

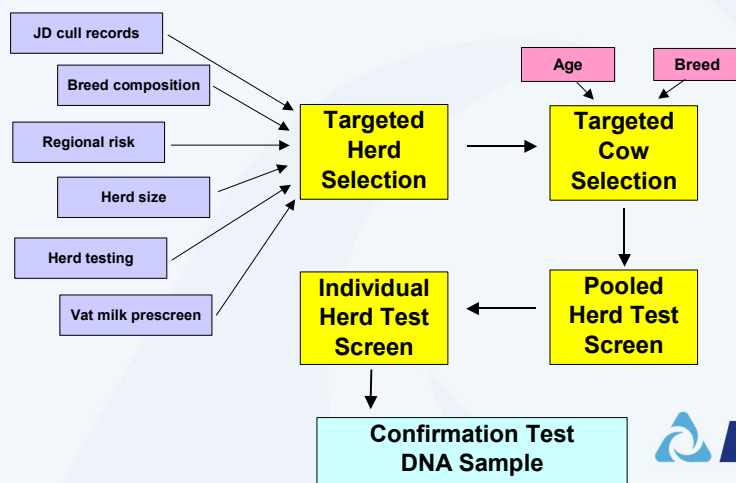
## Bovine DNA Archive – Genomics Study

- Genomics Approach

- Collect and store DNA samples from JD case animals:  
2000 Johne's positive cows identified using ELISA  
on milk sample screening, followed by serum confirmation
- Phenotype
- Existing LIC population data (4000+) used as control data
- 400 test-negative sire-matched cows from case herds as matched control
- Genomics analysis requires within-breed approach
- 50000+ SNP panel – JD case cows and matched controls



## Method of Identification of JD-case Animals



## Targeted Herd Selection

- Herd testing at least 3 times per annum (possibly 2x)
  - need access to HT samples – primarily late spring/summer
- Herd size – aim for larger herds for efficiency
  - given likely low prevalence, low reactor yield / herd
- Breed composition
  - prioritize herds with high proportion single breed to maximize 'purebred' Jersey & Friesian case cows
  - FxJ crossbreed
    - large proportion of NZ dairy population is FxJ ie relevant 'breed'
    - more difficult to target breeds at herd selection



## Targeted Herd Selection: JD Culling Data

- 10 seasons of good JD culling data ~ up to 1000 herds
- pilot bulk milk screen suggests relevant data – see later

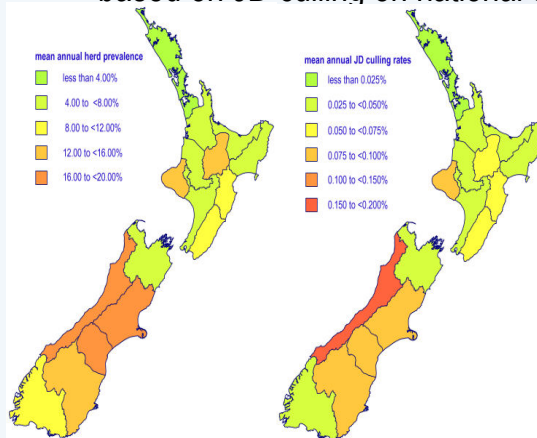
season	JD culling herds	total NZ herds	herd prevalence	RR vs 1 <sup>st</sup> 3 season mean RR	99% CI	mean herd size	
						JD cull	national
1998 / 99	1 101	14 362	7.67%	<b>0.98</b>		319	229
1999 / 00	1 079	13 861	7.78%	<b>1.00</b>		350	236
2000 / 01	1 108	13 892	7.98%	<b>1.02</b>		362	251
2001 / 02	1 168	13 649	8.56%	<b>1.10</b>	(0.99 - 1.22)	394	271
2002 / 03	1 170	13 140	8.90%	<b>1.14</b>	(1.03 - 1.27)	423	285
2003 / 04	1 018	12 751	7.98%	<b>1.02</b>	(0.92 - 1.14)	435	302
2004 / 05	995	12 271	8.11%	<b>1.04</b>	(0.93 - 1.16)	470	315
2005 / 06	1 059	11 883	8.91%	<b>1.14</b>	(1.03 - 1.27)	482	322
2006 / 07	1 107	11 630	9.52%	<b>1.22</b>	(1.10 - 1.35)	505	337
average	1 089	13 049	8.35%			415	283

- breed will limit useable herds



## Targeted Herd Selection: High Risk Regions

- based on JD culling on national dairy cow database



- target regions with higher herd & within-herd JD rates
- possibly limited application overall
- avoid Northland
- breed effect apparent
- vat test?



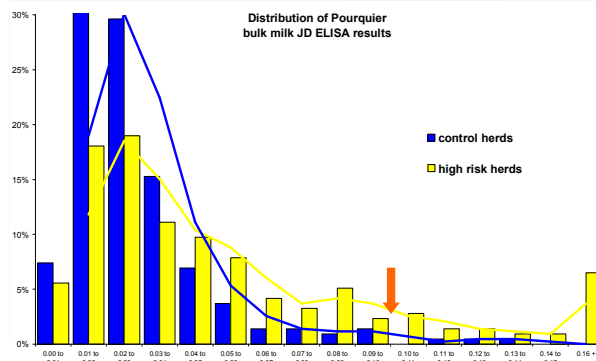
## Targeted Herd Selection: Vat Milk Pre-Screen

- Pooling experience suggests possible value (later)
- Literature indicative of possibilities (v Weering and Duthie)
- Pilot study carried out on >400 herds
  - low-risk control = low prevalence regions
  - high-risk case = JD culls (preferably 2+ seasons) plus based on high-risk region
- Alternate classification based on culling only, no region:
  - JD-cull herds vs non-JD-culling herds



## Vat Milk Pre-Screen: Pilot Study Results

- Distinct right-shift of case herds vs control herds
  - 14% case vs 1.4% controls > 0.1 ( $\cong$  5-10% sero-prev?)
  - non-culling herds from high-risk region: **no** right shift



- breed effect?  
possibly a poor predictor for Friesian herds (or low prev!) but insufficient data



## Vat Milk Pre-Screen: Pilot Study Continued

- Vat milk pre-screen is clearly a valuable tool!
- An efficient method to screen wider herd population if needed including 'low-risk' regions, smaller herds etc
- Currently using pilot study results & data to select herds for initial cow selection by pool HT screen ...
  - focus on herds with SP > 0.1
  - but also selection of herds across spectrum 0.0 to 0.1 to characterize relationship between vat test & the pool prevalence
  - may employ variable cut-off eg small herd or low prev region – raise c/o
  - any case cows identified will be sampled for project



## Targeted Cow Selection: Breed Pre-requisite

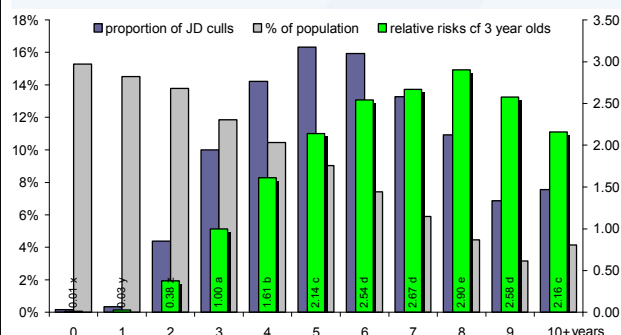
- Genomics study requires defined breeds only
  - FxJ option: large proportion of NZ dairy cow population is FxJ (33% vs 45% Friesian and 14% Jersey – wider definition)
- Selection based on national cow database
  - Friesian and Jersey: >13/16<sup>th</sup> of specified breed
  - FxJ crossbreed: F & J only and <12/16<sup>th</sup> of either breed
- Challenges – to obtain case numbers efficiently
  - Friesian: good 'purebred' cow / herd #s, but low JD cull rates
  - Jersey: higher JD culling but small total numbers
  - FxJ: intermediate

(relative JD cull risk for F : J : FxJ = 1 : 4.3 : 1.8)



## Targeted Cow Selection: Age (JD Cull Rates)

- JD deaths and cull rates increase with age according to literature and NZ dairy JD culling data
- Clinical JD in young cattle (~2y) linked to high challenge



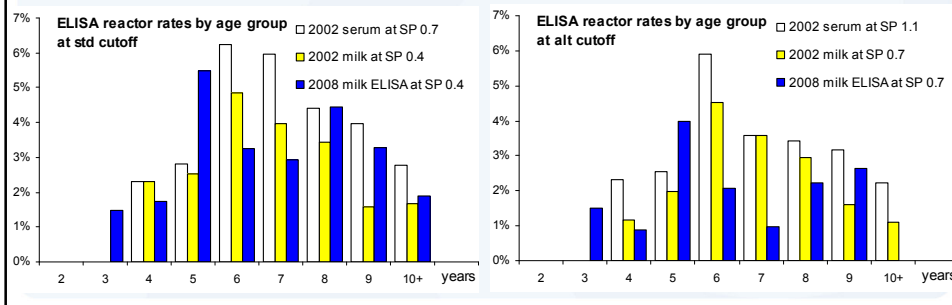
(but early clinical progression possibly affected by infection / disease susceptibility?)

- max risk 5-10 years
- max cow #s 3 - 8 years



## Targeted Cow Selection: Age (ELISA Sero)

- Similar distribution seen with ELISA serology (literature and experience of testing 2-year-old heifers vs older cows) suggests reactors amongst 2 year olds are extremely rare
- 2002 & 2008 data (excludes 2 year olds) – peak risk 5+ years:



## Targeted Cow Selection: Age

- Need to balance:
  - efficiency of low clinical / ELISA reactor rates amongst young stock
  - with larger total numbers and potential pool, as well as
  - possible genetic predispositions expressed as age of onset
- So current thinking: exclude only 2-year old heifers
- Ongoing validation work in conjunction with vat pilot trial may shed additional light on impact of 3 year old cows (in or out)

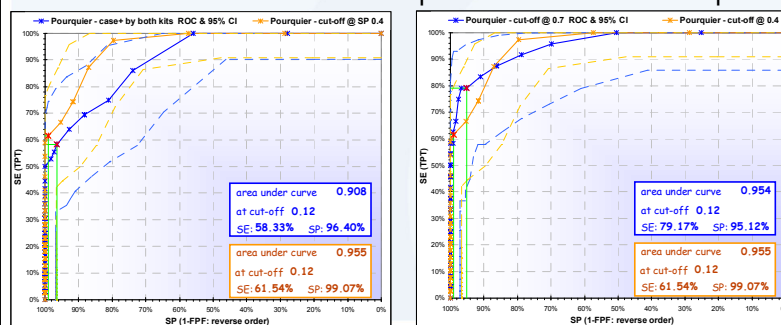
## Pool Test Strategy for HT Milk Screen

- JD ELISA kits (Prionics ex CSL & Pourquier) have poor sensitivity (SE) but near-perfect specificity (SP)
- ELISA performance as pool tests in doubt, therefore designed study to assess test performance with pooling:
  - 1400 lactation 2+ cows from 3 JD-history herds in N & S Islands
  - captured HT milk samples in autumn 2008
  - test all individual HT milks by ELISA (2 kits)
  - pool HT samples in 1:10 and tested
  - assessed ability of pool test to detect reactor cows



## Pool Test Strategy for HT Milk - Performance

- Only Pourquier ELISA kit showed potential as pool test
- Test performance assessed against individual samples
  - using combined Pourquier / Prionics interpretation
  - as well as scaled cut-off points on individual Pourquier ELISA



select cut-off for pool test to adjust SE to detect reactors vs SP to reduce retesting rate

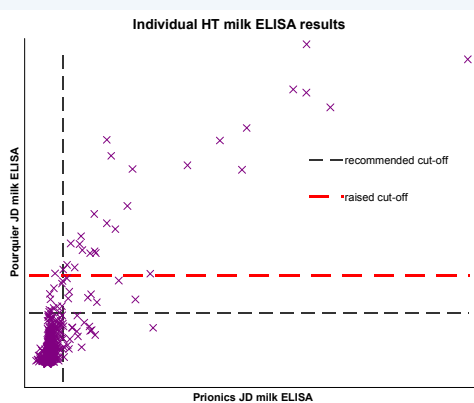


## Pool Test Strategy for HT Milk - Advantages

- ~60-70% fewer tests required than if individual testing
- Individual screening #s are highly sensitive to actual with-in herd sero-prevalence, test SE and changes to test cut-off,
- while pool → individual testing is much more robust with little impact of raising cut-off on total number test kits needed
- which allows us to better target more advanced JD cows as JD cases for the genomics study.



## Individual HT Milk ELISA Testing



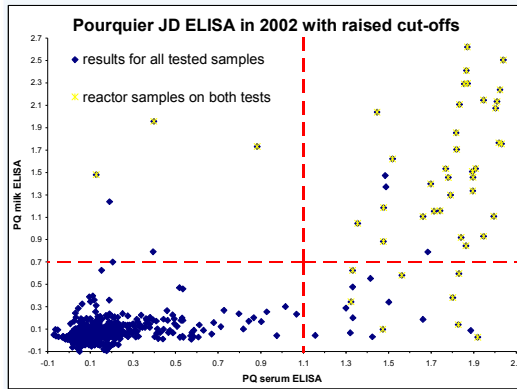
- Both kits performed similarly on single samples with good agreement amongst strong reactors
- Literature suggests very high specificity (>99.5%) of ELISAs
- Sensitivity strongly linked to stage of JD infection/disease - very poor amongst sub-clinical
- No own data – but age effect

- Raise cut-off ⇒ max SP and target advanced JD





## Confirmation Testing: Milk – Serum ELISA

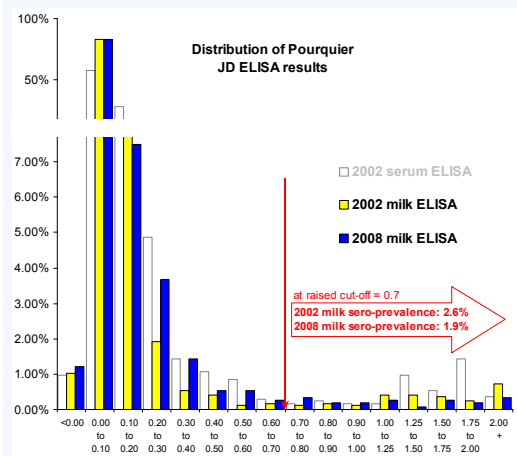


- Serum SE essentially higher, hence able to raise cut-off without significant loss of SE
- At cut-off S/P = 1.1, serum serial sensitivity reduces milk test SE by ~10%.
- Weak reactors excluded to improve targeting cows with advanced JD infection

	serum POS	serum neg		rel milk SE	67.3%	rel serum SE	88.1%
milk POS	37	5	42	rel milk SP	99.7%	re serum SP	98.9%
milk neg	18	1604	1622	serum prev	3.31%	milk prev	2.52%
	55	1609	1664	serial PQ prev	2.22%	kappa	0.756
				obs agree	0.986	exp agree	0.943
				POS agree	0.763	neg agree	0.993



## Distribution of ELISA Results at LIC



- 2002
  - 11 Taranaki herds
  - vets identified worst cases
  - targeted older cows
  - milk prev from <1 to >6%
- 2008
  - 3 herds, 2 with JD history
  - targeted older cows



## Expected Prevalence & ELISA Case Numbers

- 2002 study – JD history herds: 2.6% milk  $\Rightarrow$  2.3% serial SE  
(no breed effect on ELISA)
- 2008 study – mixed JD history: 1.9% milk  $\Rightarrow$  1.7% serial SE
- With bulk milk herd targetting and age restriction, expect ~2% sero-prevalence.
- So:
  - require at least 100 000 cows for primary HT screen
  - which will be a challenge with breed requirements



## Additional Confirmation Test (FC + PCR) ?

- All JD tests are prone to wide range of performance with very low sensitivity in pre-clinical stage, but rising with advancing stage of JD infection.
- Generally SE in clinical stages of JD very high for both faecal cultures and ELISAs (Pourquier).
- Young ELISA+ / FC- cow likely  $\Rightarrow$  heavy shedder / clinical
- Specificity is excellent – guaranteed with raised cut-off.
- Serial testing will impact on final sensitivity and costs.



## ELISA vs Faecal Culture

- What phenotype is required for the study?
- ELISA SE ranges from ~15% in early MAP to ~90% in clinical JD
  - so we expect good agreement with advanced JD phenotype  
esp with raised cut-off we already apply strong selection
  - suggestion in literature that antibody response occurs if cell immunity fails,  
ie presence of humoral immunity is reflection of raised susceptibility to JD
- Faecal culture / PCR:
  - Risk: If used as final selection tool, there is a risk if agreement with ELISA is sub-optimal (because imperfect SE of both tests), that costs escalate greatly
  - Alternatively, FC / PCR could be used as additional phenotype information for JD cases selected on ELISA only – then extra expense restricted to FC cost
- Essentially ELISA should suffice.

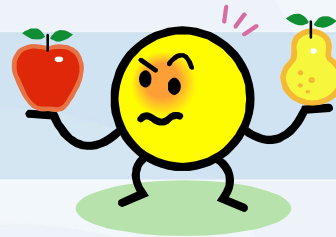


## 400 Matched Controls

- Match by:
  - herd – select from herds with JD cases
  - age – minimum age or control must be at least as old as case
  - **sire**
- Selection by:
  - serum ELISA
  - faecal culture – need to minimize chance of JD
  - long-term follow-up



## Questions



- Breed
  - may be challenging with narrow definition
  - crossbreed Friesian x Jersey
- Faecal culture options
  - not needed or
  - additional phenotype or
  - use as definitive phenotype
- Matched controls
  - any additional data required

