

# Investigation of infectious bursal disease (IBD) seropositivity on two commercial free range layer properties.

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# Infectious Bursal Disease (IBD)

## Agent

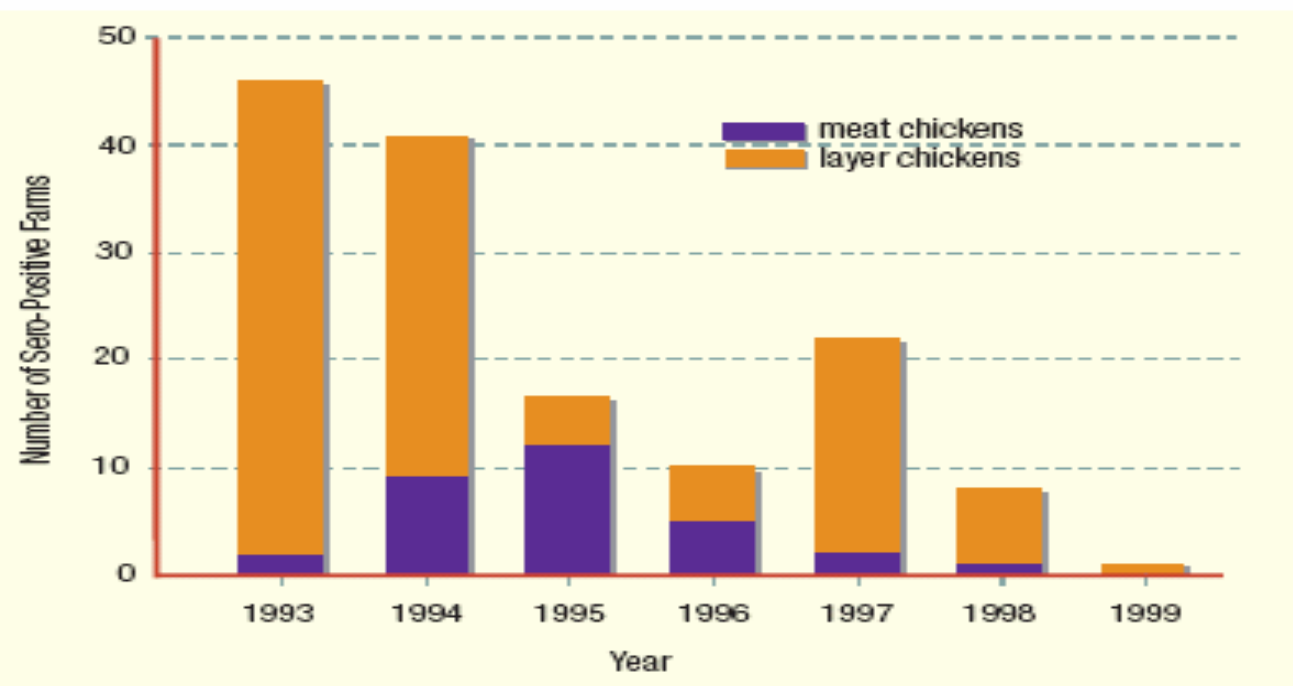
- *Birnaviridae*
  - RNA virus
- Highly contagious
- Pathogenic and non-pathogenic strains
- Low and high virulent strains
- Serotypes 1 and 2
  - Serotype 1 worldwide

## Host

- Young chickens
  - 3 weeks +
- Pathogenic strains
  - Morbidity 100%
  - Mortality (20%+)
  - Immunosuppression

# New Zealand IBD history

Figure 1: Number of IBD seropositive meat and layer farms detected each year during the period 1993 to 1999.



**1993** – non pathogenic strain

Industry eradication program

**1999** – last seropositive flock

Ryan, T., R. Leong, and B. Diprose  
Surveillance 2000

# Investigation

- March 2005 – Industry 'Country Freedom Quality Plan' reports to Investigation and Diagnostic Centre
  - 10/10 ELISA test positives (FlockChek®, IDEXX Laboratories) on two properties.
- Free range layers
- Clear tests November 2003
- Only exotic strains of IBD are notifiable in New Zealand under the Biosecurity act 1993

# Layer Results – VNT results cut off >1:16

## Property A

Age	Shed	1	0	Total	Rate
26	1	12	8	20	0.60
44-60	2//3	1	41	42	0.02
	Total	13	49	62	0.21

## Property B

Age	Shed	1	0	Total	Rate
20	1	14	5	19	0.74
40-80	2/3	1	40	41	0.02
	Total	15	45	60	0.25

# Layer Results – VNT results cut off >1:16

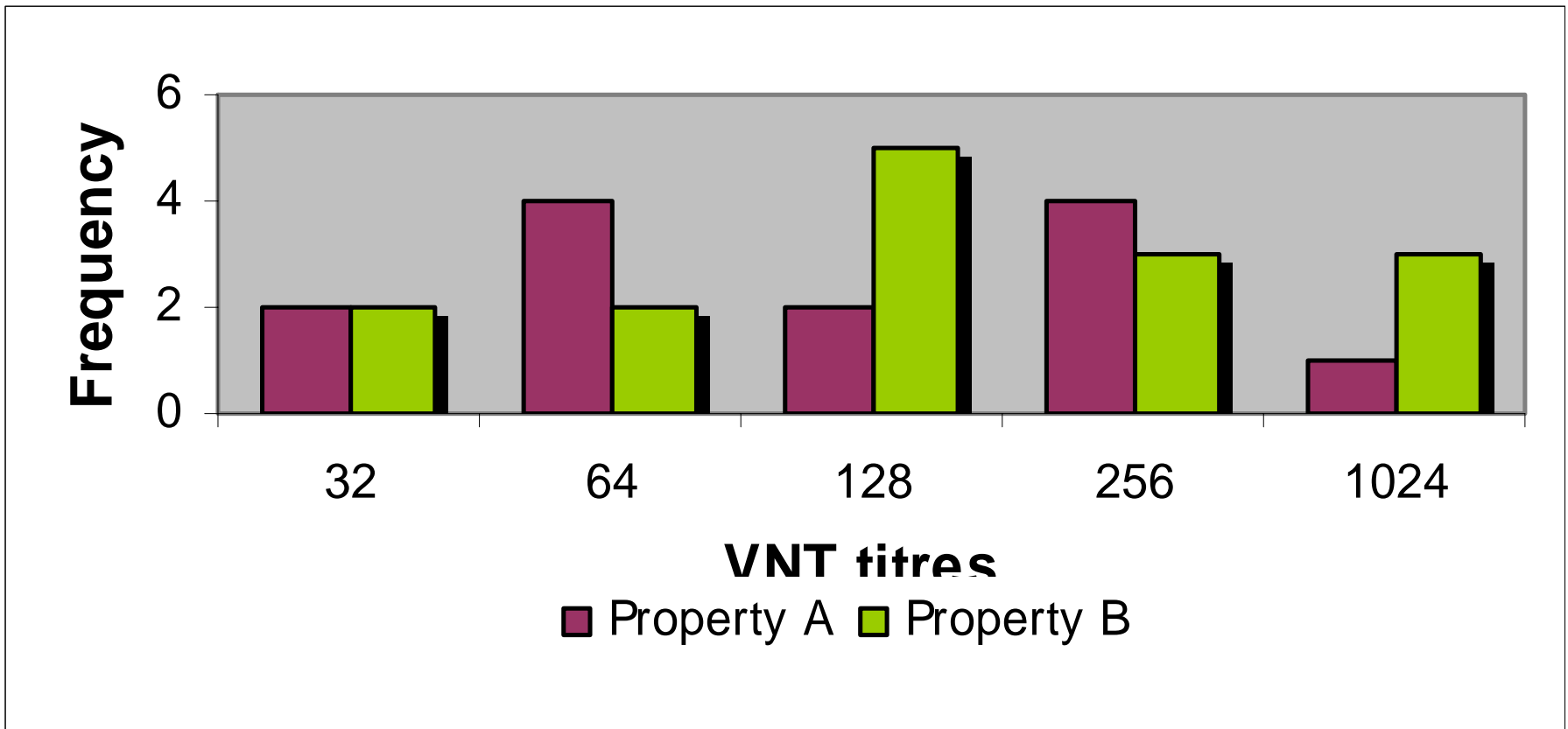
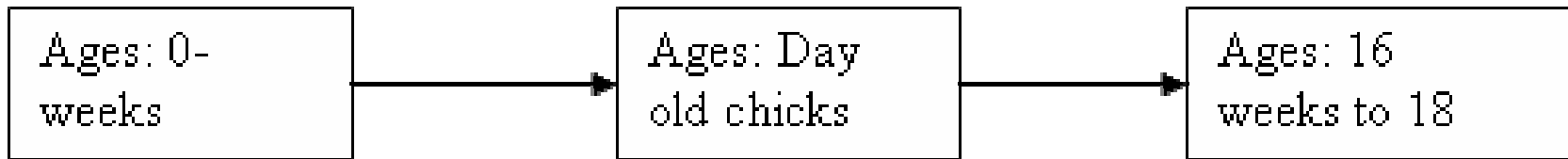
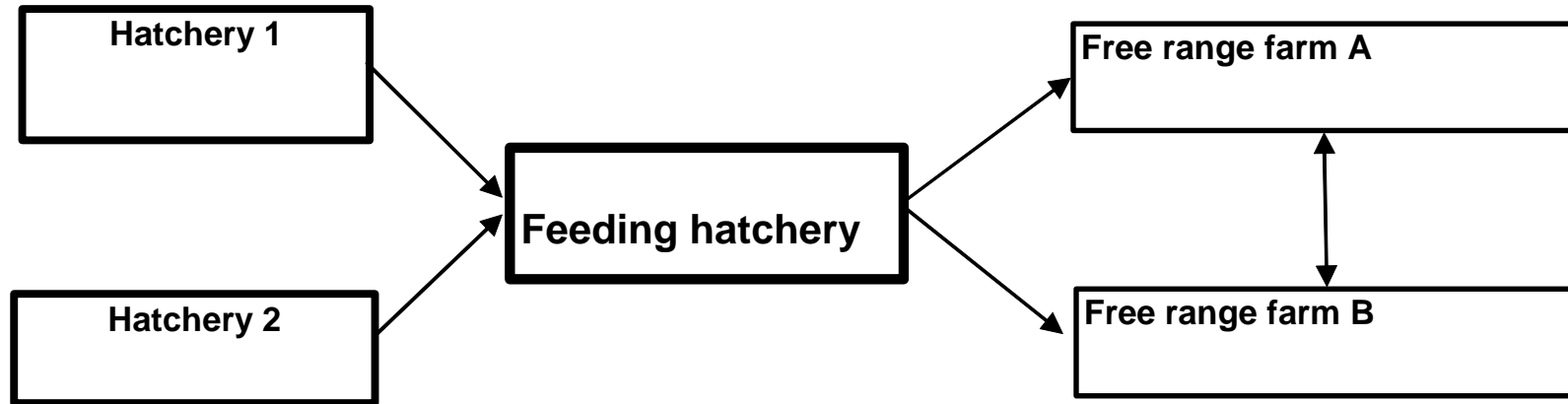


Figure 1: VNT titres layer birds from property A and property B

# Crude flow from hatch to layers

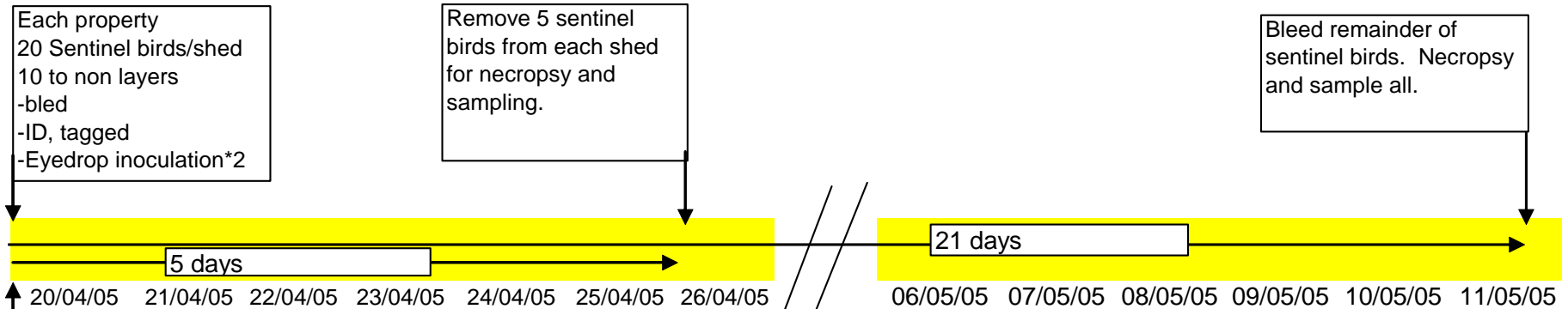


# Epidemiology

- **Source hatchery**
  - seronegative
- **Layer farms**
  - no change in production (high on A, poor on B but increased)
  - no significant mortality events or change in mortality.
  - wrong age group birds for antigen or VI
  - Epi links between the two farms
  - No obvious origin for IBD



# Sentinel bird protocol



- Bleed all non layer birds.  
- Cloacal swabs from all pedigree/non layer birds.  
- Cloacal swabs from 59 birds from each layer shed.

Sentinel birds 5 to a cage



# Sample size estimates

- **Sentinel #**

- FreeCalc (Version 2, Angus Cameron, 1998)
- Se detection 70%
- Prevalence 20%
- Type 1 and 2 errors set at 5%

Assumes random mixing

- **Litter infectivity high**

(Benton, W.J., M.S.Cover, J.K.Rosenberger, Avian Dis 1969)

- Eyedrops
- Feed from floor
- Placement of cages

- **Layers for cloacal swab**

- FreeCalc
- Se 95%
- Sp 100%
- Prevalence 5% shedding
- Type 1 and 2 errors set at 5%



# Identification and bleeding of sentinel broilers.

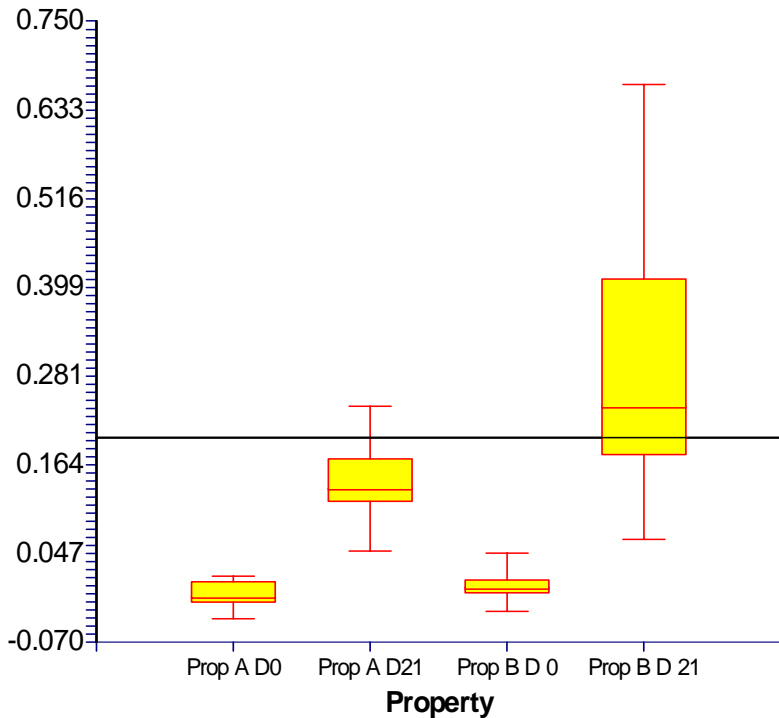


# Results Day 5

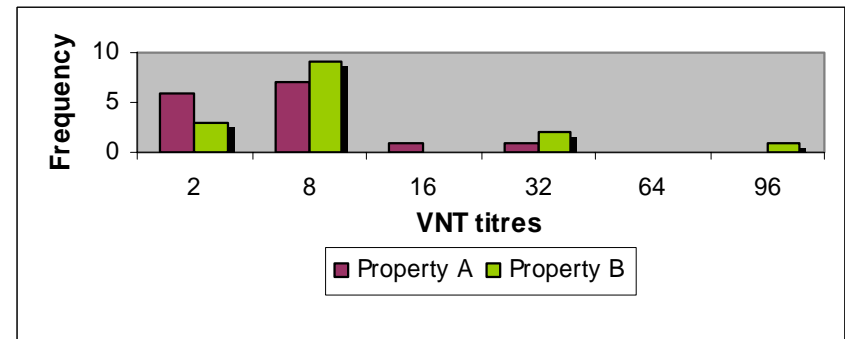
- No clinical disease
- Serology negative (at 0 days and 5 days)
- PCR Results all -ve
- Virus isolation result – Pending
- Histology --not consistent with IBD.
- Pooled PCR results from 60 layers, and in contact non-layer birds - negative.
- PCR from eye drop - Negative

# Results Day 21-Seroconversion

S\_P\_Ratio



Paired (day 0 and 21) IDEXX ELISA S/P ratios for property A and property B.



Day 21 convalescent VNT results from sentinel birds

Table 1: Summary statistics sentinel bird ELISA readings on day 21 sampling

	Number	ELISA(positive)	Range S/P	Median S/P	Mean S/P	
Property A	15	2	0.05-0.24	0.13	0.14	
Property B	Layers	11	7	0.06-0.67	0.24	0.29
	Non-layers	5	1	0.03-0.31	0.05	0.12
	Overall	16	8	0.03-0.67	0.2	0.24

# Conclusion from serology:

- Sentinel program – good sensitivity
- median values close to cut-off at 21 days this could indicate
  - The titres are still rising?- Inconsistent with lit and experience
  - Cross-reaction with another antigen? – What?
- **still require more direct evidence of IBDV**
  - RT-PCR product
  - Virus isolation
  - histological changes consistent with IBD.

# Independence of serological tests?

- Kappa ELISA day 21 and VNT 1:4 cut-off

		VNT 1:4		Total
ELISA 1/0	1	0		
1	7	3		10
0	3	17		20
Total	10	20		30

Observed	0.8
Expected	0.56
Actual agreement over chance	0.24
Potential agreement over chance	0.44
<b>Kappa</b>	<b>0.55</b>

Conclusion: Moderate agreement at VNT 1:4

Complete independence strengthens case for IBDV

ELISA – antibodies induced by

- VP3 and VP2 genes

VNT – antibodies induced by

- VP 2 gene

*Saif, Y.M., Diseases of Poultry. 11 ed. 2003,*

Conclusion:

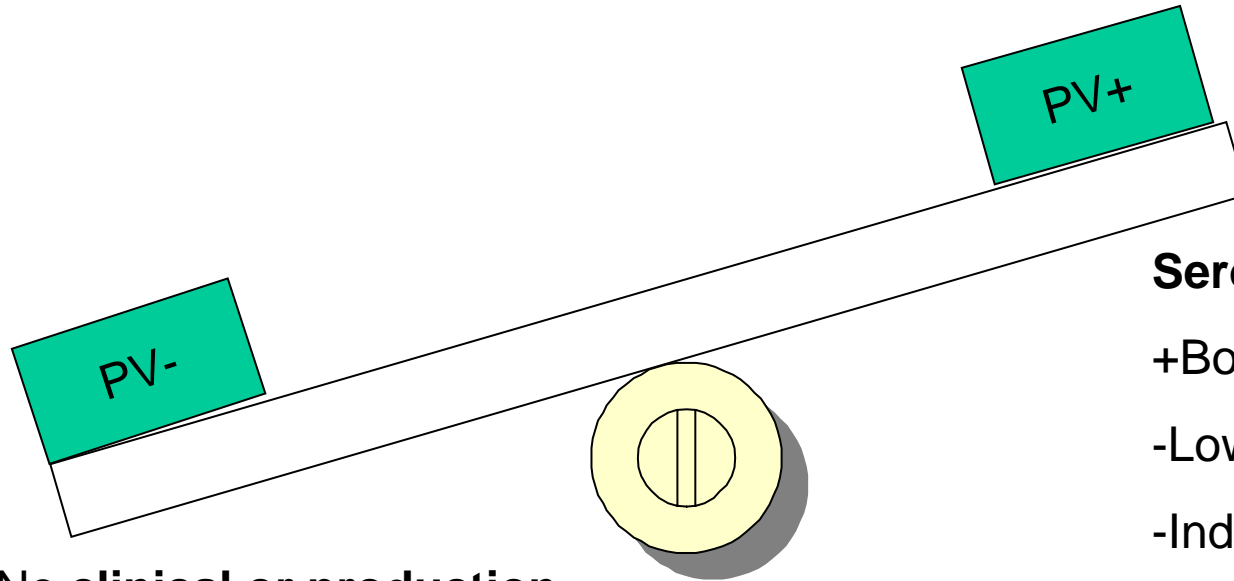
Cross reactivity likely

Not completely independent

# Further results day 21

- No disease
- RT-PCR negative.
  - More sensitive than VI (Abdel and Saif 2001)
  - Primers for the segment A gene of IBD virus.
- **Histology: Not consistent with IBD**
- **Virus isolation**
  - 5 days and 21 days still pending

# Dealing with uncertainty



## Serology:

+Both tests

-Low titres

-Independence

No **clinical or production** effect

No Epidemiology evidence

No **histology**

No **antigen/agent**



In exposed birds of  $\sqrt{\text{age}}$

(high Se at farm level  $1-(1-AP)^n$ )

# Future planning?

- **MAF**
  - No evidence of exotic IBDV – stand down
- **Industry**
  - ‘Country freedom plan’ -dealing with lack of specificity in the face of low disease prevalence (individual and farm level).
- **MAF and Industry**
  - Plan for dealing with positives (farm level) to rule in/out endemic/exotic IBDV
  - Causes of cross reactions
    - Research
    - Observational study – risk factors.