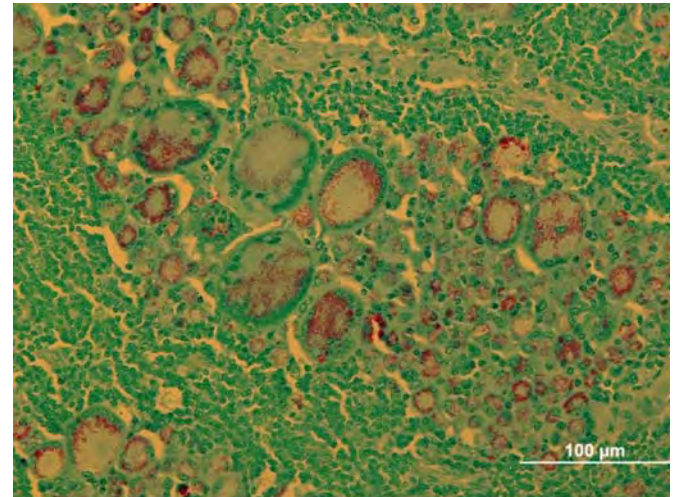
Normal gut



Bacteria (pink) in diseased gut

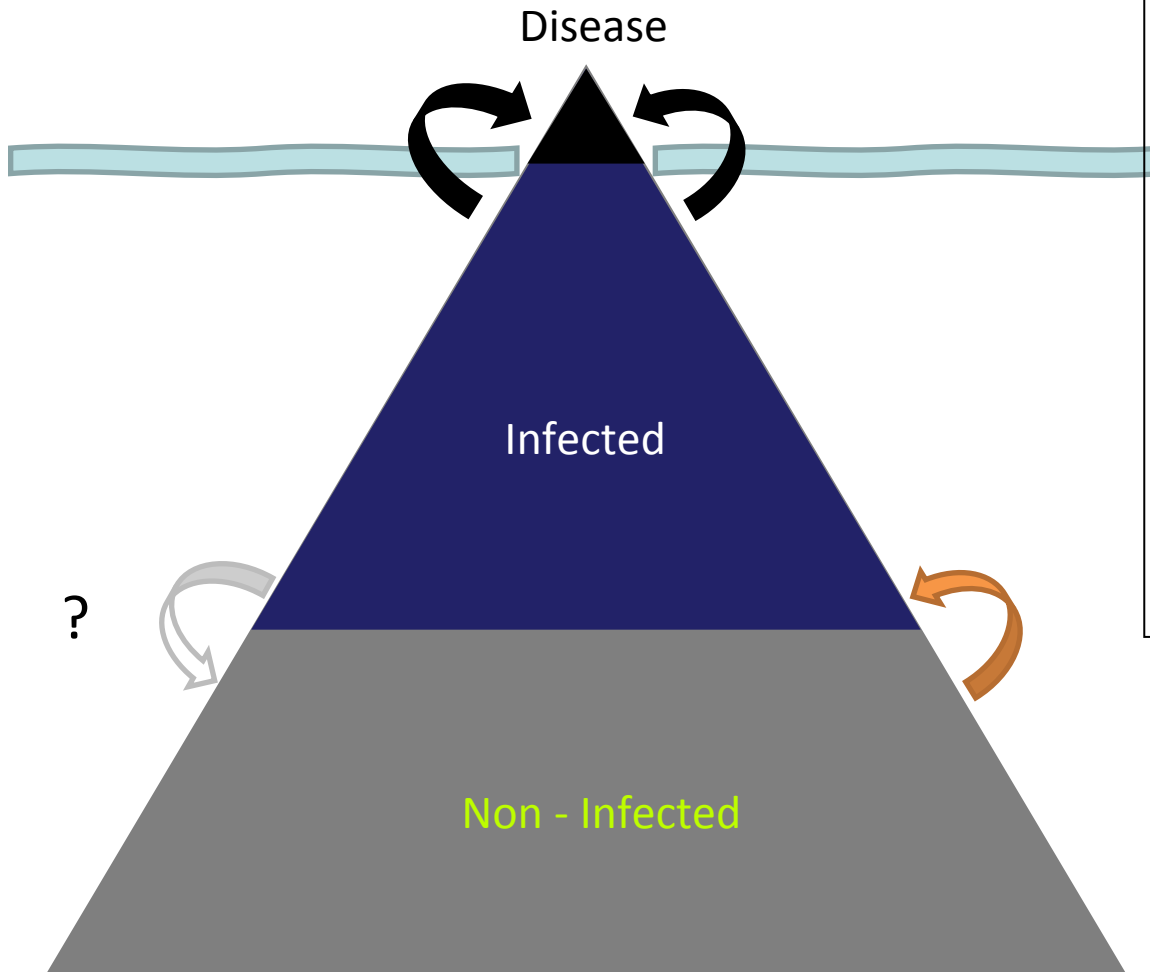


Blunted villi in Johne's diseased gut



G
i
2

Stages of *Map* Infection



Priorities for Diagnosis

Confirm JD (*Map* +)

**Predict progression
to Disease**

Detect early Infection

Mycobacterium avium* subspps *paratuberculosis **(Map)**

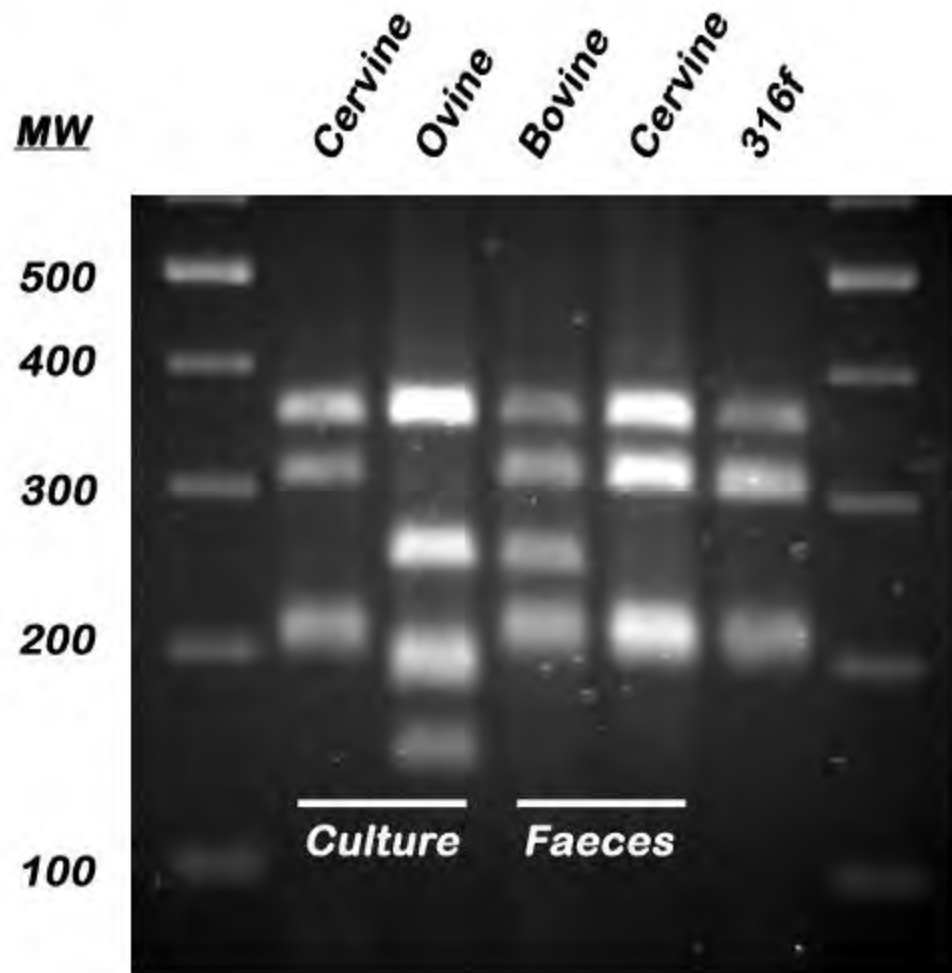
Transmission

- **Faecal - Oral**
 - Highest risk to young fawns/calves/lambs.
 - May be partially controlled by improved farm hygiene
- **Mother - Fetus**
 - **Transplacental (Mother to fetus)**
 - **Colostrum after birth - Pooled colostrum especially important in dairy herds(Mother to multiple offspring)**

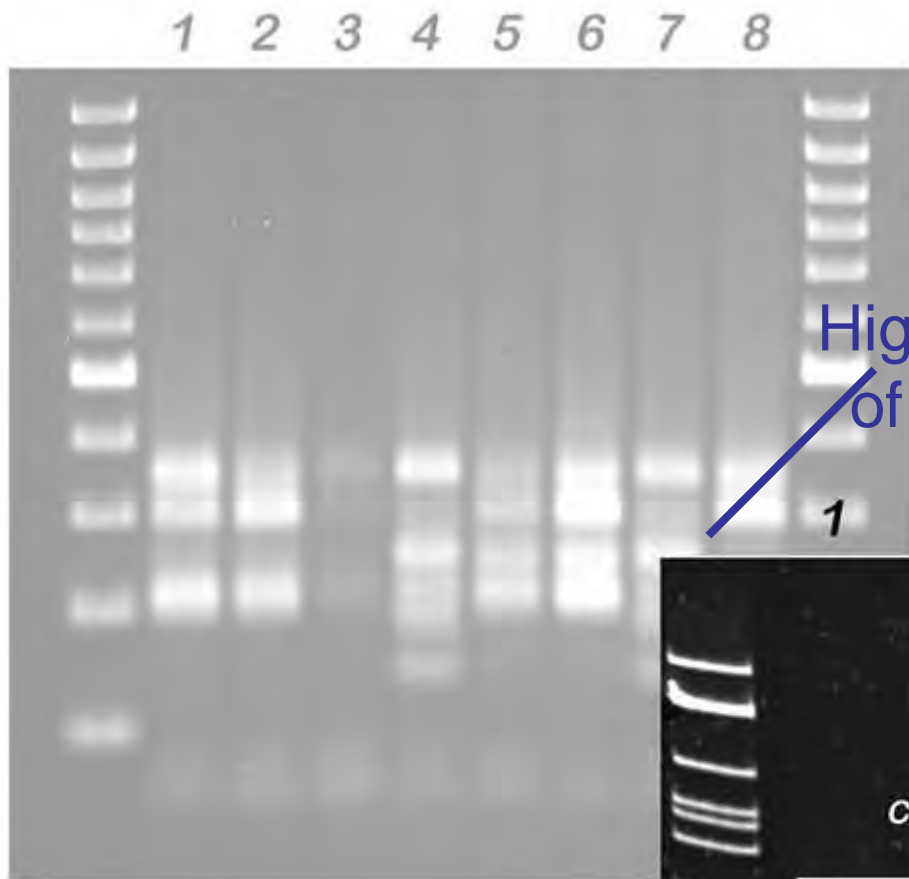
Diagnosis of Map in farmed animals

- Microbial culture
 - Slow (6-24 weeks)
 - Most sensitive with Pm tissues (***Gold standard***)
- Molecular techniques
 - PCR - issues with sensitivity (faecal inhibitors)
 - qPCR - allow for microbial quantitation
 - VNTR - strain typing
- Immune diagnosis
 - Potentially quick but questionable sensitivity (Early in Infection)
 - Specificity problems due to Cross reactivity with other mycobacteria

VNTR typing of *Map* Possible using genomic sequences

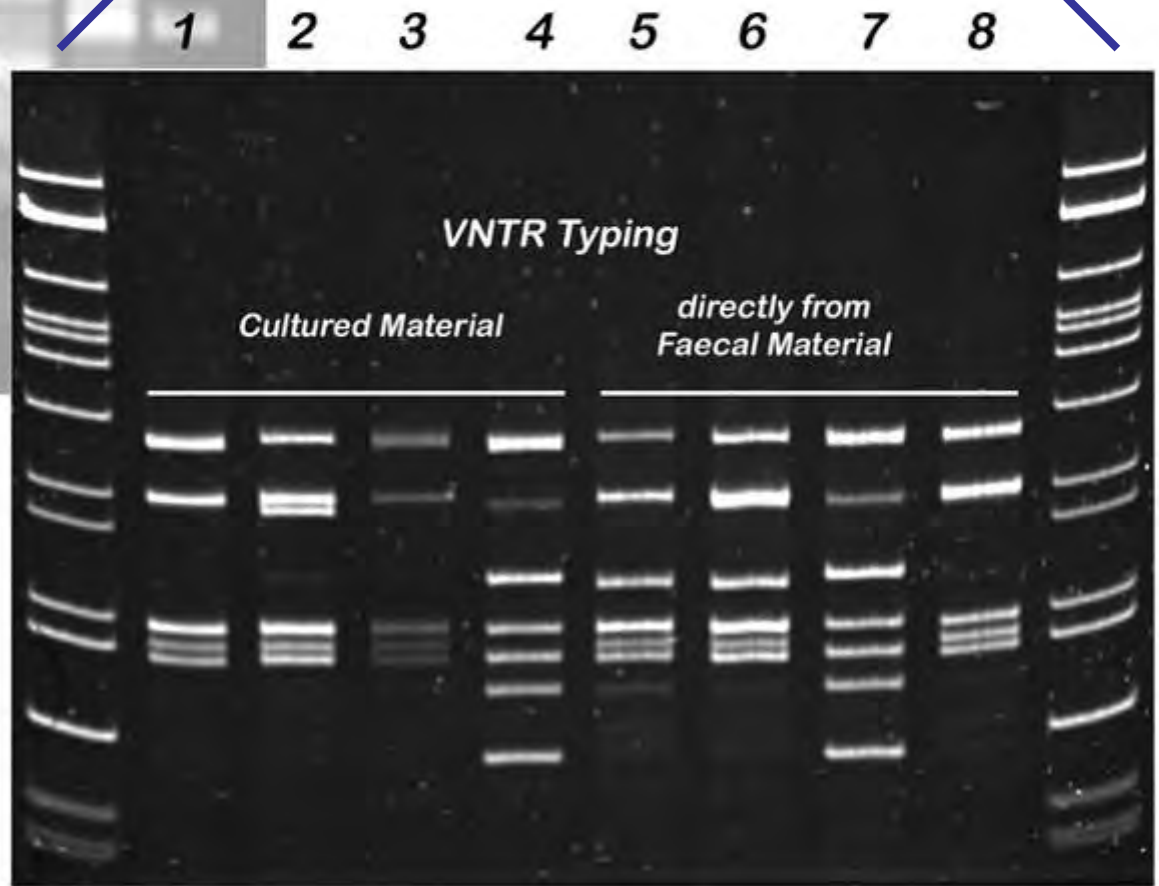


Use of 8 VNTRs allow typing of *Map* from cultures or faeces



Strain typing is feasible
(Even with faeces)

High Resolution VNTR Typing
of *Map* directly from Faeces



PAGE offers
greatly increased
resolution over
agarose gels

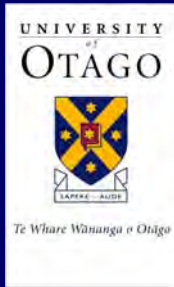
Development of An IgG1 ELISA for diagnosis of Infection

Sensitivity of Test

- No. Test (+)/Total No. *Infected*.

Specificity of Test

- No. test (-)/Total No. *Not-infected*.



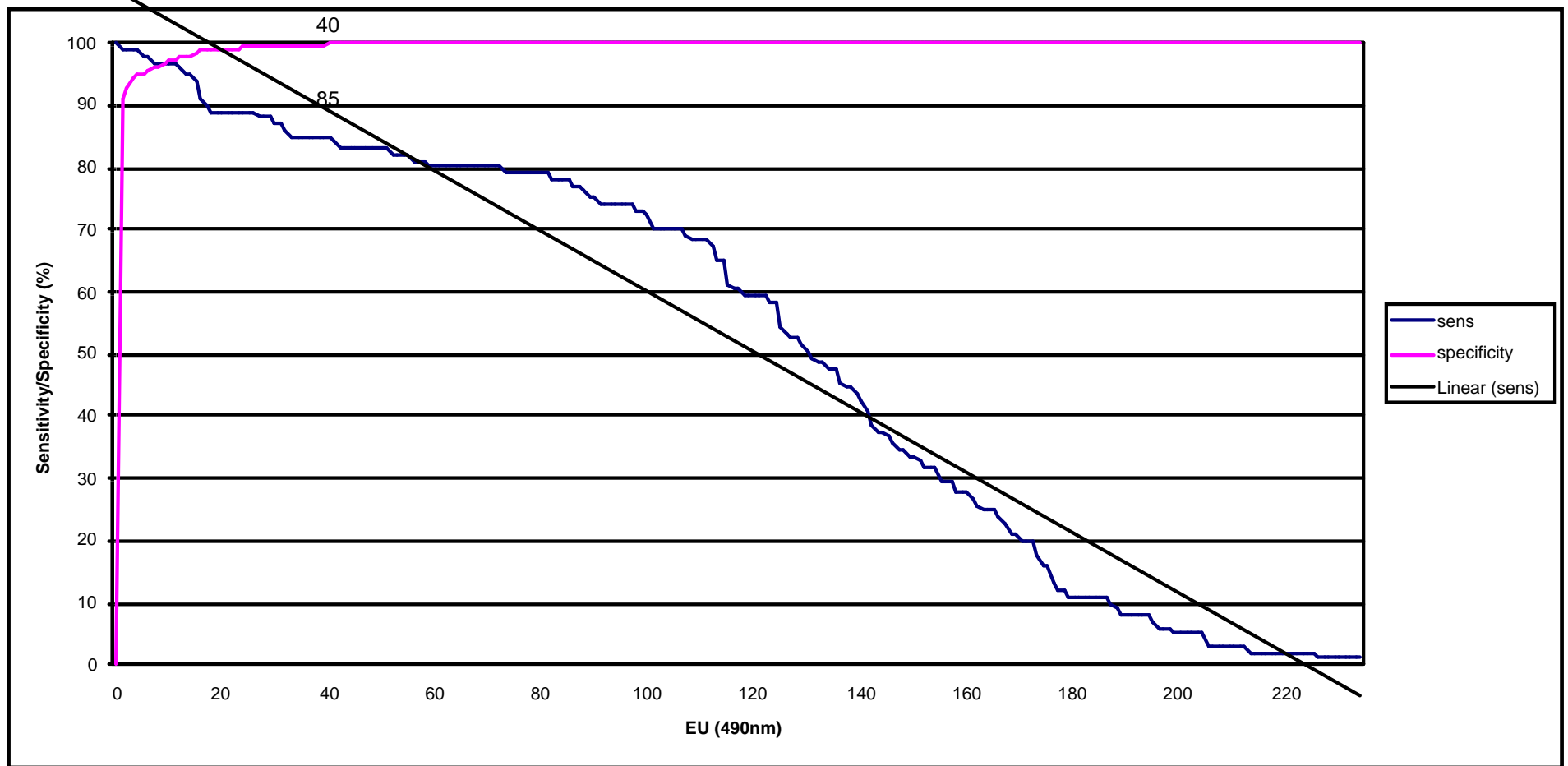
Dataset for establishing Test Parameters

Sensitivity : 300 Johne's diseased animals
(*Map positive*)

Specificity : 500 Uninfected animals

Use ROC analysis of data retrospectively
to establish cutpoints for the assay

Specificity and Sensitivity values with PpAg



There is NO “Gold Standard” for any Map test

Relationship between IgG₁ ELISA and disease severity

| | | Lesion-positive animals | | |
|-------------------|--------|-------------------------|-------|-------|
| Culture (+) | | H-1 | H-2 | H-3 |
| ELISA(+) | 77/100 | 39/43 | 37/40 | 66/67 |
| ELISA sensitivity | 77% | 91% | 93% | 98.5% |

Inverse Relationship: Antibody & Disease Severity

Existing ELISA methods are 15-25% for Culture (+) animals

Johne's Disease: The Tip of the Map Infection Iceberg

| Farm | No. Killed | Johne's disease | <i>MAP</i> +ve |
|-------|------------|-----------------|----------------|
| A | 24 | 1 | 21 |
| B | 10 | 3 | 10 |
| C | 17 | 1 | 17 |
| D | 10 | 0 | 10 |
| E | 12 | 0 | 12 |
| F | 13 | 1 | 13 |
| G | 15 | 1 | 15 |
| H | 15 | 1 | 12 |
| Total | 116 | 8 (6.9%) | 110 (94.8%) |

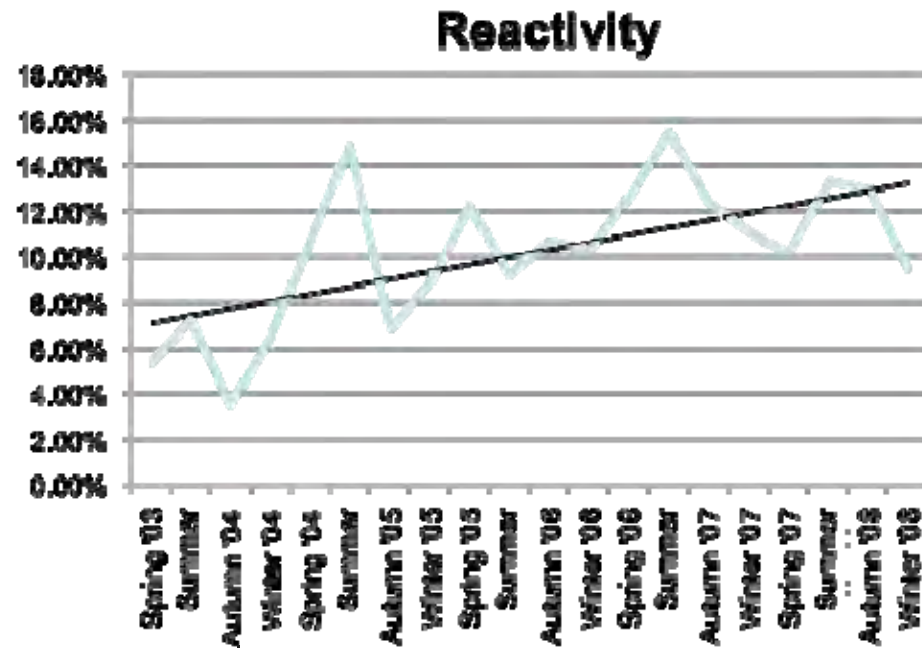
**Most JD animals would be undetectable at Meat Inspection
Paralisa test has a Positive Predictive value > 95%**

Proportion of Herds with Jd Reactors

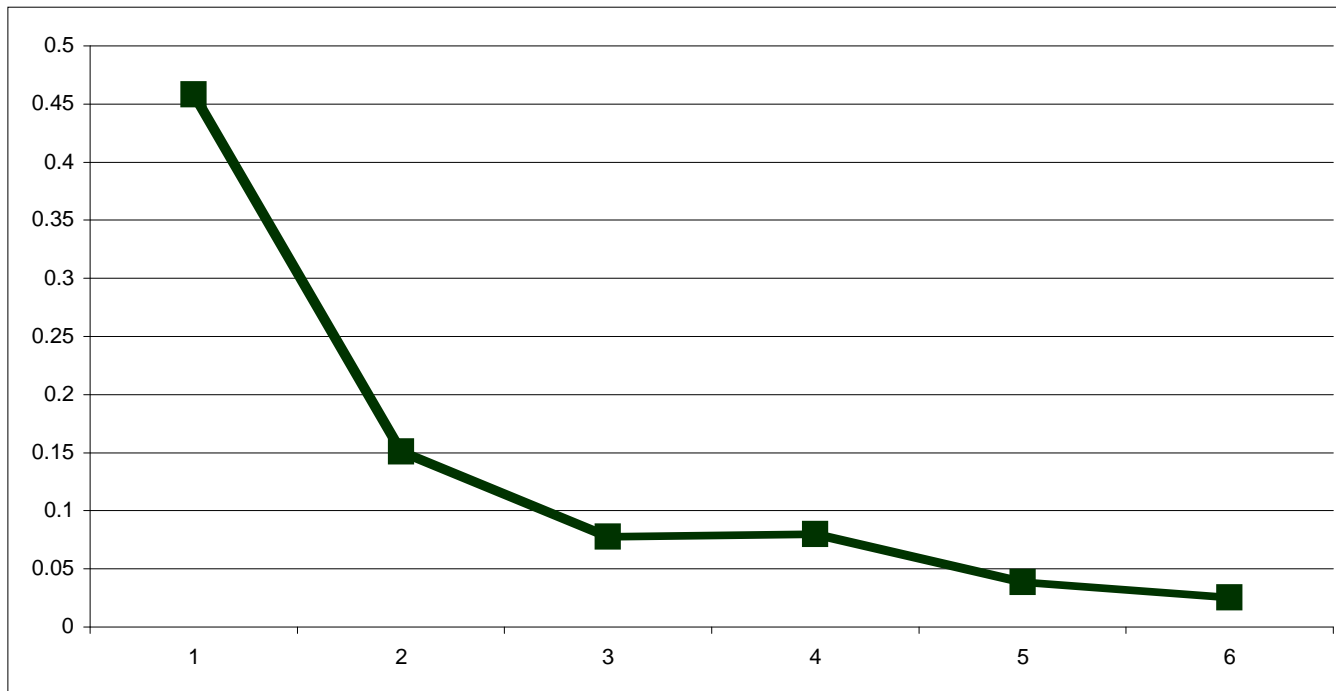
| | Herds Tested | Number <i>MAP</i> (+) | % |
|----|--------------|-----------------------|-------|
| NI | 122 | 76 | 62.3% |
| SI | 525 | 332 | 63.2% |

❖ ***Map*** infection is widespread equally throughout
New Zealand

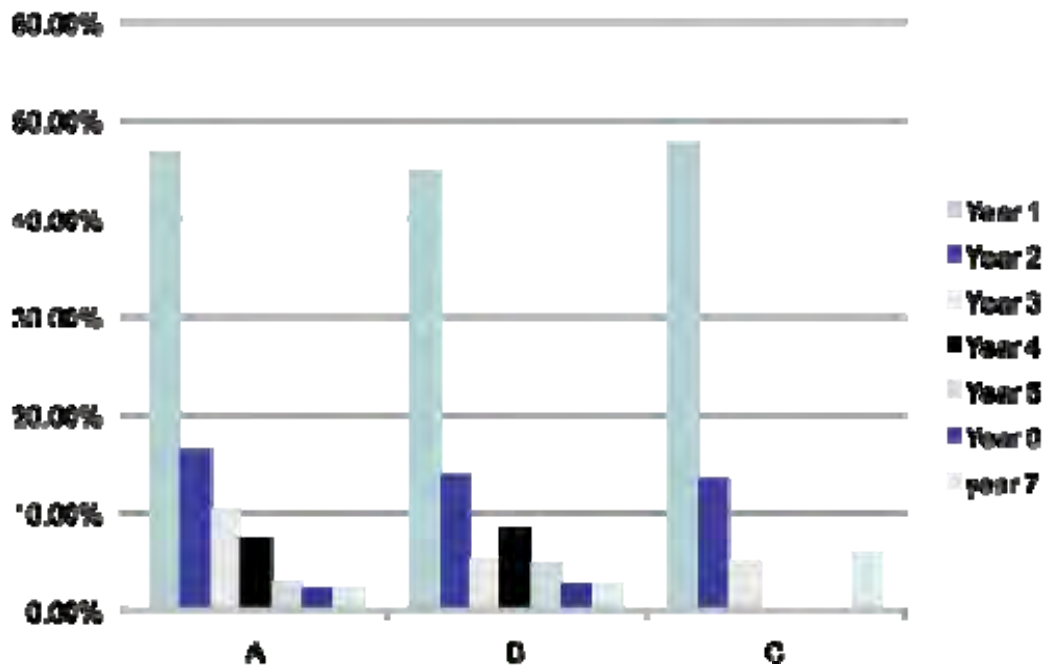
Seroreactivity has increased incrementally since 2003



Rates of seroreactivity in 5 herds (6,000 animals) with a PLAN



Test and Cull !

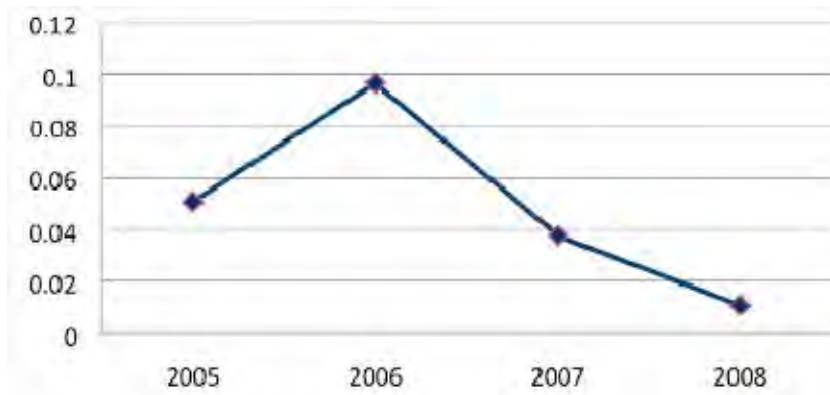


strategy works BECAUSE test sensitivity is high (80%)

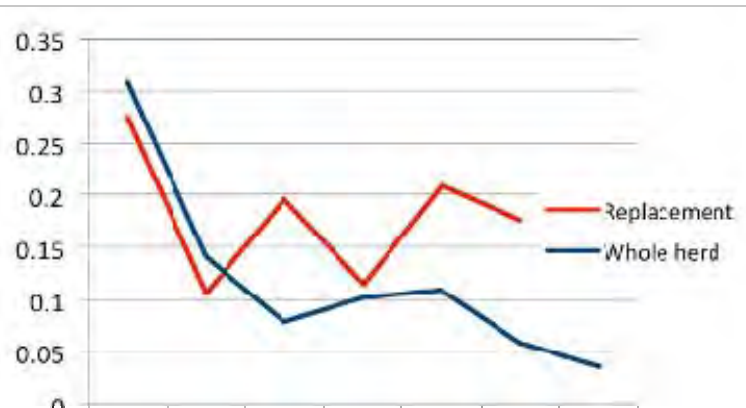
Comments by owner of Herd A

Looking back I was shocked the herd had such a high incidence of positives at the first test. This was due to self denial of the seriousness of the disease, and there was No proven remedy. The results of using the Paralisa have been fantastic considering the first year we used 100% of the cut point, and the next year we only tested the animals that were between 50 and 100% of the previous year. The velvet herd have not been redone except for 2yr olds going in. The whole hind herd was redone in 2003 and 100% replacements since. I am sure the incidence could drop to almost zero if I paralisa the whole herd and this just becomes

Control in a Low Incidence Herd



The Benefit of Whole herd testing



Contribution Diagnostics could make at the herd level

Removal of 'shedder' animals - Reduce spread of *Map*

Removal of 'subclinicals' - Minimise production losses

Accelerate removal of 'susceptible' animals

Increase proportion of 'resistant' animals

**Diagnostics will reduce disease prevalence and infection,
but may not eradicate infection**

Suggested use of Diagnostics in a Conventional Production Herd

Use your commonsense to determine if *Map* is present in your herd

Weight loss/ouring/ill-thrift

Unusually high TB test reactor rate

IF YOU ARE CONVINCED THERE IS NO PROBLEM THEN THERE'S NO PROBLEM

If you suspect *Map* is likely to be present but are unsure of relative risk

Test a subgroup (100 m/a Hinds and breeding Stags)

If reactor rates are >5% do Whole herd test

Test ALL Reactors

Repeat test R2s for at least the following year

Remember that a Healthy JD(+) reactor is worth about \$500 in the works

It will be worthless (and actively spread *Map* if it progresses to Clinical

New Experiences in a Stud Herd

1st test produced 26% Reactors

Expectation for farmer Year 2 test should yield <5% Reactors

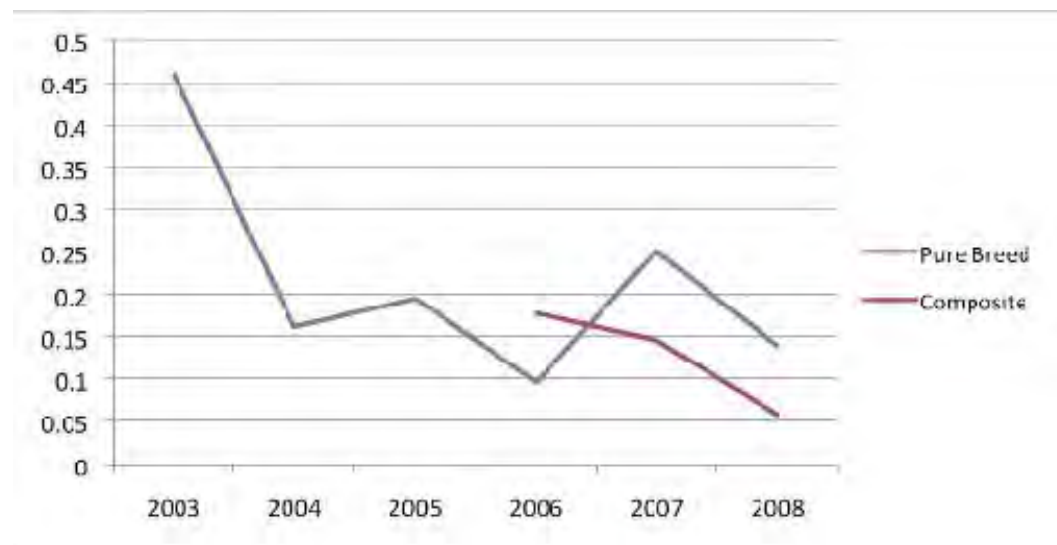
2nd Results : Subgroup of 91 animals Tested – 20 reactors (22%)

Immediate suggestions

- Griffin Con man
- Test doesn't work

Check 18/46 (39%) reactors Breed A; 2/45 (4.5%) reactors Breed B

Control in herds with Composite vs Purebred



opportunities to study Map Resistance/Susceptibility in Deer

We have Access to Breeds of deer that display polarised resistance or Susceptibility to *Map* infection

We have access to multiple embryos from individual dam-
e progeny of unique genetic purity

We have developed qPCR techniques to monitor expression
cell receptors, cytokines and activation factors

We have the credibility to access deer samples form NZs
mmer deer herds based on our diagnostic platform.

Unique opportunities studying *Map* infection in deer

Polar expression of *Resistance & Susceptibility* traits

Orid Immune & pathological responses

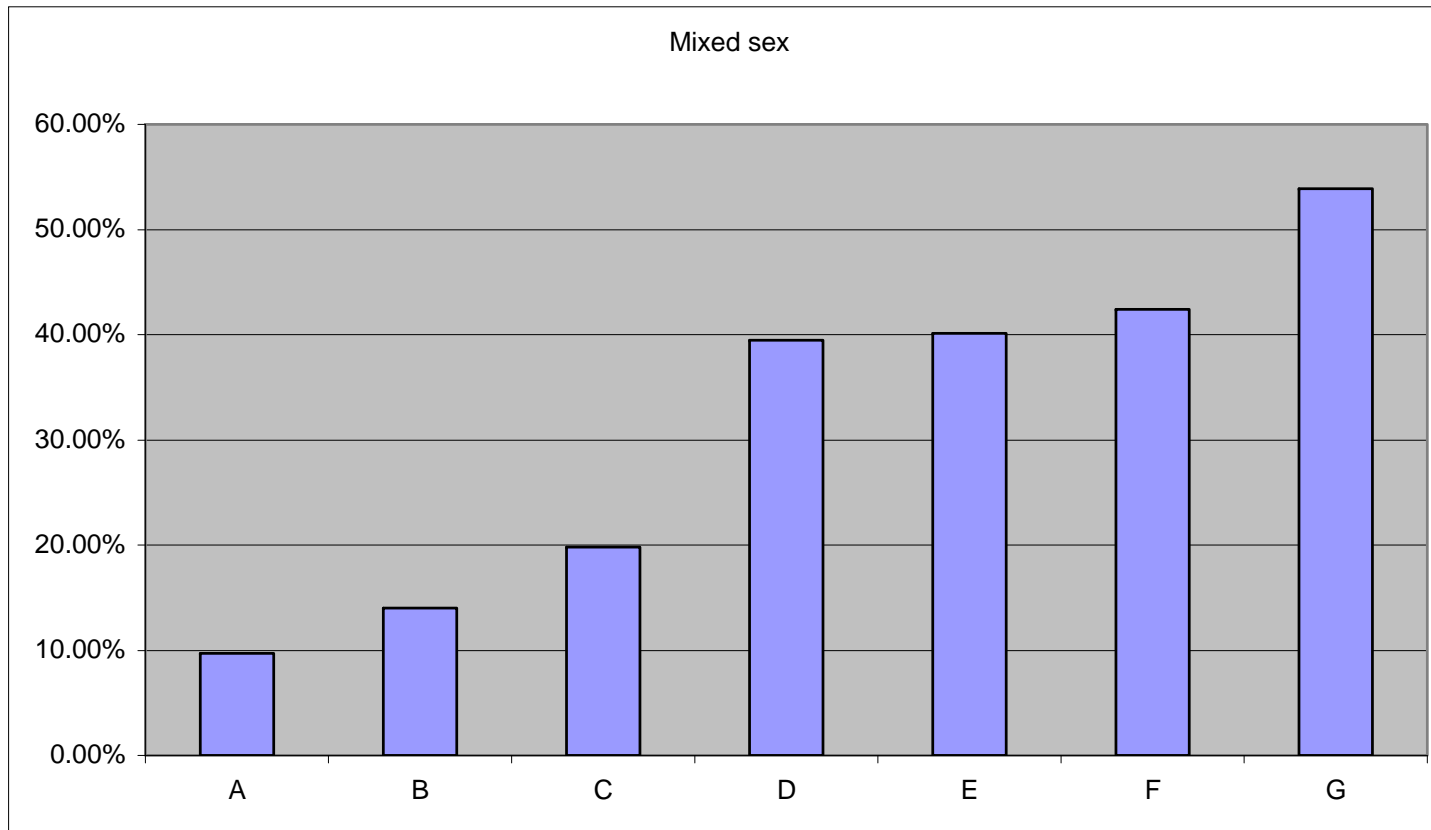
**Reproducible Experimental Model for JD , and presentation of
sease in Young animals**

**Gene breeding of Deer to select for individual traits
(elvet/Venison)**

**Multiple Embryos (1,000s) to study host genotype and provide an
excellent model to study heritable resistance**

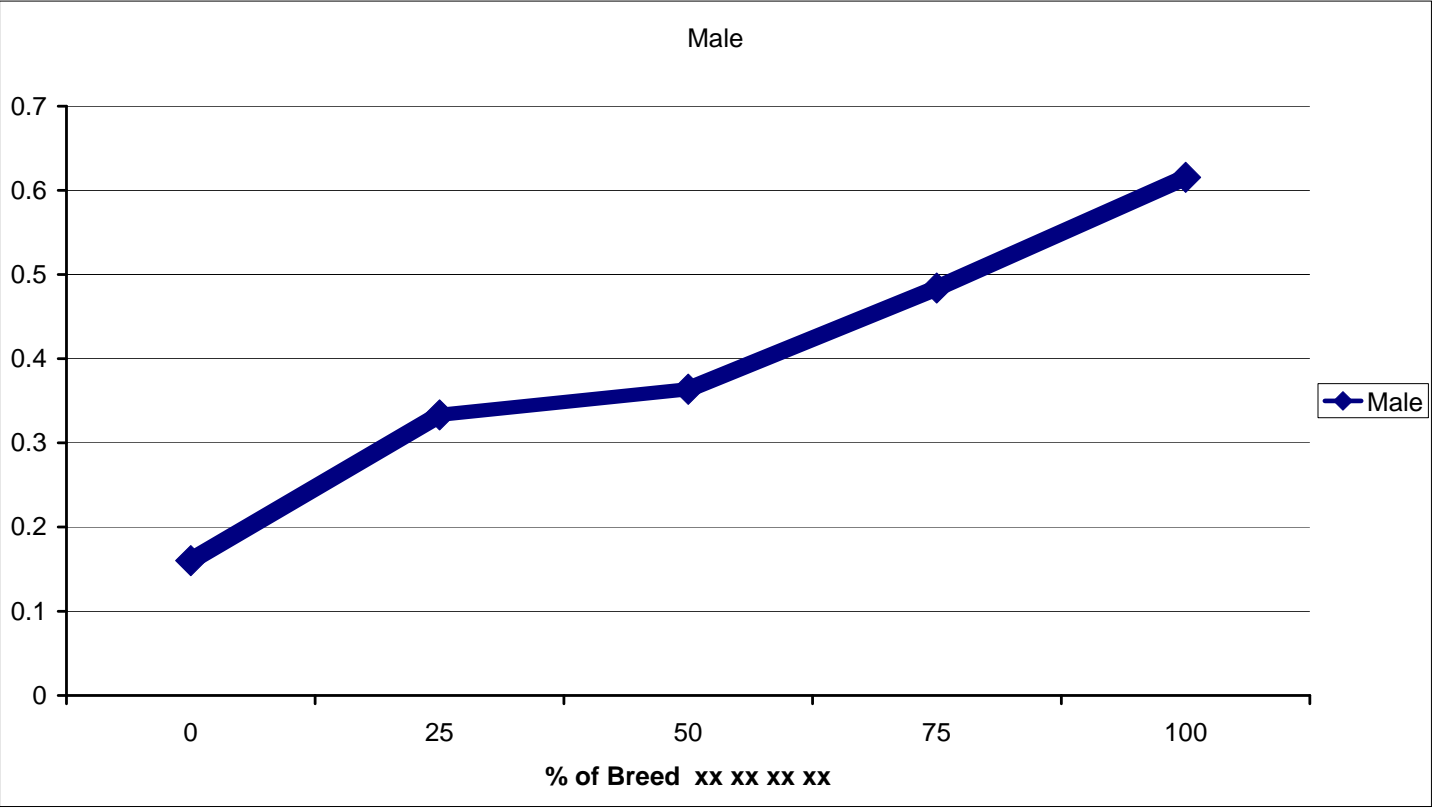
The Genome

Deer Breed (A - G) effects on susceptibility



Level of seroreactivity among deer breeds

A heritable Genome



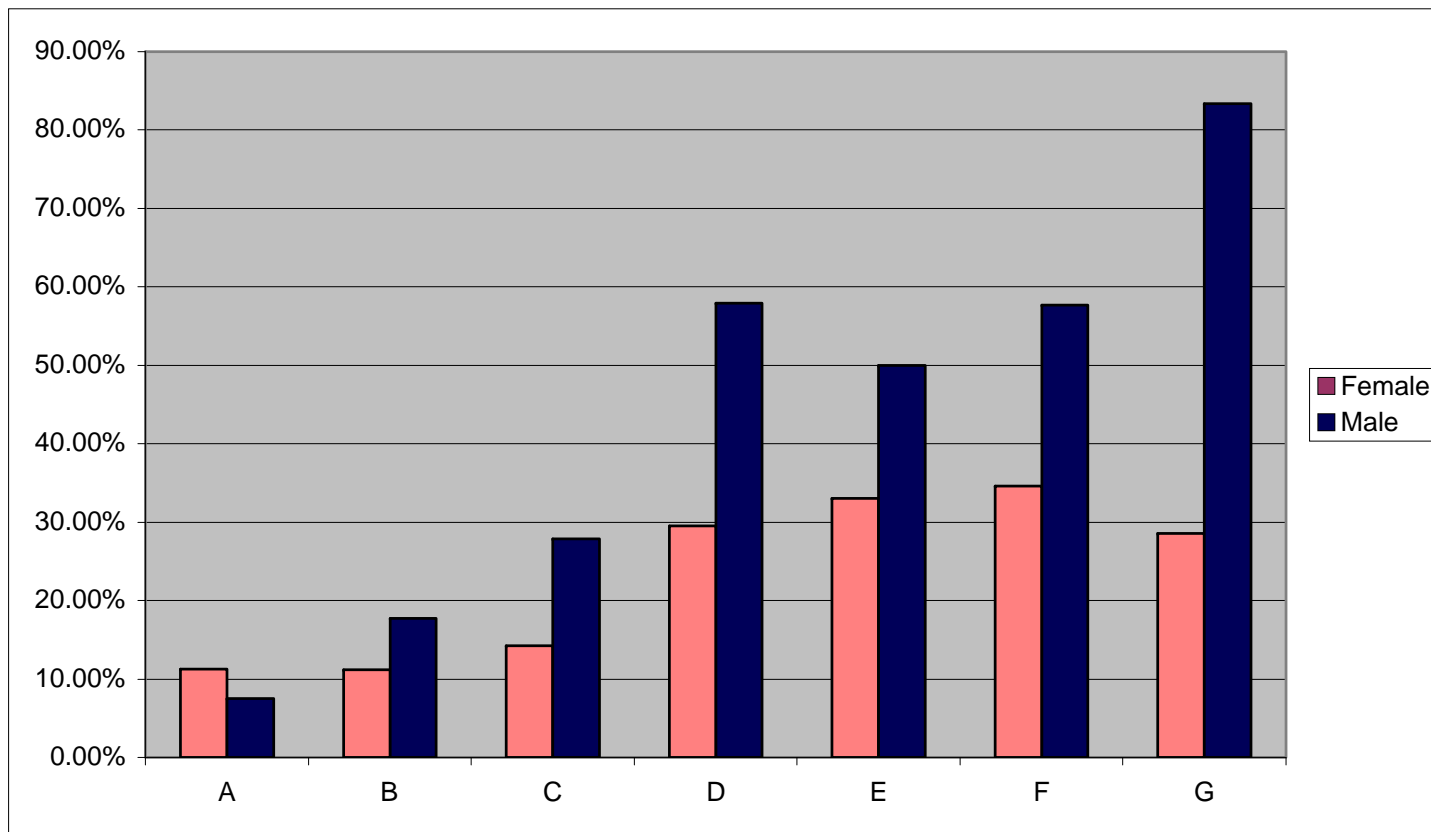
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The Phenome

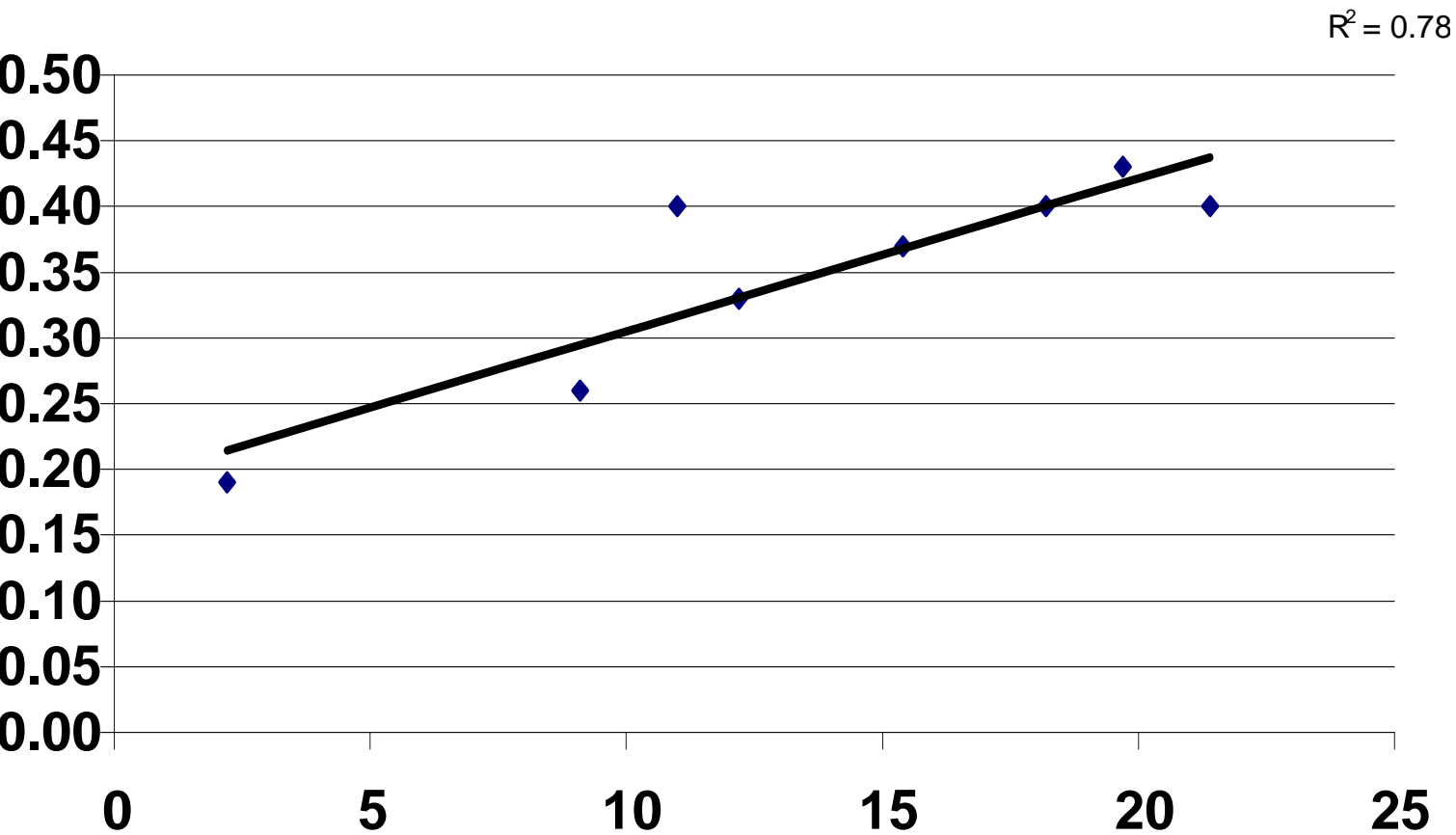
er Breed (A - G) effects on resistance (Resilience)



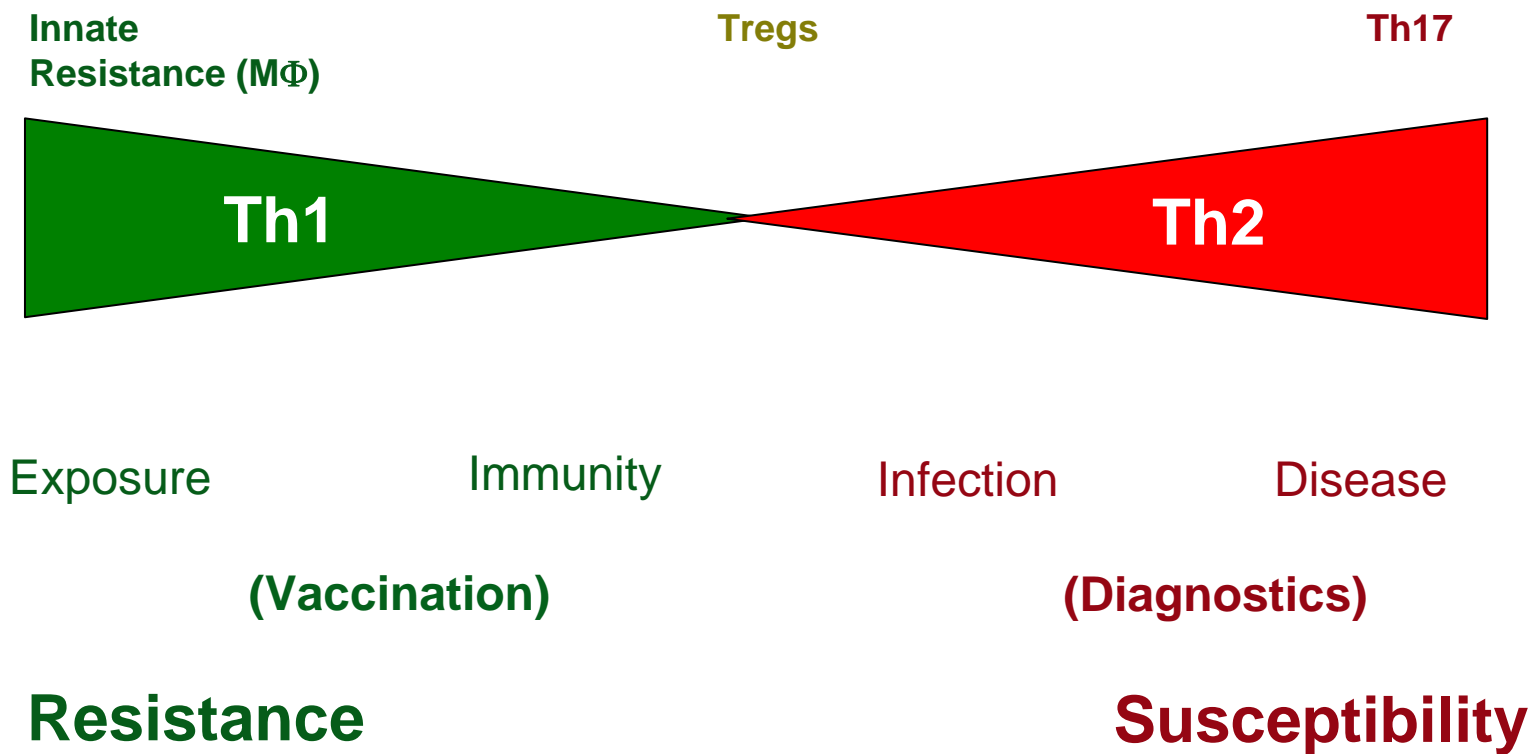
Remember NO BREED is ALL GOOD nor is any BREED ALL BAD
However, it's HARDER to pick WINNERS from Breed G than Breed A

The Physioime

relationship between reactor rate and breeding values



The Diagnostome: Diagnosis across the Infectious spectrum



Suggested use of Diagnostics in a Stud Herd or Herds involved in Live Sales

Common sense requires you establish the level of risk from *Map* within your herd

**EVEN IF YOU ARE CONVINCED THERE IS NO PROBLEM YOUR CLIENTS MAY NOT SHARE
YOUR CONFIDENCE**

Test Herd to establish risk for each Breedline

**At Annual Testing all Breeds with abnormally high reactor levels
(More than 2 times herd average)**

Test All R1 and R2 animals before sale or use as herd replacement

Conclusions ! Take Home SIX PACK

Map infection likely affects >50% of NZ Deer herds and > 10% of animals within most infected herds. Probably similar for Dairy herds.

***The Iceberg Effect:* Majority of *Map* infections do not cause covert Johne's disease (Pathology/ Clinical symptoms)**

Deer may develop more 'florid' cases of Johne's, than cattle or sheep, and are more amenable to diagnosis than infection in cattle or sheep.

Johne's Disease is the most important (& underestimated) infectious threat to deer farm (and probably dairy farm) profitability in NZ.

Fast genes for Production Traits may Negatively impact disease resistance. Ensure that high performing breeds are resilient

Could the link between *Map* and Crohn's Disease become more credible, NZ's Red meat and dairy products will be at **SERIOUS RISK !**



Acknowledgements:

Farmers and Veterinarians - field support

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