

IMMUNOLOGICAL ASSAYS TO MONITOR FLOCK EXPOSURE TO *CAMPYLOBACTER HEPATICUS*, THE CAUSE OF SPOTTY LIVER DISEASE IN CHICKENS

Thi Thu Hao Van¹, Chithra Muralidharan¹, Arif Anwar², Jiongrui Huang², Timothy B. Wilson², Peter C. Scott², and Robert J. Moore¹

¹School of Science, RMIT University, Bundoora, Australia, ²Scolexia Pty Ltd., Moonee Ponds, Australia.



Spots on the liver of an infected chicken

Spotty liver disease (SLD)

- Causes up to 15% increased mortality and up to 25% egg loss
- Mostly affects during early to peak laying period
- Characteristic cream/grey spots on liver surface

The causative agents

› Int J Syst Evol Microbiol. 2016 Nov;66(11):4518-4524. doi: 10.1099/ijsem.0.001383.
Epub 2016 Aug 5.

➤ *Campylobacter hepaticus* (2016)

Campylobacter hepaticus sp. nov., isolated from chickens with spotty liver disease

Thi Thu Hao Van¹, Eltahir Elshagmani¹, Mian Chee Gor¹, Peter C Scott^{2 3}, Robert J Moore^{3 1}

INTERNATIONAL JOURNAL OF
SYSTEMATIC AND EVOLUTIONARY
MICROBIOLOGY

TAXONOMIC DESCRIPTION

Phung et al., Int. J. Syst. Evol. Microbiol. 2022;72:005314
DOI 10.1099/ijsem.0.005314



➤ *Campylobacter bilis* (2022)

Campylobacter bilis sp. nov., isolated from chickens with spotty liver disease

Canh Phung¹, Peter C. Scott², Chaitali Dekiwadia¹, Robert J. Moore¹ and Thi Thu Hao Van^{1*}

SLD - DETECTION

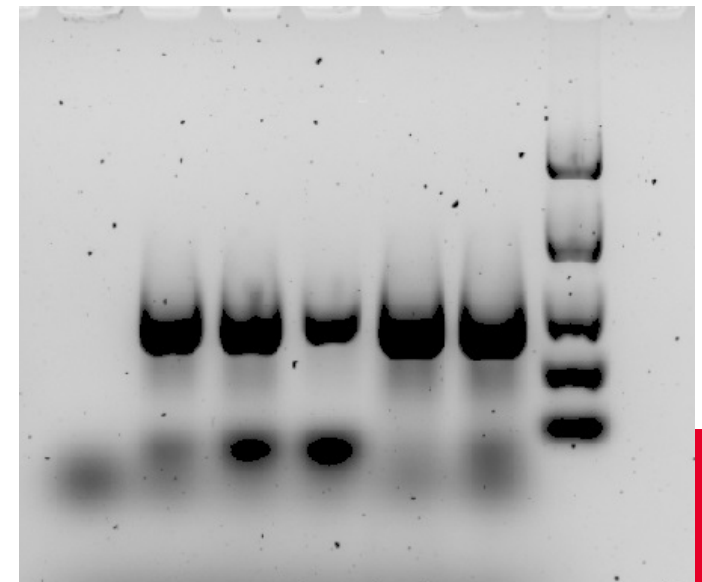
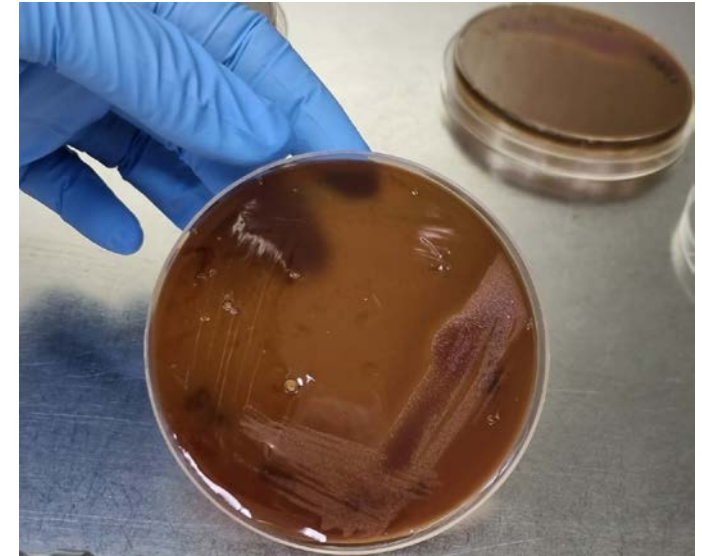
- Detection methods such as culturing and PCR can only detect a live infection



***Campylobacter hepaticus*, the Cause of Spotty Liver Disease in Chickens: Transmission and Routes of Infection**

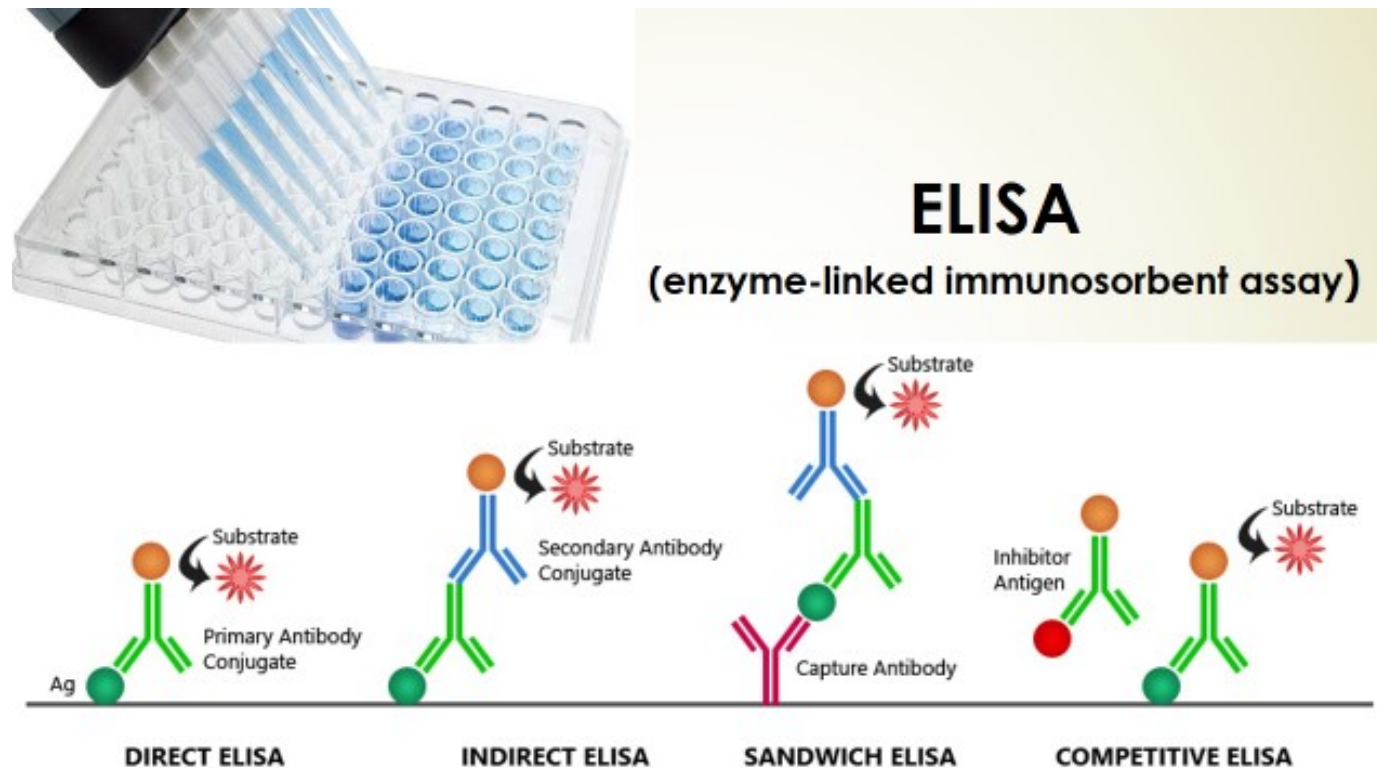
Canh Phung¹, Ben Vezina¹, Arif Anwar², Timothy Wilson², Peter C. Scott², Robert J. Moore^{1*} and Thi Thu Hao Van¹

¹ School of Science, RMIT University, Bundoora West Campus, Bundoora, VIC, Australia, ² Scolexia Pty Ltd., Moonee Ponds, VIC, Australia



SLD - DETECTION

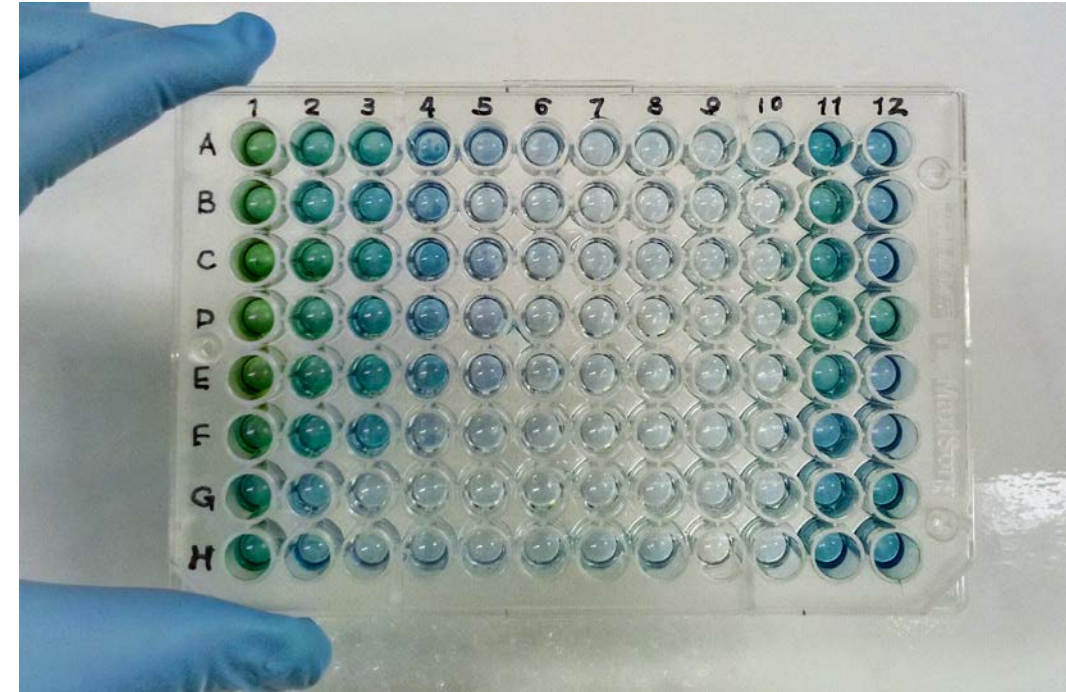
- No immunological methods were available that can give an information about a past infection



<https://www.biosciencenotes.com/elisa-enzyme-linked-immunosorbent-assay/>

Significance of an SLD immunoassay

- Study the immune responses in birds infected with *C. hepaticus*
- Better understand the epidemiology of disease
- Surveillance of SLD in farms
- Inform biosecurity
- Understand the nature of infection
- Evaluate the efficacy of bacterin vaccines



Research Aims

1. To develop an immunoassay capable of detecting *C. hepaticus* specific antibodies in chicken sera using *C. hepaticus* total proteins, **SLD-ELISA1**
2. To develop a second immunoassay using the recombinant protein with specific immunogenicity, **SLD-ELISA2**
 - To identify proteins with *C. hepaticus* specific immunogenicity using bioinformatics and molecular methods.
 - To investigate the seroprevalence of SLD in Australian free-range farms.

Development of SLD-ELISA1

- ❑ The first immunoassay developed for Spotty Liver Disease (SLD)
- ❑ Highly specific (95.5%) and sensitive (97.6%) assay
- ❑ A key tool for use in SLD bacterin vaccine development

Application:

- ❑ Used to assay over 400 sera samples from various vaccination and infection trials to evaluate the antibody responses

AVIAN PATHOLOGY
2020, VOL. 49, NO. 6, 658–665
<https://doi.org/10.1080/03079457.2020.1813252>



ORIGINAL ARTICLE

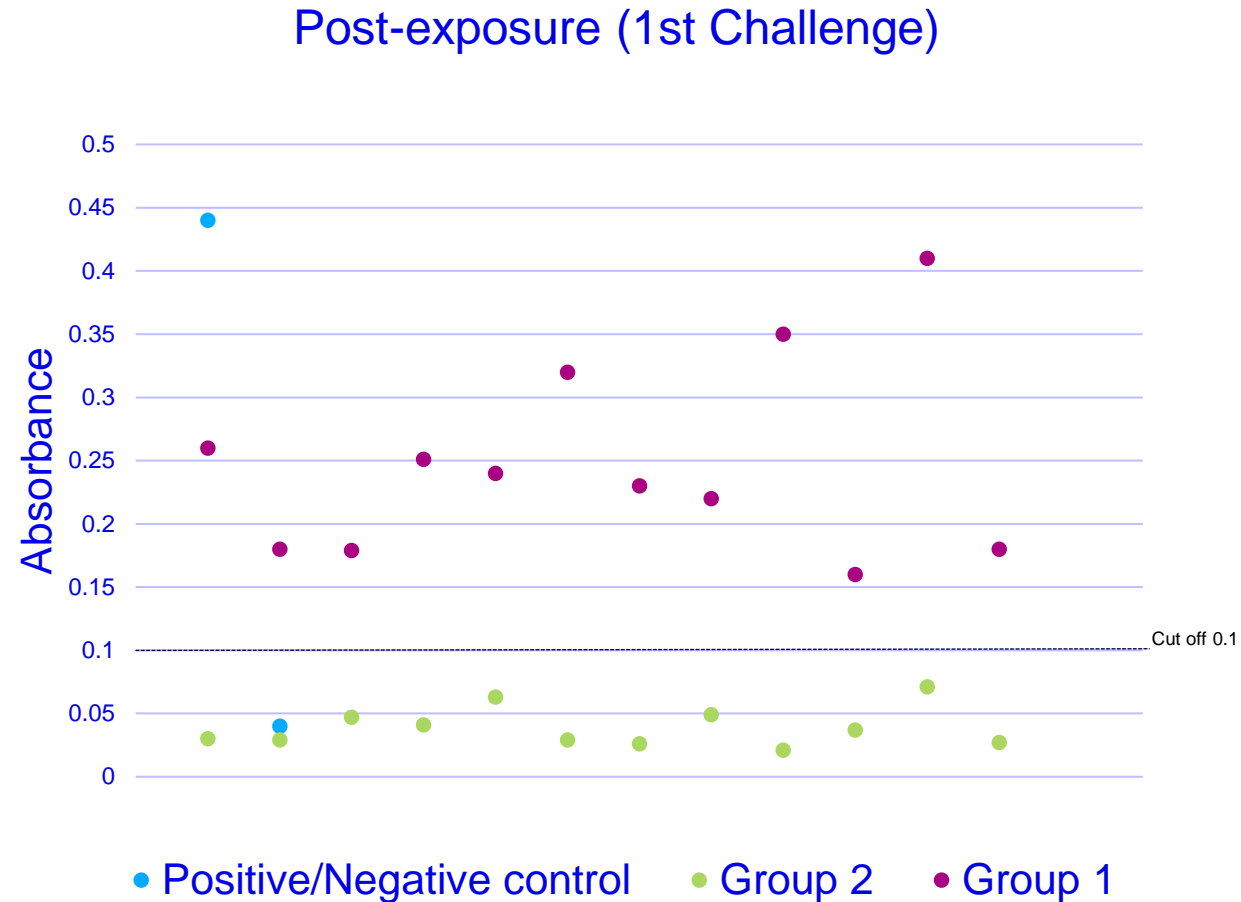
OPEN ACCESS Check for updates

Development of an enzyme-linked immunosorbent assay for detecting *Campylobacter hepaticus* specific antibodies in chicken sera – a key tool in Spotty Liver Disease screening and vaccine development

Chithralekha Muralidharan^a, Arif Anwar^b, Timothy B. Wilson^b, Peter C. Scott^b, Robert J. Moore ^a and Thi Thu Hao Van^a

^aSchool of Science, RMIT University, Bundoora, Australia; ^bScolexia Pty Ltd., Moonee Ponds, Australia

SLD-ELISA1- birds' antibody responses 3 weeks after *C. hepaticus* challenge



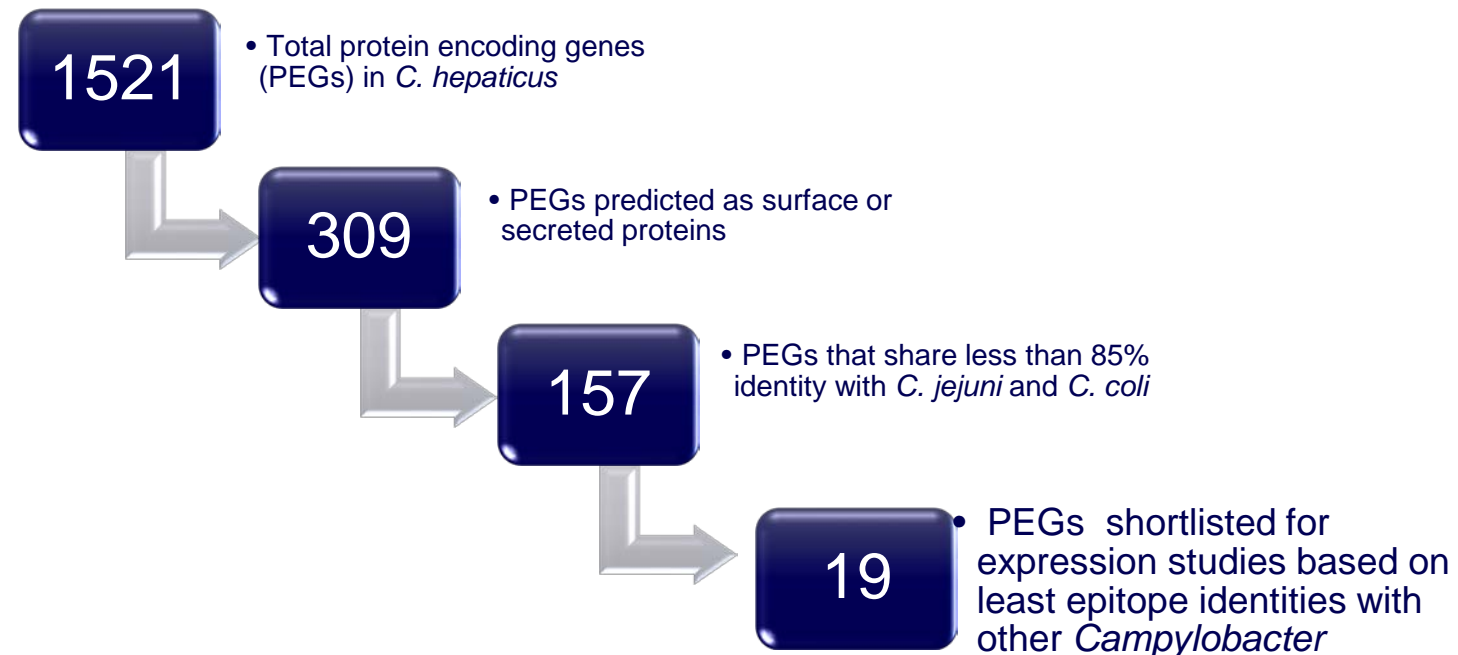
Development of ELISA 2

Why another ELISA?

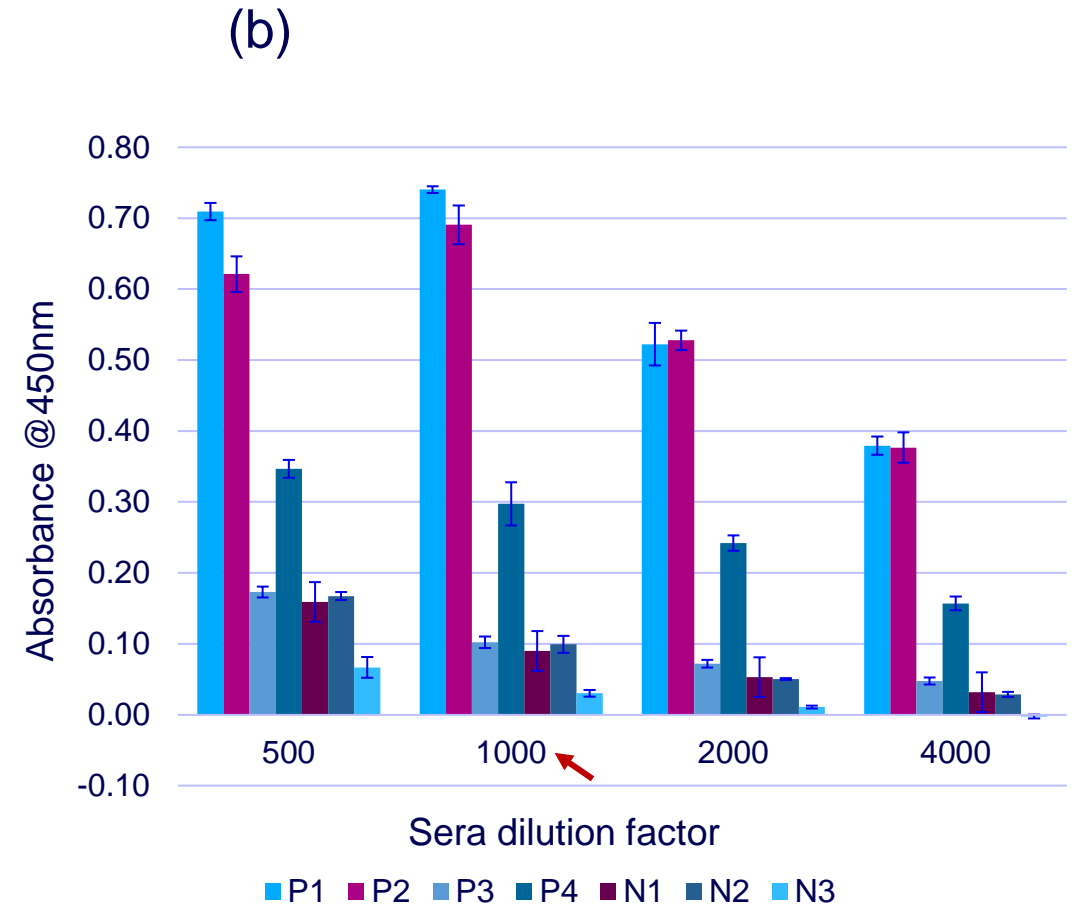
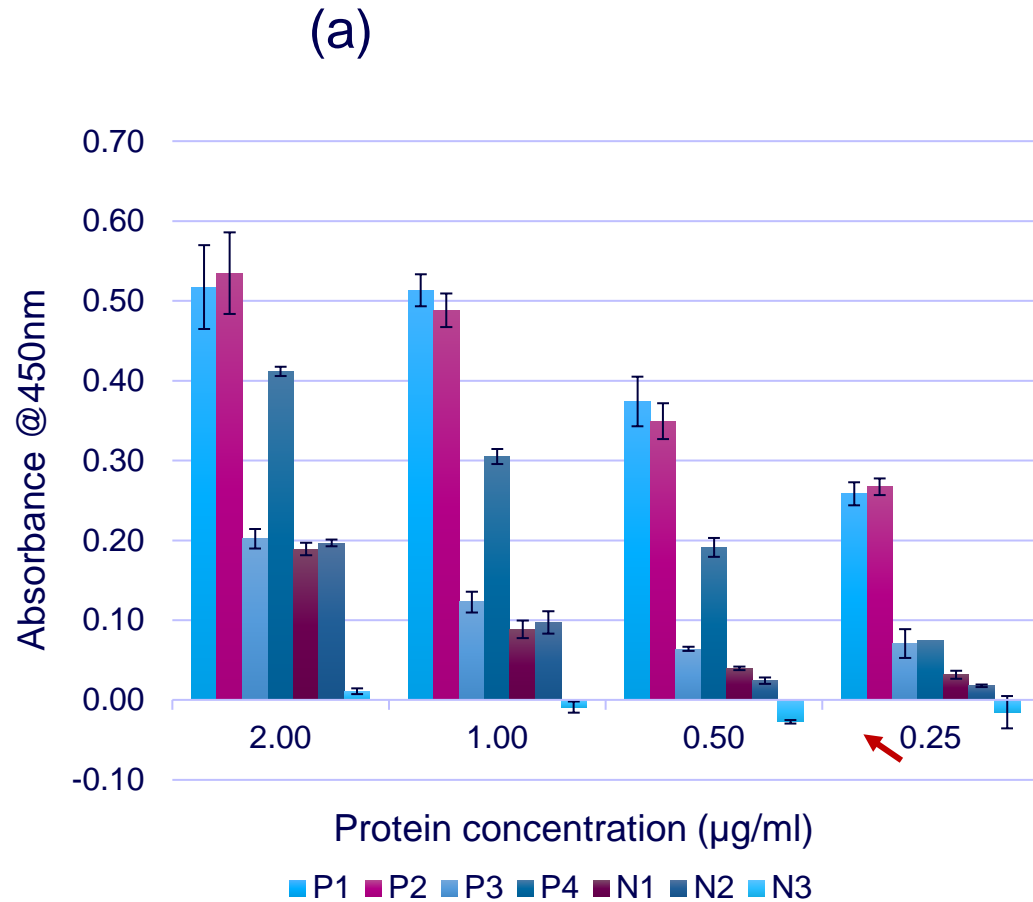
- An ELISA that uses pure protein is more standardizable
- It has better quality control characteristics than ELISA1
- More convenient than SLD-ELISA1 as it doesn't require the additional resources and time for pre-absorption

Development of ELISA 2

- Identification of *C. hepaticus* specific immunogenic proteins
 - bioinformatics
 - molecular methods
- From 1521 protein encoded genes, 19 prospective candidates for cloning, transformation, and expression studies.

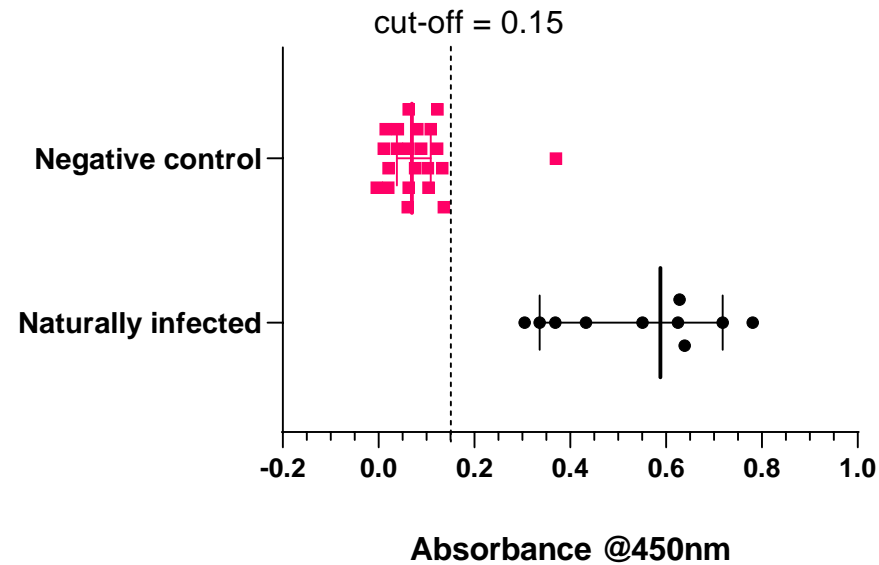


Optimisation of protein coating and sera dilution for SLD-ELISA2



ELISA 2- Absorbance value distribution

Individual values - Median with 95% CI



Ordinary one-way ANOVA pairwise comparison: $p < 0.0001$

- Assay sensitivity: 100% naturally infected birds
- Assay specificity: 95%

Validation of SLD-ELISA2 using sera samples from free range layer farms

	<i>Number of samples</i>	<i>Number of samples that were positive in SLD-ELISA1</i>	<i>Number of samples that were positive in SLD-ELISA2</i>
Samples from SLD affected farm	69	38 (55%)	38 (55%)
Samples from farm with no history of SLD	10	1	1

ELISA2

- ❑ A more convenient and standardizable ELISA using recombinant protein to detect *C. hepaticus* specific antibodies was developed.
- ❑ SLD-ELISA2 is suitable for screening *C. hepaticus* infection in farms.

Investigation of seroprevalence of *Campylobacter hepaticus* in commercial free-range layer farms in Australia

Experimental design

- 500 blood and cloacal swab sample pairs were collected from free-range egg farms in Victoria, New South Wales, and Queensland
- Sera samples were assayed using SLD-ELISA2
- Cloacal swabs were assayed using end point PCR (Van et al., 2017)
- The egg production and mortality data provided by the farms were correlated with the ELISA and PCR results

SLD-ELISA2 and PCR results

Farm location	Flock age (weeks)	SLD status reported	SLD-ELISA 2 (percentage of birds that tested positive)	PCR (percentage of birds that tested positive)
NSW	58	Positive (First SLD reported 21 weeks before sample collection, most recent outbreak reported 15 weeks ago)	44.20%	2%
VIC	34	No known SLD history	68.60%	0%
VIC	33	Positive (First SLD reported 4 weeks before sample collection)	80%	0%
QLD*	53	No known SLD history	22%	4%

*In two weeks preceding sample collection, mortality increased 2.7 times and 3.4% drop in egg production.

- Birds without SLD history but positive in ELISA assay: *C. hepaticus* infection may not be sufficient to produce clinical SLD- other predisposing factors need to be present before SLD manifests.

Overall Summary

- ❑ Two immunoassays have been developed, ready to be used in studying SLD immune responses in chickens.
- ❑ ELISA1 can be used for naturally and experimentally *C. hepaticus* infected samples.
- ❑ ELISA2 consumes less time to perform and has better quality control, as pure recombinant protein was used as the capture antigen.
- ❑ Assays can be used for surveillance of SLD in farms, inform decision making about biosecurity measures and flock management.

We would like to thank:

- PHA for financial support
- Dr Victor Alvarez (DA Hall & Co) for providing field samples
- Thanks to all for listening