Introduction

- Venue, loos, escapes, contacts.....
- Workshop outline
  - Key concepts
  - Thresholds for intervention
  - Key epidemiological aspects
  - Metrics for diagnostic test performance
  - Diagnostics available
  - Review of science
  - Case study
  - Typical testing scenarios and some estimated costs
  - Work through some examples
  - Conclude

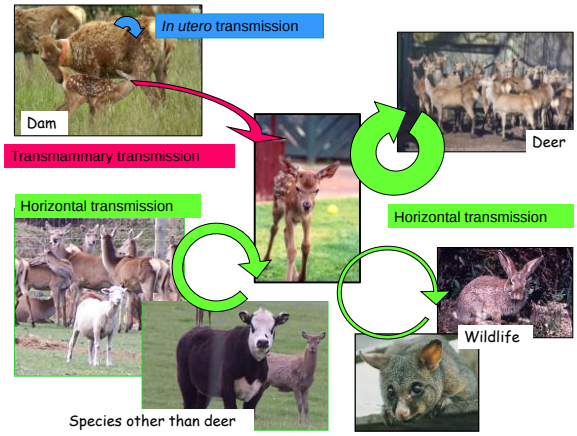
Acknowledgements

The material we cover this afternoon has accumulated over many years by a range of experts in diagnostics and Johne's disease. In particular an acknowledgement of support from

- Disease Research Laboratory, University of Otago
- EpiCentre, Massey University
- AgResearch
- Canterbury Health Laboratory
- AbacusBio Ltd
- Johne's Disease Research Consortium

Key concepts

- Youngest stock by far most susceptible, out to ~ 12 months old
  - Keep them away from MAP and there is no problem
- Keep a closed herd
  - Reality is purchase minimal risk stock
- Blood testing in diseased herds will help if used right
  - Get expert advice to ensure cost-effectiveness
- One 'super-shedding' deer can ruin a lot of effort
  - The amount of bacteria shed in some cases is hard to believe...



Nature of JD

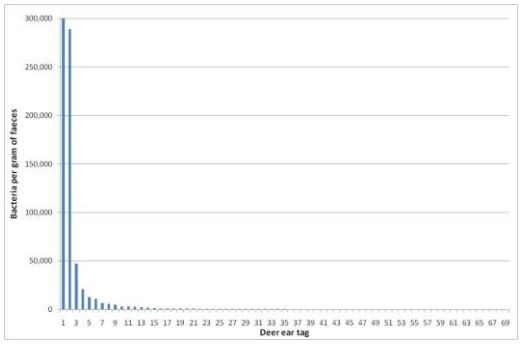
Shedding spectrum

- How much is too much?
  - Pass through
  - Mild
  - Medium
  - Max
  - Log scale increases in shedding rate
  - Consider that ~60+ of deer on a property with disease may be culture positive, irrespective of disease levels
  - 10,000 or more organisms (by qPCR) typically accepted as confident the animal is actively shedding

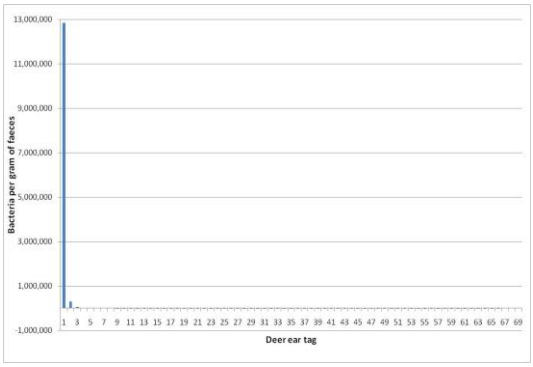
Super shedding

- Somewhat dependent on disease levels for farm in question
- 9 months old minimum age, recommended for major issue farms
  - Going into winter to minimize seasonal impact on clinical rate
- Post winter for farms with less issue – more time for disease and immune response to develop with winter
- Overall minimizing contamination
- Of course can qPCR at any time

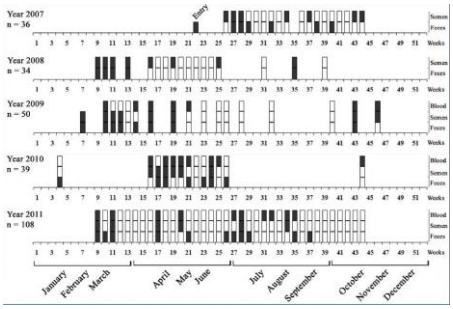
300,000bugs/g looks a lot



But it's all relative



Shedding over time



- Status depends on time and test type

Does it come back?

- If we:

Controlled clinical losses

Achieved a declining JD-suspect lesion rate in processed deer
  - Reality is purchase minimal risk stock

Keep essentially a closed herd (except stags)

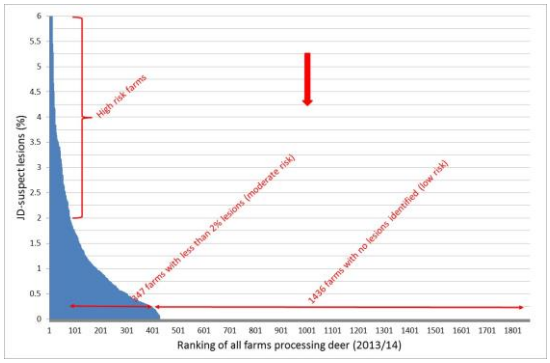
- *Would JD levels rebuild in the absence of testing?*

Where and when might we intervene

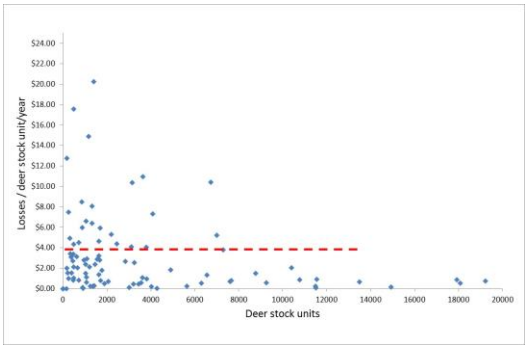
Summary of JD control in deer

- A risk based approach to managing exposure
  - Present on most farms
  - Serious issue in small proportion of herds
  - Point at which decide to intervene is personal
  - Experience shows >$4/deer stock unit losses often a trigger
  - Intervening directly in fawn management not feasible
  - Reduce contamination by removing highly infectious deer
  - Substantial reduction in losses usually achieved in 1-3 years
  - Depends on intensity of intervention
  - Trickle on effect of JDSLN can extend years

JDSLN rate by farm



On-farm economic cost



Key question: seeking what?

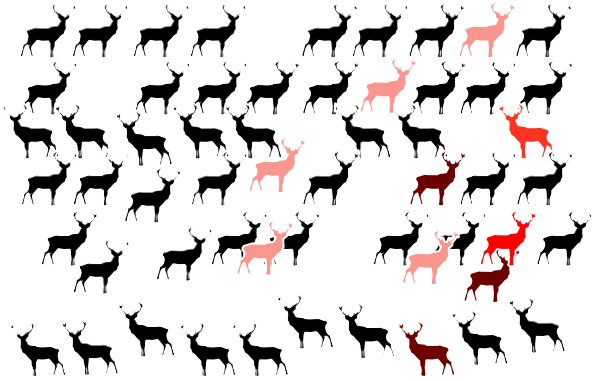
- Defining condition of interest is essential
  - MAP all but ubiquitous
  - Deer with MAP?
  - Normal looking deer infected with MAP?
  - Sub-clinical Johne's disease
  - Clinical Johne's disease
  - Differential diagnoses?
  - Direct costs of…
  - Repercussions of results…

Metrics 1

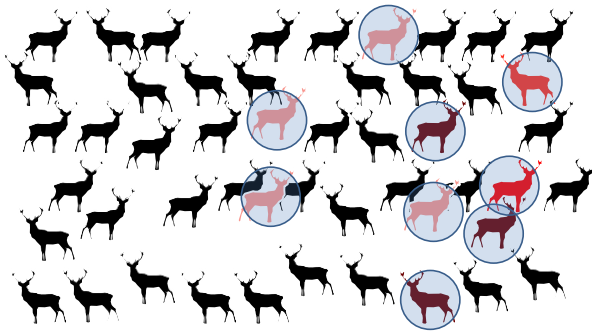
- Sensitivity
- Specificity
- Predictive values: influenced by prevalence, Se & Sp are not.

Metrics 2

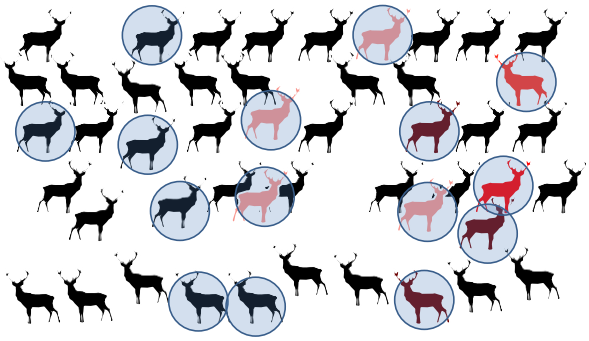
- Effect of combining tests
- Effect of repeat testing



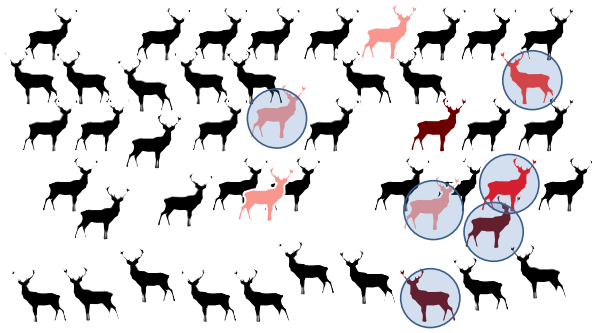
Sensitive & specific



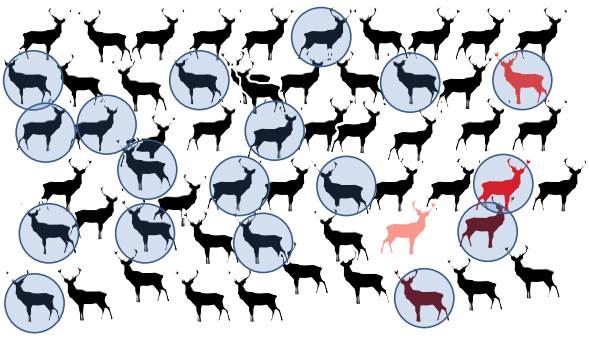
Sensitive but not specific



Specific, not sensitive



Paralisa picking 10,000+



As a selection process

- Contend with imperfect specificity
- Get equivalent money for test pos and neg
  - Provided source of replacements to maintain capital stock
  - Use as last step in selection process of R2 hinds
  - Test during peak schedule if possible
  - Test pregnant R2s up until September 30 to enable transport – schedule is high then
- CRITICAL QUESTION: Opportunity cost of culled false positives
- Stag pre-sale testing over 3 years
  - Condition of interest: minimal chance of developing disease

Diagnostic options

Diagnostic options

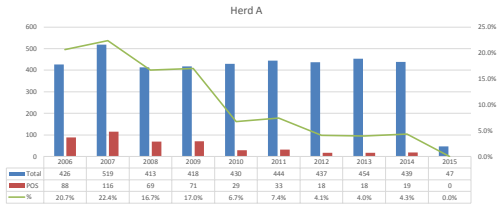
- Diagnostics – a little theory but mostly practicalities
  - Types of diagnostics
  - Pros and cons
  - Reading test results
  - Using appropriate tests
  - Useful materials and resources
  - Biological limitations of testing

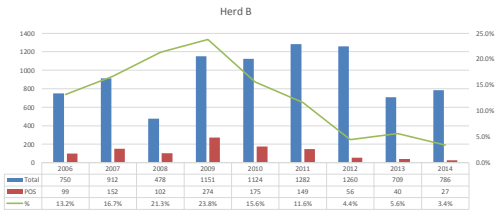
On-farm post mortems

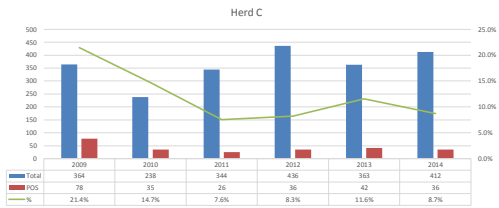
- Cheap for farmers
- Easy to organise
- Timely
- Moderate sensitivity
- Easily increase sensitivity with vet and/or lab input
- Valuable first step in surveillance

ELISA

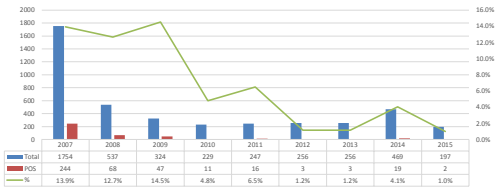
- Well established in deer industry and beyond
- Low cost
- Quick
- Measures immune response as proxy for disease/exposure
- Useful performance when used appropriately
- Subjective interpretation of results
- Offered by Disease Research Lab, Otago University
- Offered by Canterbury Health Laboratories
- Gribbles and NZ Vet Path
- DRL ~ $15 per test plus collection and shipping
- Following graphs are from DRL





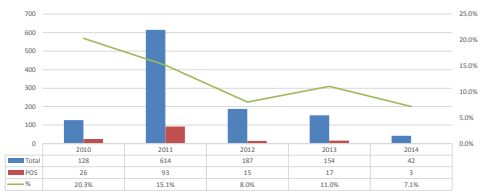


Herd D



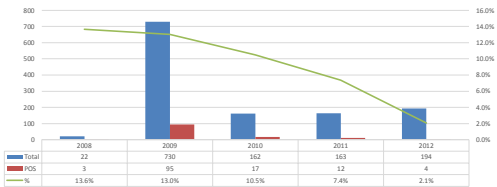
	2007	2008	2009	2010	2011	2012	2013	2014	2015
Total	1754	537	324	229	247	256	256	469	197
POS	244	68	47	11	16	3	3	19	2
%	13.9%	12.7%	14.5%	4.8%	6.5%	1.2%	1.2%	4.1%	1.0%

Herd E



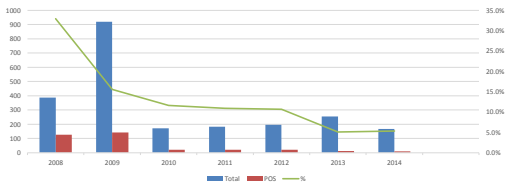
	2010	2011	2012	2013	2014
Total	128	614	187	154	42
POS	26	93	15	17	3
%	20.3%	15.1%	8.0%	11.0%	7.1%

Herd F



	2008	2009	2010	2011	2012
Total	22	730	162	163	194
POS	3	95	17	12	4
%	13.6%	13.0%	10.5%	7.4%	2.1%

Herd G



qPCR

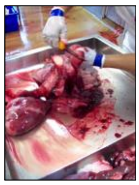
- Well established but seldom used
  - Quick
  - High specificity, high sensitivity
  - Measures actual shedding directly
  - Requires standardized methodology
  - Ideal back-up test for high value animals or uncertain Paralisa results
  - Pooled option available
  - $50 + collection and shipping
  - Offered by DRL
  - Massey developing their own

Culture

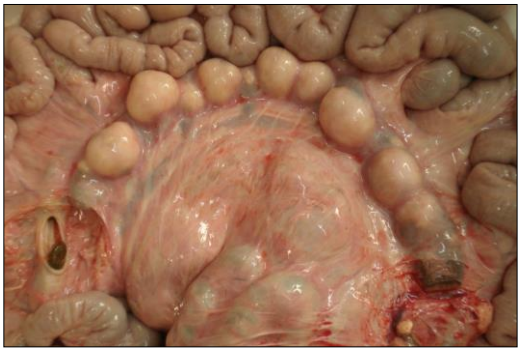
- Traditionally the definitive Gold Standard for MAP
- High sensitivity, very high specificity
- Comparatively slow (up to 16 weeks for low CFUs)
- Valuable research tool
- Limited supplies of BACTEC media
- General transition from culture to qPCR
- Offered by AgResearch Wallaceville

Surveillance data

- JML surveillance database
- 3.7 million deer, >99% of production since 2006
- AsureQuality routine inspection/recording of JDSLN
- Johne's disease suspect lesions
- Not confirmed as MAP
- Very low sensitivity, low specificity
- Low cost – built in to national JD control programme
- A useful component of an on-farm JD investigation



JD-like lesions



Review of science

- Several papers and projects on diagnostics for JD in deer.
- Each one an important part of the optimized testing puzzle
- Take a quick look a the contribution of each one and some of the strengths and weaknesses
- Good demonstration of the evolution of the thinking on this topic
- Converge to a practical understanding of test performance

Review: Griffin et al 2005

- Methods: positives: 102 suspected JD deer from >10 farms
  controls: 508 deer from 5 farms with no history of JD

Analysed with on-line ROC curve programme

- Results: Se=85%, Sp=99.8% when PPDj and PPAg use in series at cut point of 50
- Case study (434 hind herd) using ELISA test-and-cull for 4 years, reactivity dropped from 40% to >3%, production increased and deaths reduced in young deer twice tested negative

Review: Griffin et al 2005

- In support -
  - Shows the test can identify clinical JD
  - Specificity appears high in control group
  - Evidence for performance increase in test negative young deer
- In critique -
  - Tested population not representative of wider deer industry
  - Nature of test population leads to exceptional performance of test
  - No measures of variability around test performance estimates
  - No measures of variability around animal performance estimates

Review: Stringer et al 2012

- Methods: cross sectional design, 38 herds NZ wide, 20 clinically normal yearling deer per herd, 757 samples, Bayesian absence of gold standard approach

Test	*Sensitivity (%)*	*Specificity*
Paralisa	19 (10 - 30)	94 (93 – 96)
Faecal culture	77 (61 - 92)	99 (99 - 100)

- Conclusion: limited application as a herd classification tool. FC Se high?

Review: Stringer et al 2012

- In support -
  - Valid, well defined approach
  - Reasonable estimates of performance in population of interest
  - Good counterpoint to paper 1
- In critique -
  - Herd classification scheme is unlikely to be implemented by Deer Industry
  - External sourcing of young replacement hinds is not that common
  - Se of FC is high suggesting 'balance of results' may be a little out

Review: Rendel et al 2012

- ***Aim:*** develop a protocol for JD control in Landcorp's ~60,000 hind deer herd
- ***Methods:*** DEERSelect and Paralisa data for 4 studs (hinds and stags)
- ASREML model to quantify value of test and cull at a variety of test positive rates and JD influenced weaning rates, and growth rate depressions
- ~8,000 hind test results, ~3,500 stag test results
- Initial positive rates to Paralisa were high - most at least 15% and up to 37%
- Rates then tended to drop sharply in the following year or 2
- ***Recommendations***
- T&C where JD ↓ weaning rate by >6% & ↓ offspring growth rate
- Where positive rate >20%, T&C if
  - weaning rate ↓ 8% due to JD
  - weaning rate ↓ 6% and growth rates ↓ 30g/day due to JD
- Breeding values for JD heritability of limited value

Review: Rendel et al 2012

In support:

- Shows substantial drop in positive rates in 1-3 years, like DRL suggest
- Suggests intervention with blood testing is worthwhile when prev is high
- Acknowledges lost production must be due to JD to get gains from control

- ***In critique***
- Measurement criteria difficult to define as due to JD
- Does not consider JD-related death rate which is important
- Methodology not clear

Review: O'Brien et al 2013

- ***Aim:*** Define performance criteria for qPCR and Paralisa
- **Method:**
  - Compare qPCR against bovine proficiency panel samples from US National Vet Services Laboratory
  - Correlate qPCR and histopathological lesion score
  - Correlate Paralisa values with qPCR measured shedding levels
  - Estimate sensitivity and specificity of Paralisa against qPCR
- 72 proficiency panel samples
- 40 qPCR & histo matched samples
- 663 qPCR & paralisa matched samples

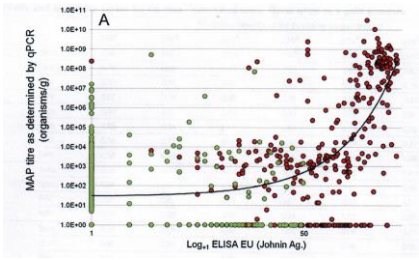
Review: O'Brien et al 2013

- Results
  - qPCR essentially equivalent to culture (correlation of 0.93)
  - qPCR and histopathology less correlated (0.73)
  - Paralisa sensitivity: 62 – 98% depending on shedding level
  identifies most deer shedding MAP, virtually all high shedders
  - Paralisa specificity: 70 – 58% depending on shedding level
  Pays to back up test suspicious results in high value animals with qPCR

Review: O'Brien et al 2013

- In support -
  - Valid, well defined approach
  - Performance stats in the population the test is usually used in
  - Good counterpoint to previous papers
  - Practical application informed by these results
- In critique -
  - Lacks confidence intervals
  - 'True' estimate of specificity *under these conditions*

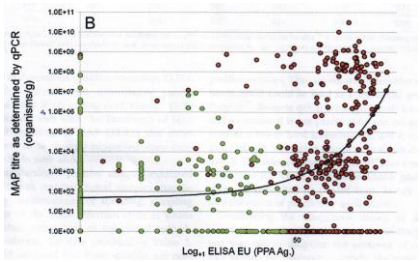
qPCR & Johnin



O'Brien et al, 2013, BMC Vet Research

- 663 bloods submitted for routine JD testing by Paralisa

qPCR & PPA



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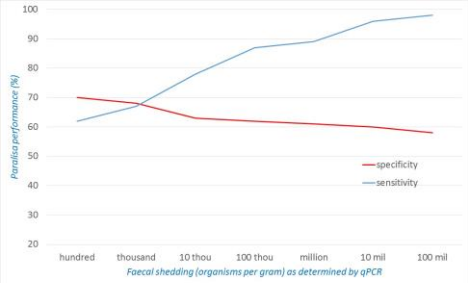
Paralisa



O'Brien et al, 2013, BMC Vet Research

- 663 bloods submitted for routine JD testing by Paralisa

JDRC study

JOHNE'S DISEASE RESEARCH CONSORTIUM

- Aim:

  Compare the ability of the Paralisa and Parachek 2 serum ELISA test to detect faecal shedding as measured by qPCR under conditions typical in typical venison production herds.

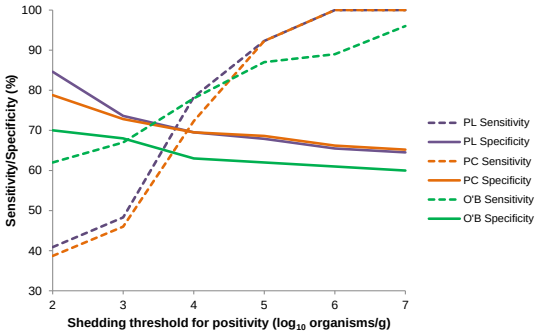
JDRC study

- Materials and methods
  - 8 herds identified by JML with potentially high rates of JD
  - 883 (2013) and 1354 (2014) deer tested, virtually all R2 hinds
  - Screened with Paralisa test, positives tested by Parachek2, qPCR & Paralisa
  - Overall screen test prevalence of 8.6% (2013) and 7.8% (2014)
  - Farm-level screen prevalence range 1.4% - 49.6%

JDRC study

- Sensitivity results
  - Paralisa consistently higher than Parachek2
  - But difference was small and not statistically significant
  - Range from ~40% at 10^2 to 100% at 10^6 and 78% at 10^4
- Specificity results
  - Paralisa usually slightly higher than Parachek2
  - But difference was small and not statistically significant
  - Range from ~79% at 10^2 to 66% at 10^6 and 69% at 10^4

JDRC study



JDRC study

- Conclusion
  - Function of the two blood tests under these conditions is similar
  - Both good at picking up highly infectious deer
  - Both have false positive rates that require consideration –
  - Specifically, is the cost of culling those animals low?

JML database validation

- **Aim**
  - Quantify relationship between JDSLN, on-farm JD and farmer concern

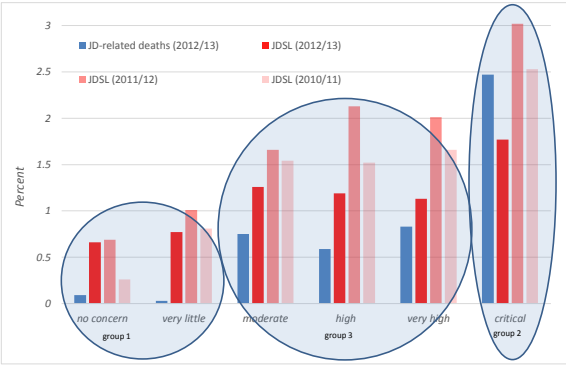
JML database validation

- **Method**
  - Phone interview of 121 farmers
  - Full spectrum of JDSLN rates
  - Demographic and farm information
  - 1000minds data – multiple criteria decision analysis tool
    - Indirectly measures farmers value of JD relative to higher weaning rate and higher venison schedule
  - Economic estimate of impact of JD on-farm

JML database validation

- **Results: respondents fell into three distinct groups –**
- Little or no concern about JD
  - low JD-related death rates on-farm and low JD-suspect lesion rates
- Critical concern about JD –
  - highest JD-related death rates on-farm and highest JD-suspect lesion rates
- Moderate, high or very high concern regarding JD –
  - intermediate JD-related death rates on-farm and moderate JD-suspect lesion rates

JML database validation



JML database validation

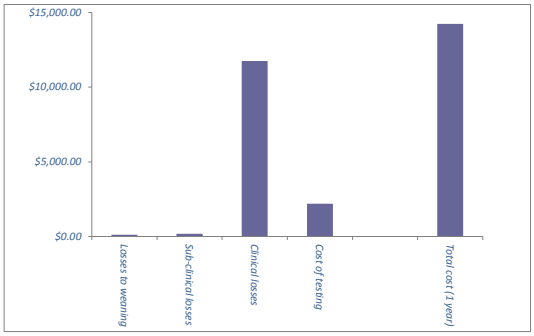
- **Conclusion:**
- Database can be used to identify high-risk farms
- Focus on a JDSLN rate of 2% of higher will prioritise high risk farms
- 77% of farmers felt the impact of JD in their deer was declining or already low
- 64% of farmers felt parasites were an equal or larger issue than JD
- The cost of JD per farm averaged $3,215, peaked at $53,015
- Meat inspectors record ~ 70% of JDSLN (previous study)

Case study

Case study background

- Farm background
  - 500 hind breeding and finishing unit in south Canterbury
  - First noticed deaths in 2000
  - Deaths peaked in 2005 at 25/yr (5%)
  - Beatrix diagnosed JD with 5 post mortems in that same year
  - Lesion positive carcasses 8% lighter than those without
  - Loss of $14,000/yr or $7.31 per deer stock unit in 2005

Peak losses

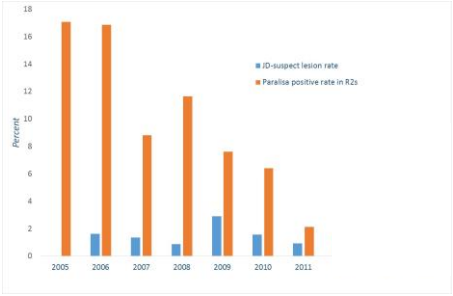


Intervention

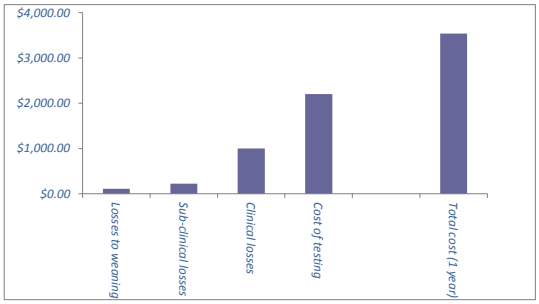
- Implemented Paralisa testing of R2s in 2005
- No other significant initiatives to control JD
- Concurrent decline in clinical rate
- After 7 years deaths down to 1 or 2, loss of $1,000
- Cost of testing @$2,200/yr

Impact of blood testing

- Decline in Paralisa positive rate in R2s over 7 years



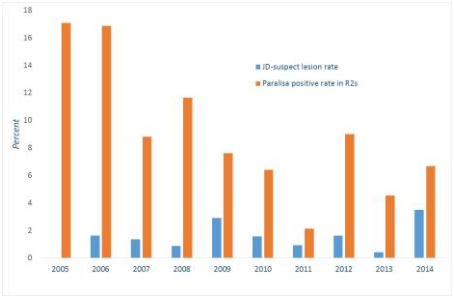
Annual cost after 7 years



- Cost-benefit? – how bad would disease have got?
- What cost for a culled positive animal?

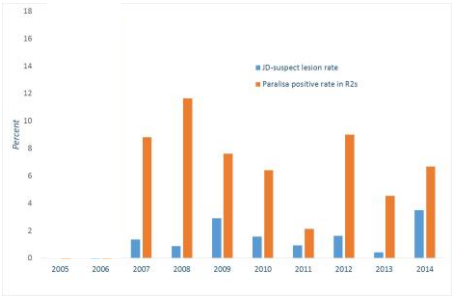
Continued blood testing

- Decline in Paralisa positive rate in R2s over 10 years



Post peak test results

- Annual Paralisa positive rate post peak in disease
  Draw a trend line through this data



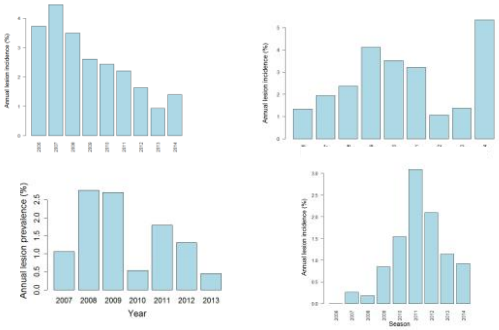
What have we seen?

- Has there been enough shedding to transmit infection?
- How many false positives would we expect?
- Depends on prevalence – as p↓ False positives ↑
- Expect deer infected during the peak to go clinical over some years
- Herd owner:

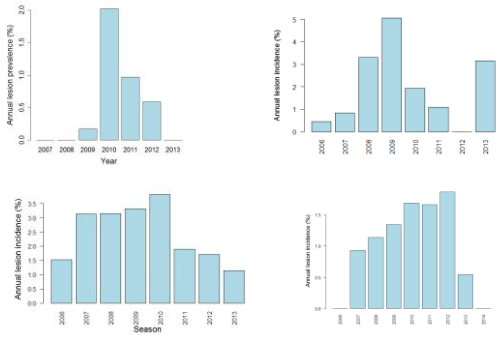
  "Clinical JD was worst in 2005. I've seen a downward trend since then with only one hiccup in 2012 (couple of clinical cases after a long wet spell of weather). As the test positives declined, so did the number of clinical cases".

On-farm trends in JD

Trend in JDSLN rate

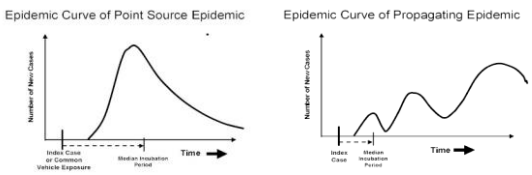


Trend in JDSLN rate



Nature vs intervention

- Characteristic epidemic outbreak to some degree
- Impact reduced by intervention
- Cost-effectiveness needs case-by-case analysis
- Heavily dependent on extent of initial outbreak



Practical application

- Informing about
  - The very first step…any lesions?
  - Ones and twos as **surveillance**
  - Diagnosing the cause of a tail end mob
  - Routine selection criteria for replacement hinds - **intervention**
  - Status of animals in trade
  - Whole herd testing
  - Stag testing prior to sale
  - Diagnosing a high non-specific Tb reactor rate
  - Industry level reporting

Scenario 1: surveillance

- Monitoring to ensure no issue emerging
  - On-going activity as part of animal health management
  - JDLSN rate
  - Offal pit side post-mortem inspections
  - Productive and reproductive performance of deer unit

  - No additional cost
  - Part of good farm management
  - Early detection hugely influential in overall outbreak severity
  - Sensitive and specific with combined vet and farmer input

Scenario 2: routine R2 testing

- Reduce chance of infected hinds entering breeding herd
  - Selection criteria for replacement hinds
  - Common activity as part of animal health management
  - After all other selection criteria have been met to minimize cost
  - Valuable for several years following an outbreak

  - Additional cost
  - Important to consider **opportunity cost** of false positives
  - Won't detect all infected hinds
  - At what rate do we consider less frequent testing?
  - In combination with surveillance to ensure low prevalence remains

Scenario 3: pre-sale testing

- Blood testing to reduce between farm transmission

  - Moderately effective
  - Stock usually in good health – so low prev, low PPV
  - More value in trading stock from previously diseased farms
  - Quarantine/isolate and monitor following transport
  - Combine with other measures of JD risk – JML or other testing data
  - Response to a positive result
    - qPCR to quantify shedding
    - Only take negative animals (given other infected deer may well remain)
    - No sale or reduced price

Scenario 4: pre-sale stag testing

- Small numbers of high value young animals
  - Seeking maximum test performance - combine ELISA and qPCR (pooled?)
  - Price less of an issue
  - Stock usually in fine health with minimal stress
  - Young age means disease unlikely to have developed
  - May be more merit in testing them following their first rut
  - Combine with other measures of JD risk – JML or other testing data
  - Valuable supporting info from the history of existing on-farm JD control

Scenario 5: stock class level testing

- Blood testing to reduce impact of outbreak
  - Early diagnosis and intervention will minimize length and severity of outbreak
  - Focus on stock class and mobs with highest losses
  - Work outward from there comparing each subsequent lot of results
  - Plot pattern of the focus of infection in the herd
  - Accept removing some false negatives to minimize risk as far and fast as possible
  - Immediate removal from herd of test positive animals

Scenario 6: youngest stock

- Somewhat dependent on disease levels for farm in question
- 9 months old minimum age, recommended for major issue farms
  - Going into winter to minimize seasonal impact on clinical rate
- Post winter for farms with less issue – more time for disease and immune response to develop with winter
- Overall minimizing contamination
- Of course can qPCR at any time
- Case of a fawn in April shedding 2 million bugs/g (qPCR)

Scenario 7: Tb reactors

- Test non-specific Tb reactors for JD
- Good opportunity for surveillance
  - Likely reactors have been exposed to something – focused way to check for JD
- Potential selection criteria in some cases

Estimated cost model

Class	Year	Prev	Test(s)	Cost ($400/deer)	Per high shedder	Cost ($100/deer)
1000 hinds	1	5 → 1.1	Paralisa	$171,000	$4,400	
	2	1.1 → 0.2	Paralisa	$165,000	$17,400	
1000 hinds	1	5 → 1.1	P & qPCR	$45,000	$1,165	
	2	1.1 → 0.2	P & qPCR	$33,000	$3,807	
200 R2s	1	5 → 1.1	Paralisa	$34,000	$4,390	$10,810
	2	2.5 → 0.6	Paralisa	$33,000	$7,800	$10,605
	3	1 → 0.2	Paralisa	$33,000	$9,185	$10,482
200 R2s	1	5 → 1.1	P & qPCR	$9,000	$1,165	$6,748
	2	2.5 → 0.6	P & qPCR	$7,500	$1,873	$6,294
	3	1 → 0.2	P & qPCR	$6,700	$3,330	$6,060

Working through examples

- Routine testing of R2s over 5 years
  - Condition of interest:?
  - How many deer tested
  - How many positives
  - How many false positives
- Stag pre-sale testing over 3 years
  - Condition of interest: minimal chance of developing disease
  - Sensitivity
  - Specificity
  - Likelihood removed genuinely
- A single whole herd test
  - Condition of interest: ?
  - Sensitivity and specificity
  - How many positive identified, how many false positive?

Conclusion

- All available tests are useful in the control of JD when used appropriately
- Get the mix of tests right for best cost-benefit ratio
- Use scenarios as a guideline, advice always available from JML and DRL
- For maximum benefit, integrate with wider herd health and farm management
- Booklet of suggested guidelines to follow