

**‘Investigation of the diagnostic value of ELISAs using
colostrum derived immunoglobulins for targeted production
animal diseases’**

by

Caitlin J Jenvey BSc. (Animal Science) Hons.

A thesis submitted for the fulfilment of the
requirements of the Doctor of Philosophy

March 2015

The University of Adelaide
Faculty of Sciences
School of Animal and Veterinary Sciences
Roseworthy Campus

Table of Contents

Abstract.....	6
Declaration of Originality	8
Acknowledgements	9
Chapter 1: The diagnostic value of colostrum	10
1.1 Introduction.....	10
The mammary gland.....	10
The role of the mammary gland	10
The local immune system.....	11
Immunoglobulins.....	14
Immunoglobulin G (IgG)	17
Immunoglobulin A (IgA)	19
Immunoglobulin M (IgM).....	21
Secretions of the mammary gland	22
Colostrum	22
Milk	27
Whey	28
Passive transfer.....	31
Neonatal protection	31
IgG specific transport	33
Monitoring of disease.....	33
Measures of diagnostic utility	34
Sensitivity and specificity	34
Likelihood ratio	37
Diagnostic odds ratio.....	38
Predictive values	39

Receiver Operating Characteristic (ROC) curve and Area Under the Curve (AUC)	40
Youden's index	43
Diagnostic accuracy	43
Enzyme-linked immunosorbent assay (ELISA).....	44
Types of ELISA	45
Disease-specific ELISAs	48
Utilisation of colostrum and whey in ELISA	55
Thesis outline	57
References	59
1.2 Letter: Role for colostrum and whey in testing for bovine TB and Johne's disease?	76
Chapter 2: The coagulation potential of rennet.....	79
2.1 Improving the performance of disease antibody tests: Immunoglobulin G concentrations in colostrum and whey produced using different rennet coagulation protocols	80
2.2 Investigation of the impact of oral rennet supplementation on the serum globulin concentration in neonatal piglets	96
Original Article: Investigation of the impact of oral rennet supplementation on the serum globulin concentration in neonatal piglets.....	97
Chapter 3: Swine Erysipelas and Enzootic Pneumonia	104
3.1 <i>Erysipelothrix rhusiopathiae</i> and <i>Mycoplasma hyopneumoniae</i> : The sensitivities of enzyme-linked immunosorbent assays for detecting vaccinated sows of unknown disease status using serum and colostrum, and the correlation of the results for sow serum, colostrum, and piglet serum	105
Short Communication: <i>Erysipelothrix rhusiopathiae</i> and <i>Mycoplasma hyopneumoniae</i> : The sensitivities of enzyme-linked immunosorbent assay for detecting vaccinated sows of	

unknown disease status using serum and colostrum, and the correlation of the results for sow serum, colostrum, and piglet serum	124
Chapter 4: Bovine Viral Diarrhoea Virus	132
4.1 Performance characteristics of ELISA to detect Bovine Viral Diarrhoea Virus (BVDV) antibodies in colostrum	133
Short Communication: Performance characteristics of ELISA to detect bovine viral diarrhoea virus (BVDV) antibodies in colostrum.....	150
4.2 Optimising the measurement of colostrum antibody concentrations for identifying BVDV persistently infected calves	159
Short Communication: Optimising the measurement of colostrum antibody concentrations for identifying BVDV persistently infected calves	160
Chapter 5: Johne's disease.....	168
5.1 Investigation of the comparative diagnostic sensitivity of serum, colostrum and whey for the detection of specific antibodies in a flock of South Australian Merino-cross ewes vaccinated against Johne's disease	169
Short Communication: Investigation of the comparative diagnostic sensitivity of serum, colostrum and whey for the detection of specific antibodies in a flock of South Australian Merino-cross ewes vaccinated against Johne's disease	182
5.2 The diagnostic performance of an antibody enzyme-linked immunosorbent assay using serum and colostrum to determine the disease status of a Victorian Jersey dairy herd infected with <i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>	187
Short Communication: The diagnostic performance of an antibody enzyme-linked immunosorbent assay using serum and colostrum to determine the disease status of a Victorian Jersey dairy herd infected with <i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>	200
Chapter 6: Discussion and Conclusions.....	212
Appendix 1: Supporting Publications- Conference Papers	221

Oral Conference Presentations	222
Poster Conference Presentations	223
Bibliography	224

Abstract

Due to the process of colostrogenesis, maternal antibodies are selectively transferred from the serum and are concentrated in the colostrum. The concentration of IgG in cow colostrum can be up to 10 times greater in concentration compared to serum, while the concentration of IgG in the sow and the ewe may be up to 2.5-3 times greater than serum. In dairy cows and sows in farrowing crates, the collection of colostrum is simple and non-invasive. Following fractionation of colostrum into whey and curds, the concentrations of Igs in the whey are often higher than in the colostrum. Due to these higher concentrations of Igs, colostrum and colostrum whey may improve the diagnostic utility of antibody ELISAs used for the diagnosis of important infectious production animal diseases.

There are a number of commercially available antibody-ELISAs for important production animal diseases. These tests are inexpensive and easy to perform, and are used extensively. Tests with a high sensitivity correctly identify a higher proportion of infected animals within a population, increasing the assurance of absence or presence of disease. Tests with a low sensitivity correctly identify a lower proportion of infected animals, increasing the incidence of false-negative test results.

This thesis investigated the diagnostic value of colostrum derived immunoglobulins for targeted production animal diseases. Using vaccinated animals, the diagnostic sensitivities of commercially available ELISAs for erysipelas (*Erysipelas rhusiopathiae*) and enzootic pneumonia (*Mycoplasma hyopneumoniae*) in pigs, Bovine Viral Diarrhoea Virus in dairy cattle and Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*) in sheep were determined when using colostrum compared

to serum. Additionally, the diagnostic specificity was also determined for Bovine Viral Diarrhoea Virus in dairy cattle using samples collected from an unvaccinated, bulk tank milk negative herd. The diagnostic utility of the ELISA for Johne's disease was also further investigated using samples collected from a dairy cattle herd with a history of previous infection.

A model using sow disease-specific antibody levels for the prediction of piglet serum disease-specific antibody levels was also developed, as well as the use of colostrum in ELISA for the detection of heifers carrying a calf persistently infected (PI) with Bovine Viral Diarrhoea Virus. The relationship between rennet dilution and IgG concentration in colostrum whey and the benefit of oral rennet supplementation during the neonatal period on the serum globulin concentration of piglets was also investigated.

Overall, the diagnostic sensitivities of the ELISAs were improved when using colostrum compared to serum, whilst also maintaining diagnostic specificity. Sow colostrum and serum were useful predictors of piglet serum disease-specific antibody levels, while colostrum collected from heifers carrying PI calves had significantly higher disease-specific antibody levels. The experiments that explored the coagulation potential of rennet were unable to demonstrate any improvements in Ig concentrations.

Colostrum may be a useful sample type for the detection of other important livestock diseases. The sensitivity of the ELISA for bovine tuberculosis when using serum is too low for control policies to be effective, while the analytical sensitivity of the ELISA for *Neospora caninum* when using bulk tank milk could still be improved.

Declaration of Originality

I certify that this work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or tertiary institution without the prior approval of the University of Adelaide and, where applicable, any partner institution responsible for the joint award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Caitlin J. Jenvey

Acknowledgements

First and foremost, I must thank my supervisors, Professor Peter Cockcroft and Professor Michael Reichel for giving me the opportunity to conduct this PhD. Three years ago, when I began this PhD, I felt that I was in way over my head and it all seemed a bit daunting. Three years on, and a career in research is something that I am very interested in, which ultimately comes down to the unwavering guidance, help and support from my supervisors.

Secondly, I must also thank the people, on and off campus, who were able to help me with sample collection and lab work, in particular, John Matheson and the Roseworthy Farm staff, the Roseworthy Piggery staff, Greg and Brian Wilson, Kevin and Margie Tesselaar and Peter Younis and The Timboon Vet Group. Special thanks must go to Dr Andrew Weir, Dr Sasha Lanyon, and Caitlin Evans who were able to make their time available to assist me with my experiments, and all my endless questions.

Most importantly, I must thank my family for their support and encouragement during the last eight years. I know that my parents would love if I did not live quite so far away. I want to thank them for their support and understanding when I moved interstate for my undergraduate degree and their continued support and understanding when I decided to stay to complete my Honours degree and PhD. A special thank you must go to my future partner in crime, Brenden Johansson, who I am sure is just as glad as me to see everything finished. It is due to all this support that I was able to complete this PhD. So, thank you.