

**‘Control and Mitigation of Bovine Viral Diarrhoea in
Australian Cattle Populations’**

by

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2 Abstract

Bovine viral diarrhoea (BVD), more commonly in Australia as Bovine Pestivirus, is an economically important disease of cattle. The causative agent, BVD virus (BVDV), is a member of the genus Pestivirus in the family Flaviviridae, closely related to Border Disease Virus and Classical Swine Fever Virus. An increased incidence and severity of secondary disease and potentially dramatic reproductive loss associated with BVDV infection results in ongoing financial impacts in infected herds. Fortunately, the epidemiology of BVD is such that the disease can be effectively controlled, and losses mitigated, by identification and removal of persistently BVDV infected (PI) cattle. Regional or national control schemes have been shown to be economically beneficial. In Australia, however, no regional schemes are active for the control of BVD.

The first clinical case of BVD was reported in Australia in 1957. Recent serological evidence suggests that BVD may be the most prevalent infectious disease of cattle in Australia today. Despite this, BVD fails to be acknowledged as a major animal health priority. A postal survey of 631 South Australian cattle farmers showed that while interest in BVD was high, many producers did not believe their herds to be infected and failed to acknowledge the true impact the disease may have in an affected herd. The survey results revealed that farmers that practiced disease management through quarantine procedures, regular vaccination, participation in disease control and attendance at seminars were most likely to have high knowledge and perceived understanding of BVD. The survey results suggest that a BVD education program (which could be targeted to farmer demographics that were observed to have the lowest knowledge of BVD) and subsequent control scheme would likely be well received.

Control schemes rely on accurate diagnosis of BVD, with rapid, inexpensive tests (such as ELISA and RT-PCR) available for detection of specific antibody, viral antigen and viral RNA. A thorough understanding of the pathogenesis of BVD allows veterinarians and diagnosticians to appropriately select diagnostic samples and tests that are most appropriate and cost-effective for a particular diagnostic goal. Milk samples represent an alternative for testing of lactating

animals for BVDV-specific antibodies, with test performance observed to be very good compared to serum testing. Furthermore, bulk milk may be tested to produce an estimate of seroprevalence within the milking herd and, in turn, the likelihood of the herd being actively infected. In non-milking cohorts, including beef animals and young stock, pooled serum can be tested for a similar result. These bulk samples are a highly cost-effective testing option.

An experimental trial investigated diagnostic opportunities in pregnant females and their resultant calves. In pregnant females, very high antibody levels should cast suspicion of fetal PI, while low positive results may coincide with neurological deformation (hydrocephalus and cerebellar hypoplasia) in the developing calf, resulting in clinical signs such as ataxia, astasia and wide-based stance. In the calves, ingestion of colostrum interfered with diagnosis of PI status until maternal antibody levels waned. Ear notch samples were least affected by interference, while serum and swab samples were similarly affected.

3 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.

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Sasha R. Lanyon

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5 Introduction

Bovine viral diarrhoea (BVD) is a highly prevalent, economically important disease of cattle which has been the subject of a significant amount of research. Indeed, a Web of Science search using search term ‘BVD’ returns more than 8,000 results, spanning early reports of clinical disease and isolation of the virus in the late 1950’s to recent communications of successful control efforts. The underlying motivation of this substantial body of research is simple: the opportunity to increase productivity and profitability in cattle industries. The same motivation underlies the research presented in the nine manuscripts contained within this thesis.

This first manuscript, a review published in Springer Science Reviews (Vol.1, 2013), begins by exploring the intricacies of the various ways in which BVD impacts cattle populations. The paper then proceeds to outline the options for systemic BVD control, and the status of some current control efforts.

Review: Understanding the Impact and Control of Bovine Viral Diarrhoea in Cattle Populations

SR Lanyon, MP Reichel (2013)

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6 Bovine Viral Diarrhoea in Australia: Perceptions and Perspectives

Having appreciated the impact of BVD and acknowledged that not only are there options for control of the disease, but that control has been successfully achieved in certain regions, the question arises: what is the BVD situation in Australia? The following review and discussion paper, published in the Australian Veterinary Journal (Vol. 92, Pp. 277-282), examines the available literature relating to BVD in Australia and discusses the feasibility of a control scheme of similar structure to those operating in Europe.

***Review and Discussion: Bovine Viral Diarrhoea (“Bovine Pestivirus”) in Australia:
To Control or Not To Control?***

SR Lanyon, MP Reichel (2014)

Bovine Viral Diarrhoea (“Bovine Pestivirus”) in Australia: To Control or Not To Control?

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6.1 A Postal Survey Investigation of the Attitudes and Awareness of South Australian Cattle Farmers Towards Bovine Viral Diarrhoea

It is clear that stakeholder education and compliance is a key factor in any control program. In Australia, where BVD is not recognised as an animal health priority, assessment of the attitudes and awareness of stakeholders – primarily cattle farmers – seems prudent. The following two manuscripts present the results of a postal survey of South Australian cattle farmers. The first paper (accepted for publication in the Australian Veterinary Journal) reveals the apparent current attitudes towards control of endemic diseases, in particular BVD, in South Australia. Meanwhile, the second paper presents the characteristics of farmers and their production systems that are associated with high interest, knowledge and perceived understanding of BVD, such that educational efforts may be effectively tailored and targeted.

Original Article: A Survey of Farmer Knowledge and Attitudes to Endemic Disease Management in South Australia, with a Focus on Bovine Viral Diarrhoea (Bovine Pestivirus)

SR Lanyon, ML Anderson, MP Reichel (2014)

A Survey of Farmer Knowledge and Attitudes to Endemic Disease Management in South Australia, with a Focus on Bovine Viral Diarrhoea (Bovine Pestivirus)

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A survey of farmer knowledge and attitudes to endemic disease management in South Australia, with a focus on Bovine Viral Diarrhoea (Bovine Pestivirus)

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Abstract

Objective This study aimed to establish the attitudes of South Australian cattle farmers towards endemic animal disease prevention and control, with a particular focus on the awareness of and attitudes towards Bovine Viral Diarrhoea (BVD).

Design Cross-sectional postal survey

Procedures A questionnaire mailed to all South Australian cattle owners with 35 or more head of cattle.

Results Worms and lice were the most common animal disease concerns. Less than half of responding farmers were ‘adequately’ vaccinating their herds against clostridial diseases, while 53.0% stated that they utilised quarantine procedures. Less than 20% of respondents had actively taken part in BVD educational opportunities, or had vaccinated or tested their herd for BVD. Similarly, less than 20% of respondents were actively involved in any systematic control of Johne’s Disease. Overall, actual knowledge of BVD was lower than the perceived understanding, while interest in BVD and its control was high.

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Conclusions Disease prevention measures such as vaccination, quarantine and participation in systematic control schemes employed by a minority of respondents. The results suggest that respondents acknowledge BVD as an important and relevant disease, despite many believing BVD is not a problem in their herd. Interest in BVD appears high, and it is likely that an education program would be well received.

Key words: Disease Control; Preventative Health Care; Bovine Viral Diarrhoea; Pestivirus; Farmer Attitudes; Survey

Introduction

The South Australian cattle industry consists of more than 5,000 beef and 300 dairy herds, and produces around 93,000 tonnes of beef and 606 million litres of milk annually (South Australian Farmers Federation, 2009). This industry is free from several of the major infectious diseases such as Bovine Tuberculosis and Brucellosis, Bovine Spongiform Encephalopathy and Foot-and-Mouth Disease (World Organisation for Animal Health (OIE), 2013) that affect cattle industries on other continents. This may affect the perceived risk of infection which, if not severe enough to motivate disease control (Santarossa et al., 2005, Toma et al., 2013), may contribute (along with other factors such as ignorance, time constraints and perceived cost-effectiveness) towards a tendency for attitudes towards preventative disease control to lapse. However, endemic infectious diseases of cattle that are present in South Australia (such as Bovine Johne's Disease, Campylobacteriosis, Leptospirosis, Bovine Viral Diarrhoea and several clostridial diseases) may incur substantial associated costs (Sackett et al., 2006). Farmers who have better knowledge of, or place more value on biosecurity are more likely to exhibit stronger biosecurity behaviour (Toma et al., 2013), and thereby more effectively protect their herds from endemic disease. Therefore, it is important to know and understand the attitudes of farmers towards disease prevention and control so that the prevalence and associated costs of disease can be reduced.

Of particular focus in this study is Bovine Viral Diarrhoea (BVD), an infectious viral disease of cattle that is highly prevalent in the South Australian cattle industry. A report by Sackett et al. (2006) found that the economic impact of BVDV in Australia could not be accurately modelled due to a lack of data. However, based on a serological survey in 2008 that indicated that 97% of dairy and 85% of beef farms in South Australia have been exposed to BVD virus (Anderson et al. unpublished data, 2008), New Zealand costs estimates (Reichel et al., 2008) can be adapted to provide a conservative estimated annual cost to the South Australian cattle industry of \$5.6 million. Control and eradication programs have been put in place across much of Europe, including national campaigns in Switzerland (Presi and Heim, 2010), Sweden (Hult and Lindberg, 2005) and Norway (Valle et al., 2005). One of the key observations from the European BVD programs is that education and farmer compliance is vital to success (Lindberg and Alenius, 1999, Heffernan et al., 2009). Therefore, the present survey aimed to establish the current levels of awareness, knowledge and interest in BVD and its control and attitudes towards preventative animal health management amongst South Australian cattle farmers.

Materials and methods

Questionnaire design

A four page questionnaire was designed to collect information regarding disease management practices and attitudes (see supplementary material). Questions were separated into six sections: “*Your involvement in the cattle industry*”; “*Personal details*”; “*Your biosecurity and disease management*”; “*Your understanding of BVD*”; “*Your awareness of BVD*” and; “*Your interest in controlling BVD*”. Sections *your involvement in the cattle industry* and *personal details* contained questions regarding the cattle operation and the farmer, respectively, including but not limited to age, gender, education, herd size and breed(s) of cattle. The “*Your biosecurity and disease management*” section consisted of questions pertaining to the use of preventative health measures such as vaccination, quarantine, animal introductions, disease reporting and participation in a systematic Johne’s Disease program. Finally, the sections “*Your understanding of BVD*”, “*Your awareness of BVD*”, and “*Your interest in controlling BVD*”

targeted the respondent's self-perceived understanding of BVD, their actual knowledge of BVD and their interest in BVD and its control, respectively. The understanding and interest sections consisted of multiple statements to which respondents answered on a scale of 1 to 7 from strongly disagree to strongly agree. The "*Your awareness of BVD*" section contained sixteen statements which respondents were asked to classify as either "true", "false" or "don't know". The questionnaire underwent an extensive consultative process with feedback received from farmers, veterinarians, students, researchers, academic staff and government personnel.

Target population and mail out

In June 2011, hard copies of the questionnaire were posted to all (n = 4,165) South Australian cattle producers with 35 or more head of cattle, as recorded in the Primary Industries Information Management System (PIIMS) database. Respondents managing herds of fewer than 35 animals were excluded in an attempt to focus on commercial producers (and hence exclude part-time or hobby farmers). A reply paid envelope was included with each questionnaire and anonymous responses were received over a period of approximately five months. It was not possible to follow-up initial non-responders as the survey was conducted anonymously.

Incentive

As an incentive to return the questionnaire, all respondents were offered the chance to enter the draw to win free herd profile testing for BVD.

Statistical analyses

Answers to the "*Your understanding of BVD*", and "*Your interest in controlling BVD*" sections were recorded on a scale of 1 to 7, ranging from "strongly disagree" to "strongly agree". Answers of 1, 2 or 3 were considered to indicate disagreement, 4 was considered neutral and 5, 6 or 7 were considered to indicate agreement with the statement.

For the "*Your understanding of BVD*", and "*Your interest in controlling BVD*" sections, understanding and interest scores were generated by calculating the mean response (on the scale of 1 to 7) to all statements in the given section that the respondent answered. Responses from

one statement in the understanding section (“I do not know how to protect my herd from BVD”) were subtracted from 8 prior to calculation of the score to reflect the negative nature of this statement. Scores were rounded to the nearest integer (from 1 to 7), with a high score indicating higher self-perceived knowledge or higher interest in BVD.

An aggregate knowledge score was calculated from the “*Your awareness of BVD*” section, with a correct answer contributing +1, an answer of “don’t know” or an unanswered statement contributing 0 and an incorrect answer contributing -1. The knowledge score has a theoretical distribution from -16 to 16.

Ethics

This survey was approved by the University of Adelaide Human Research Ethics Committee (Project No: H-091-2011).

Results

Respondents

Response rate

Of the 4,165 questionnaires mailed out, 631 responses were received, giving a response rate of 15.2%.

Respondent demographics

The majority of respondents were male (86.4%). The mean age of respondents was 54.4 years (median: 55, mode: 60), varying from 15 to 86 years. There were no significant differences in mean age or age distribution between males and females. On average, respondents had been involved in the cattle industry for 30.7 years, more than half the mean age.

The vast majority (95.8%) of respondents had completed education to Year 10 level or higher, with more than half (58.5%) having completed Year 12 or higher and 40.8% (95% CI: 37.0 - 44.7) holding a post-school qualification such as a Technical and Further Education (TAFE) certificate or university degree.

A portion of respondents (14.2%) maintained a primary occupation outside of the agricultural industry (for example: doctor, teacher or tradesman), while the remainder worked in a variety of roles in the agricultural industries, including farmer, grazier, livestock transport, fencing contractor or stock agent. Nearly all respondents (96.3%) were the owners of the cattle herd with which they worked, while the remainder filled roles such as farm worker or manager. All respondents (100%) were fully or partially responsible for making management decisions regarding the herd.

Farm demographics

The cattle herds managed by the respondents were primarily beef operations (90.5%), with dairy operations representing 5.4% of responses and 4.1% running mixed beef and dairy operations. Most (87.6%) of these were commercial operations, with the remainder primarily consisting of studs, feedlots and trading or fattening operations. Mean herd size was 282 head, with a range from 5 to 14,000. The median herd size was 100, indicating a positive skew in the distribution of herd size.

The most common cattle breed was Angus, with 17.4% of respondents running straight bred Angus herds and a further 39.5% running mixed breed herds with an Angus influence. An additional 20.5% of respondents ran mixed breed herds with no Angus breeding. British breeds were the most abundant with common breeds (other than Angus) including Murray Grey, (Poll) Hereford, (Poll) Shorthorn and Holstein Friesian.

Disease management

Disease concerns

Worms and lice were the most common responses to the question ‘What are your top three disease or parasite concerns?’ with 58.6% and 45.8% of respondents mentioning these conditions, respectively. The third most common disease concern was BVD (26.2%), with bovine Johne’s disease fourth most commonly mentioned (13.0%). Other disease concerns mentioned included grass tetany, scours and respiratory disease.

Disease reporting

When asked which health events they would report, more respondents would report deaths than sickness, abortions or lameness (Figure 1). More respondents would report the health event if more of their herd was affected (Figure 1). With any given proportion of the herd affected, approximately 10 to 20% fewer respondents would report lameness than any other condition, while similar proportions of respondents would report abortions and sickness.

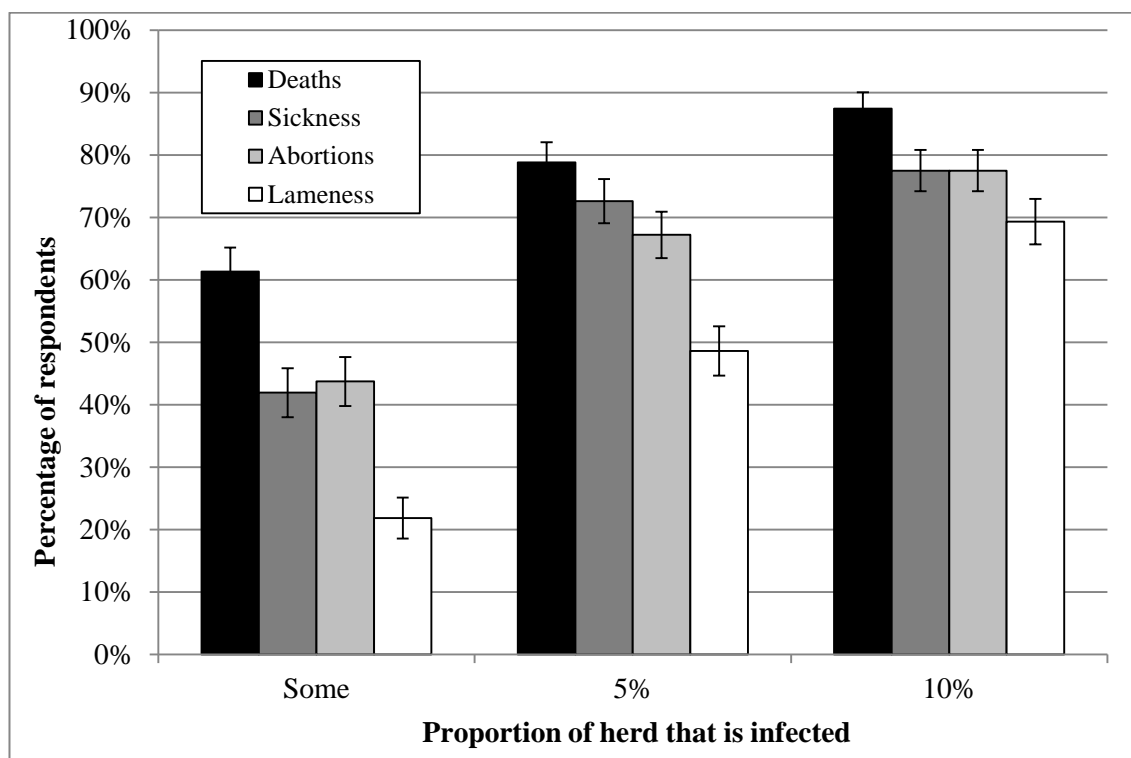


Figure 1. Proportion of respondents (n=613) to a questionnaire surveying with 4,165 South Australian cattle farmers for their attitudes towards preventative disease control that would report each health event (deaths, sickness, abortions, lameness) when it affected some, 5% or 10% of their herd. Error bars show 95% confidence intervals.

The majority of respondents (68.3%) would report health events to a veterinarian, while 9.9% would report to the Department of Primary Industries and Resources of South Australia (PIRSA). A further 20.4% would report to both, a veterinarian and PIRSA. Furthermore, 1.4% of these respondents would report to another authority in addition to a veterinarian and/or PIRSA and an additional 1.4% would report only to another authority, such as a stock agent, extension officer or online database or website.

When asked what would prevent them from reporting unexplained cattle deaths, cost was the most common response (25.8%), although nearly a quarter (23.7%) of respondents answered that nothing would stop them reporting, 15.7% answered time and 19.3% answered ‘fear of quarantine’ would prevent them reporting unexplained cattle deaths. A further 22.9% answered that they did not know who to report unexplained cattle deaths to, while 12.8% provided some other reason for not reporting. A selection of these other reasons for not reporting are displayed in Table 1.

Table 1. Selected answers provided by respondents (n= 631) to a questionnaire of South Australian cattle farmers surveying farmer attitudes towards preventative disease control when asked to specify the ‘other’ reasons for not reporting unexplained cattle deaths

‘Other’ reasons for not reporting unexplained cattle deaths
Not all deaths relate to disease
Can’t do anything for them once they’re dead
Unsure of benefit
1 or 2 deaths are normal
Unsure if it is a problem which is relevant off farm
Problems not serious enough
Bureaucracy involved
Difficulty determining cause

Disease prevention

Quarantine and biosecurity

More than half (53.0%) of respondents answered ‘Yes’ when asked if they used quarantine procedures when introducing new cattle or to isolate sick cattle. When asked to specify, respondents’ answers varied from “drench on arrival” to “in yards for 3 days” to “about 3 months isolation in designated paddocks”. When asked ‘do you ascertain the vaccination or health status of cattle entering your property?’, 64.3% answered ‘yes’.

Johne’s disease program involvement

The majority (65.0%) of respondents were aware of the systematic Johne’s Disease market assurance program (CattleMAP) operating in South Australia. The remaining 35.0% were

unaware such a program exists. Of those that were aware of the Johne's program, 26.8% were involved in the program, 7.6% had previously been involved but were no longer, and 65.5% had never been involved.

Vaccination

When questioned about their routine use of either 5in1 or 7in1 vaccines for the protection against clostridial diseases, nearly half (46.8%) of respondents never used either vaccine. Around a third (30.1%) of respondents vaccinated their herd annually with either 5in1 or 7in1, while 10.1% gave two doses of vaccine to all calves. A further 9.4% of respondents gave a single vaccine dose to all calves. A small proportion (3.5%) only vaccinated introduced stock, with no other routine vaccine use.

If one considers two doses of vaccine as calves, or annual vaccination throughout life as adequate to confer protection from disease (annual boosters are recommended by the manufacturer to maintain protection against black disease and malignant oedema), then less than half (40.2%) of respondents were adequately vaccinating their herd against clostridial diseases.

Bovine Viral Diarrhoea management

The following results relate specifically to the management of BVD.

Pestigard™ use

In total, 13.2% of respondents stated that they used Pestigard™, Australia's only licensed vaccine against BVD. The remaining 86.8% of respondents had never used Pestigard™. Of those that used Pestigard™, the majority (52.7%) did so annually, as well as vaccination of new stock before introduction. A further 20.4% of respondents gave two doses of Pestigard™ to calves, while another 20.4% vaccinated only introduced stock.

BVD testing

The majority (86.6%) of respondents had never tested their cattle for BVD. Of the portion (13.4%) that had tested for BVD, a minority (25.9%) tested on a regular basis.

BVD education

Around a fifth (20.3%) of respondents had attended a seminar or education session about BVD.

When asked to specify, no one session or program was well represented, with most information seemingly gained through veterinarians or vaccine company representatives.

Attitudes towards Bovine Viral Diarrhoea

Perceived understanding of BVD

Table 2 shows the proportion of respondents who agreed, disagreed or gave a neutral response and the mean response (on a scale of 1 to 7 from strongly disagree to strongly agree) to each of nine statements, indicating how well they felt they understood BVD. The most strongly agreed with statement was “I have heard of the disease known as Bovine Viral Diarrhoea, BVD or Bovine Pestivirus”. This statement also had the highest mean response, while the most strongly disagreed with statement and that with the lowest mean response was “I take measures to protect my herd from BVD”.

The perceived understanding scores generated from these nine statements are approximately normally distributed with a mean of 4.19 and median of 4.

Table 2. Percentage of respondents (n=631) to a questionnaire of South Australian cattle farmers surveying their attitudes towards preventative disease control conducted that agreed, disagreed or gave a neutral response to each of nine statements relating to their understanding of bovine viral diarrhoea (BVD), and the mean response (on a scale of 1 to 7 from strongly disagree to strongly agree) to each statement.

Statement	Percentage of respondents			Mean response
	Agree	Neutral	Disagree	
I have heard of the disease known as Bovine Viral Diarrhoea, BVD or Bovine Pestivirus	67.3	8.2	24.5	5.16
I feel I understand the risks BVD poses to my herd	50.4	11.9	37.7	4.21
I do not believe my herd is infected with BVD	63.5	12.4	24.1	4.92
I take measures to protect my herd from BVD	38.8	9.0	52.2	3.60
I do not know how to protect my herd from BVD	41.5	8.5	50.0	3.74
Protecting my herd from BVD is not a priority for me right now	42.9	17.4	39.4	3.98
I believe I understand the financial impact of BVD on infected herds	54.4	12.7	11.8	4.49
I feel I understand how BVD is transmitted	42.8	11.8	45.4	3.85
I know where to find clear information on BVD	45.9	11.7	42.4	4.06

Actual knowledge of BVD

Table 3 shows the proportion of respondents who correctly or incorrectly designated each of sixteen statements as true or false, as well as the proportion that responded with “don’t know”. The most frequently correctly answered statement was “BVD can cause abortions, still births, reduced conception rates and abnormal calves”, while the most frequently incorrectly answered statement was “vaccination will prevent persistently infected animals spreading BVD”. However, the authors are aware of some ambiguity in this statement. The next most frequently incorrectly answered statement was “an animal which has previously been infected will be protected from BVD for life”. At least 30% of respondents answered “don’t know” to each statement, with nearly 80% responding “don’t know” to the statement “various European countries have BVD elimination or control programs in place”.

The mean knowledge score calculated from this series of questions was 5.15, with a range from -2 to 16.

Table 3. Percentage of respondents (n=631) of 4,165 South Australian cattle farmers to a questionnaire surveying their attitudes towards preventative disease control conducted that responded correctly, incorrectly or with “don’t know” to each of 16 statements relating to bovine viral diarrhoea (BVD).

Question	Correct Answer	Percentage of respondents		
		Correct	Incorrect	Don't Know
BVD can cause abortions, still births, reduced conception rates and abnormal calves	TRUE	66.5	1.6	31.9
BVD can be passed between animals with mild or no signs of disease	TRUE	63.5	0.6	35.9
No vaccine against BVD is available in Australia	FALSE	56.2	1.9	41.9
Persistently infected cattle can appear normal and healthy	TRUE	53.7	6.7	39.6
Persistently infected cattle spread large amounts of virus and infect large numbers of other cattle	TRUE	52.5	4.4	43.1
BVD does not affect profitability unless there are signs of disease	FALSE	51.6	9.6	38.7
When a pregnant animal is infected, the resultant calf can be born infected for life (persistently infected)	TRUE	48.7	3.4	47.9
Persistently Infected cattle often have a greatly reduced life span	TRUE	45.2	4.8	49.9
BVD can be eliminated from a herd or region	TRUE	38.0	7.7	54.3
Persistently infected cattle can be cured	FALSE	34.3	9.4	55.9
BVD can infect humans	FALSE	29.8	4.5	65.6
BVD does not affect the occurrence of mastitis, respiratory infection or other disease in a herd	FALSE	25.7	14.1	60.2
An animal which has previously been infected will be protected from BVD for life	TRUE	25.1	22.0	52.9
Testing for BVD is highly accurate	TRUE	23.6	5.4	71.0
Various European countries have BVD elimination or control programs in place	TRUE	18.9	1.3	79.8
Vaccination will prevent persistently infected animals spreading BVD *	FALSE	16.5	32.0	51.4

Interest in BVD and its control

Table 4 shows the proportion of respondents who agreed, disagreed or gave a neutral response to each of eight statements, indicating how interested they were in BVD and its control. The most strongly agreed with statement was “I am interested in learning more about BVD”, while the most strongly disagreed with statement was “I am concerned about BVD in my herd”.

The distribution of the interest score which was calculated from these eight statements is negatively skewed with mean interest score of 5.13, median of 5 and mode of 6.

Table 3. Percentage of respondents (n=631) of South Australian cattle farmers to a questionnaire surveying their attitudes towards preventative disease control conducted that responded correctly, incorrectly or with “don’t know” to each of 16 statements relating to bovine viral diarrhoea (BVD).

Question	Percentage of respondents			Mean response
	Agree	Neutral	Disagree	
I believe BVD is a serious disease	68.9	21.7	9.4	5.40
I believe BVD is relevant to me	61.2	21.2	17.6	4.97
I am concerned about BVD in my herd	50.0	20.6	29.9	4.42
I am interested in testing my cattle for BVD	51.6	18.8	29.6	4.48
I would be interested in a free BVD control program	74.2	9.8	16.0	5.54
I would be interested in a BVD control program at a small cost	58.2	16.6	25.2	4.68
I would be interested in a BVD control program at a small cost, if I can be shown that the long term benefits outweigh the short term costs	71.3	12.6	16.1	5.36
I am interested in learning more about BVD	78.7	12.0	9.2	5.71

Discussion

The results of the present survey show that the respondents were primarily occupied in the agricultural industries, were the owners of their cattle herds and were responsible for the management decisions regarding those herds. This indicates that the target population (commercial South Australian cattle farmers) was reached by this survey. The response rate achieved in this survey (15.2%) was lower than the >60% that can be achieved for this type of survey (Brennan, 1992), but in line with that achieved for a postal survey of United Kingdom veterinarians on the subject of biosecurity by Gunn et al. (2008) and nearly double the response rate achieved after a single mail out for a similar survey conducted in Victoria (Smith, 2014). As with any survey of this nature, there is a question of response bias. The survey was distributed effectively to all cattle producers in South Australia through the use of a government database. Due to confidentiality and privacy considerations (upon which the human ethics approval for this project was dependent), and because of the perceived political nature of some of the questions regarding farm husbandry practices, the survey responses were anonymous, preventing reminder notices being distributed to non-responders to increase the response rate, or to follow up with them to ascertain why they did not wish to submit to the questionnaire. Thus, there is no way of knowing with certainty how representative the survey responses were. In an

attempt to provide confidence in the results, the demographics of respondents were compared to the demographics of the national Australian farmer population, as reported by the Australian Bureau of Statistics (ABS, 2012b). All demographics were similar between respondents and ABS demographics: a majority of farmers are male (72% in the ABS report, 86% in this survey), and approximately three-quarters are over 45 years of age (71% in the ABS report, 78% in this survey). According to the ABS (2012a), 38% of Australian farmers had non-school qualifications while 41% of respondents to this survey had that same level of education. There is no evidence of substantial response bias. Although absence of evidence does not guarantee a lack of response bias, it does provide some additional confidence. Furthermore, the response rate achieved in this survey was sufficient to generate nearly double the minimum desired sample size of 353 required to estimate proportions with 95% confidence and 5% precision (calculated at <http://epitools.ausvet.com.au>). While large sample size does not eliminate the question of response bias, it does provide for some expectation that responder bias effects are being diluted by numbers. Nonetheless, the results presented here are interpreted only within the confines of the respondent population, without extrapolation to the wider population of South Australian farmers. In future, alternative methods of data collection such as focus groups, delivery of questionnaires at large events or through trusted intermediaries (perhaps veterinary clinicians), or consideration of how non-responder follow-up can be conducted within the confines of privacy laws should be considered to ensure a representative sample.

The responses to the disease reporting questions show that respondents generally considered cattle deaths the most serious health event. Lameness seems to be the disease event that was considered least serious, and least likely to be reported. While this may not be surprising, it could represent an animal welfare (and possibly a biosecurity) concern. It is evident from the results presented here that most respondents are more likely to report health events to a private veterinarian rather than to the government agency (PIRSA). Gunn et al. (2008) identified veterinarians (more than government sources) as farmers' primary source of information regarding biosecurity. It seems that farmers have a more comfortable or trusting relationship with their veterinarian than with government agencies. As such, it is crucial that communication between private veterinary practitioners and PIRSA is maintained to ensure effective disease

reporting. It is concerning to note that while 87.4% of respondents would report cattle deaths if it affected at least 10% of their herd or more, more than 1 in 10 respondents claim they would not report the death of 10% or more of their herd.

With respect to disease management, the results of our survey show that less than half of respondents are adequately vaccinating their cattle herds against clostridial disease. Living in a relatively disease-privileged country such as Australia, where diseases such as Bovine Tuberculosis, Brucellosis, Bovine Spongiform Encephalopathy and Foot and Mouth Disease are absent (World Organisation for Animal Health (OIE), 2013), there may be a tendency to allow farm level biosecurity to lapse. Other factors including time constraints, farmer ignorance, socio-economic demographics and perceived cost-effectiveness may also contribute to farmer decision making (Toma et al., 2013). The results of this survey provide some evidence to support this, with only around half of respondents stating that they use some quarantine practices, and only 64.3% determining the health or vaccination status of cattle prior to introducing them to their herd. In addition, the quality of the quarantine practices in place seems to be highly variable. This behaviour of respondents is in contrast with a United Kingdom study where the majority of farmers involved in focus groups claimed to implement several biosecurity measures, such as disinfecting trailers, utilising foot baths, quarantine or testing of introduced stock (or maintaining a closed herd)(Gunn et al., 2008). However, that survey was conducted after the 2001 Foot and Mouth Disease outbreak (Scudamore and Harris, 2002) which is likely to have affected farmer attitudes to biosecurity.

The results of the survey show worms and lice as the primary endemic disease concerns of respondents. The most prevalent non-parasitic disease concerns are reported as BVD and Bovine Johne's Disease. However, a Meat and Livestock Australia report (Sackett et al., 2006) showed that bloat, gastrointestinal disease, pink eye and grass tetany are the diseases with the highest economic impact on the cattle industry in southern Australia, suggesting that farmer perceived losses and actual losses may be misaligned. On the other hand, concerns reported here may reflect not only this economic impact, but welfare, labour and social costs of disease as well (Toma et al., 2013). The presence of BVD as the third most common disease concern in the

present study may likely be an overrepresentation as a result of the present survey being labelled a 'Bovine Viral Diarrhoea Awareness Survey'. The results show BVD to be mentioned around twice as often than Johne's Disease, and more than five times more often than the next most commonly mentioned infectious disease, Pink Eye. As such, it may be fair to claim BVD is deserving of this position as one of the two most common infectious disease concerns of respondents. However, given the low response rate, it is possible that these prevalences are exaggerated by response bias, despite no evidence of such. This is in line with results from Ireland where BVD was considered by farmers (and animal health experts) to be one of the three highest impact animal diseases. Johne's Disease appears to be of higher priority to respondents than was reported in Ireland, where it ranked below Salmonellosis and Infectious Bovine Rhinotracheitis (More et al., 2010). It is interesting to note that the figure of 13.0% of respondents who listed Johne's Disease as a disease concern in the present survey, is not dissimilar to the 17.4% of respondents who claimed to be involved in the systematic Johne's market assurance program that is active in South Australia (Animal Health Australia, 2013). The CattleMAP program was specifically chosen for reference in the questionnaire as it is the most widely applicable program relating to disease management in South Australia and is inclusive of both beef and dairy cattle, unlike the ManaJD program which is applicable only to dairy producers and the BJD control program which applies only to infected herds. These results may suggest not only that systematic disease management is poorly utilised by respondents, but that the value of such programs is not recognised by all participants, with a substantial drop out rate (over a quarter of respondents who have been involved in the CattleMAP program either currently or historically, are no longer involved). These results may reflect poor perceived efficacy or economic returns from control schemes resulting in poor uptake, in line with the relationship between perceived importance of biosecurity and biosecurity behaviour observed by Toma et al. (2013)³³³³. Alternatively, as farmer motivation is considered crucial to participation in control (Heffernan et al., 2009), these results could reflect low perceived impact of Johne's Disease infection resulting in poor motivation to control.

With respect to the specific management of BVD, both vaccination with Pestigard™ and testing for BVD were only practiced by small portions (less than one-fifth) of respondents. Around

20% of respondents had attended a seminar or education session on BVD, however, the organisers of these sessions varied greatly. Lindberg et al.(2006) notes that the efficiency of farmer education is crucial and that a consistent message is valuable to progress towards control of BVD.

This survey provides evidence that the majority of respondents had heard of BVD (by one of its various names). However, 63.5% of respondents did not believe their herd to be infected with BVD. Contrary to that, evidence from a serological survey conducted in South Australia in 2008 (Anderson et al. unpublished data) suggests that, in fact, around 97% of dairy and 85% of beef herds in South Australia have had some exposure to BVD, either recent or historically.

The mean perceived understanding score (4.19 on a scale of 1 to 7; 59.9%), possibly only indicating moderate perceived understanding. However, this is contrast with the respondents' actual knowledge regarding BVD, as is apparent when one examines the results of the TRUE/FALSE questions where at least 30% of respondents answered "don't know" to every statement. When the results from these statements were combined into a knowledge score, the mean score (5.15; maximum possible score = 16; 32.2%) indicates that, on average, respondents were only capable of answering 5 of 16 questions correctly.

The most correctly answered question was 'BVD can cause abortions, still births, reduced conception rates and abnormal calves', indicating that BVD is acknowledged by respondents as a reproductive disease. However, the survey also revealed some misconceptions regarding BVD. The high rate of incorrect answers to the statement regarding the vaccine may represent misconception of the ability of vaccination to control BVD in an infected population. The results also suggest that the impact of BVD is being underestimated, while the measures required to protect cattle are being overestimated. An apparent failure to acknowledge the long-term immunity gained by cattle following natural acute infection, may be leading to overestimation of the number of susceptible cattle and, hence, the costs of protecting a herd against BVD. The costs of controlling BVD infection are a barrier to effective control (Heffernan et al., 2009), as farmers are believed to be unable or unwilling to invest in biosecurity (Gunn et al., 2008). Therefore, the lack of understanding of the disease in South

Australia may present an issue, with farmers potentially avoiding BVD control due to high perceived costs (Heffernan et al., 2009).

Despite poor knowledge of BVD and only moderate perceived understanding, interest in BVD and its control appears to be high (mean interest score 5.13 on a scale of 1 to 7; 73.3%). At least half of all respondents agreed with each statement regarding interest in BVD. There appears to be a prevalent attitude that BVD is interesting and important, but not directly relevant, with respondents not believing BVD is a problem in their herd, or something they need to take action against. Nonetheless, 74.2% of respondents still claim they would be interested in a free BVD control program. While this figure falls to 58.2% when the program is 'at a small cost', it is restored to 71.3% if respondents could be shown that 'the long term benefits (of a control program) outweigh the short term costs'. However, conversion of the positive intentions exhibited by respondents in this survey into positive action is influenced by many factors, which may include physical and economic constraints, social demographic factors, access to information and strength of the advice received (for example, from veterinarians or government) (Toma et al., 2013).

Therefore, it seems that an education program about BVD would be well received in South Australia, and a BVD control program would, it seems, receive good participation. However, as has been seen in European control programs, education is key to farmer compliance and successful BVD control (Heffernan et al., 2009). Therefore, a thorough, farmer-friendly and consistent education program would be the first step towards systematic BVD control in South Australia. The veterinary sector may be best placed to lead such an initiative (as it does in New Zealand (<http://www.controlbvd.org.nz/>)), as the results of this survey imply that veterinarians are a trusted source of information and advice on animal disease. Further analysis of the dataset created by this survey will be reported subsequently and may reveal relationships between the variables discussed here. In particular, it may be possible to elucidate opportunities to target educational efforts.

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Supplementary Material

South Australian Bovine Viral Diarrhoea Awareness Survey

Your involvement in the cattle industry

Are you: Cattle Owner / Farm worker / Vet / Other: _____

Are you the person responsible for the majority of management decisions?
Yes / No / Part responsibility

Are you involved in (circle all that apply): Dairy / Beef

How long have you been involved in the cattle industry?

What type of operation (circle all that apply):

Stud cattle / commercial cow-calf / commercial dairy / feedlot / other

If other, please specify: _____

What breed(s) of cattle?

How many head of cattle/breeding females (circle appropriate)?

Personal details

PIC: SA _ _ _ _ _

Gender: Male / Female

Age: _____

Primary Occupation: _____

Highest level of education: Primary School
 Year 10 or equivalent
 Completed Year 10, continued at school but did not complete Year 12
 Year 12 or equivalent
 Post-school qualification (eg. associate degree, diploma, TAFE/VET certificate) - not agriculture or animal science related

- Bachelor degree – not agriculture or animal science related
 - Post-school qualification or bachelor degree – agriculture or animal science related
 - Post-graduate qualification (eg. graduate diploma, masters degree, PhD)
- Other, please specify: _____

Your biosecurity and disease management

Are you aware of the Johnes Cattle MAP program? Yes / No

Are you currently involved in the Johnes Cattle MAP program? Yes / No
What is your status? _____

Have you previously been involved in the Johnes Cattle MAP program? Yes / No

What are your top three disease or parasite concerns with regard to your herd?

Do you ascertain the vaccination or health status of cattle entering your property?
Yes / No

Do you use quarantine procedures when introducing new cattle or to isolate sick cattle?
Yes / No If yes, please specify: _____

Have you ever attended an educational session about or related to BVD? Yes / No
If yes, please specify: _____

Do you test cattle for Bovine Viral Diarrhoea (Pestivirus)? Yes / No
At what age(s)? _____

How often do you administer the following vaccines to your cattle? (tick all that apply)

	Never	Single dose as calves	Two doses as calves	Annually for life	Before introduction to the herd
5 in 1 (Clostridial)					
7 in 1 (Clostridial and Lepto)					
Lepto					
BOvac (E.coli)					
Vibrovax (Vibrio)					
Botulism					
Bovilis S (Salmonella)					
Pestigard (Pestivirus)					
Other, please specify					

Would you report the following:

- Several cattle died
- 5% cattle died
- More than 10% cattle died
- A few cows were “sick”
- Lots of cattle were “sick”
- More than 10% cattle were “sick”
- Other, please specify: _____

(tick for yes, leave blank for no)

- A few cows aborted
- 5% cows aborted
- More than 10% cows aborted
- Some cattle were lame
- 5% cattle were lame
- More than 10% cattle were lame

What would prevent you reporting unexplained cattle deaths?

- Cost Fear of quarantine Time Don't know who to contact
 Other, please specify: _____

If you were to report unexplained cattle deaths, who would you report to?

- Vet PIRSA Animal Health Other, please specify: _____

Your understanding of BVD

I have heard of the disease known as Bovine Viral Diarrhoea, BVD or Bovine Pestivirus

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I feel I understand the risks BVD poses to my herd

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I do not believe my herd is infected with BVD

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I take measures to protect my herd from BVD

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I do not know how to protect my herd from BVD

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

Protecting my herd from BVD is not a priority for me right now

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I believe I understand the financial impact of BVD on infected herds

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I feel I understand how BVD is transmitted

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I know where to find clear information on BVD

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

Your awareness of BVD

Please answer true or false to these statements. This is not a test! Just a survey of your knowledge.

BVD can be passed between animals with mild or no signs of disease True/False/Don't know

An animal which has previously been infected will be protected from BVD for life True/False/Don't know

BVD does not affect the occurrence of mastitis, respiratory infection or other disease in a herd True/False/Don't know

BVD can cause abortions, still births, reduced conception rates and abnormal calves	True/False/Don't know
When a pregnant animal is infected, the resultant calf can be born infected for life (persistently infected)	True/False/Don't know
Persistently infected cattle spread large amounts of virus and infect large numbers of other cattle	True/False/Don't know
Persistently infected cattle can be cured	True/False/Don't know
Vaccination will prevent persistently infected animals spreading BVD	True/False/Don't know
Persistently infected cattle often have a greatly reduced life span	True/False/Don't know
Persistently infected cattle can appear normal and healthy	True/False/Don't know
BVD does not affect profitability unless there are signs of disease	True/False/Don't know
Testing for BVD is highly accurate	True/False/Don't know
No vaccine against BVD is available in Australia	True/False/Don't know
BVD can be eliminated from a herd or region	True/False/Don't know
Various European countries have BVD elimination or control programs in place	True/False/Don't know
BVD can infect humans	True/False/Don't know

Your interest in controlling BVD

I believe BVD is a serious disease

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I believe BVD is relevant to me

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I am concerned about BVD in my herd

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I am interested in testing my cattle for BVD

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I would be interested in a free BVD control program

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I would be interested in a BVD control program at a small cost

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I would be interested in a BVD control program at a small cost, if I can be shown that the long term benefits outweigh the short term costs

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

I am interested in learning more about BVD

Strongly disagree 1 2 3 4 5 6 7 *Strongly agree*

Contact details (optional)

Name : _____

Postal Address: _____

Contact Phone Number: _____

Email Address: _____

Any further comments?

About BVD: _____

About this survey: _____

Thank you for your participation!

Original Article: Associations Between Farmer Demographics, Management Practices and Attitudes Towards Bovine Viral Diarrhoea and Its Control

SR Lanyon, ML Anderson, MP Reichel (2014)

Associations Between Farmer Demographics, Management Practices and Attitudes Towards Bovine Viral Diarrhoea and its Control

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis

Name of Principal Author (Candidate)	Sasha R Lanyon		
Contribution to the Paper	Designed questionnaire, collated questionnaire responses, entered, analysed and interpreted data, drafted and edited manuscript, acted as corresponding author.		
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Associations between farmer demographics, management practices and attitudes towards bovine viral diarrhoea and its control

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Abstract

Economic losses associated with bovine viral diarrhoea (BVD) can effectively be mitigated using a test-and-cull disease eradication approach. Farmer participation in such control schemes is crucial to their success. Therefore, successful control programs often involve an educational component. This study aimed to identify producer groups most likely to benefit from BVD education by assessing the relationships between demographic and management variables, biosecurity behaviours and BVD awareness. A postal survey of South Australian cattle farmers was conducted, with 631 responses received and analysed. Being a stud producer, being a dairy producer and being concerned with BVD in the herd were associated with positive BVD-specific behaviours: use of BVD vaccine (Pestigard), BVD seminar attendance and testing for BVD. Strong general biosecurity behaviours (adequate use of 5in1 or 7in1 vaccine, use of quarantine, participation in a Johne's Disease market assurance program and ascertaining the vaccination or health status of cattle prior to introduction) were also positively associated with being a stud producer, being a dairy producer and being concerned about BVD in the herd, as well as positive associations with BVD-specific behaviours. Strong general biosecurity and BVD-specific behaviours, and being a stud producer, being a dairy producer and being

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concerned about BVD in the herd were associated with high perceived understanding and high demonstrated knowledge of BVD, while concern about BVD in the herd, BVD testing, Pestivirus use and use of quarantine were associated with high interest in BVD. Stud producers and dairy producers may be ideal candidates to provide peer support to educational programs in the role of ‘champions’, while commercial beef producers may be the cohort that would most benefit from an increase in BVD awareness.

Keywords Survey; Awareness; South Australia; Pestivirus

Introduction

Bovine viral diarrhoea (BVD), caused by a Pestivirus of the family Flaviviridae, has a significant financial impact in infected cattle populations. Structured control programs, generally based on a test and cull approach, have been shown to be highly effective and economically beneficial (Valle et al., 2005, Häslér et al., 2012). Stakeholder awareness is acknowledged as a primary factor crucial to the success of control and mitigation schemes (Lindberg and Alenius, 1999, Barrett et al., 2011). As such, control schemes, including those in Switzerland (Presi et al., 2011) and various American states (Ridpath, 2012), have often incorporated an educational component. An understanding of the relationships between demographic and management factors and farmer awareness of BVD may allow identification of producer groups that are most likely to benefit from educational programs, such as those that have the poorest awareness of BVD, and implement the fewest biosecurity procedures. In turn, this may allow education schemes to be effectively targeted to those producers ensuring the greatest positive impact and improving the likelihood of producer support of BVD control efforts. Therefore, this study aimed to assess the relationships between demographic and management factors, biosecurity behaviours and knowledge of, perceived understanding of and interest in BVD and its control.

Methods

Survey

As previously reported (Lanyon et al., 2014) 4-page questionnaire was mailed to all (n = 4,165), South Australian cattle farmers registered in the Primary Industries Information Management System (PIIMS) database as managing a herd of 35 or more head of cattle. Farmers managing herds of fewer than 35 animals were excluded in an attempt to focus on commercial producers (and hence exclude part-time or hobby farmers). A total of 631 responses were received (response rate 15.2%).

Ethics

This survey was approved by the University of Adelaide Human Research Ethics Committee (Project No: H-091-2011).

Statistical Analysis

A perceived understanding score and an interest score were calculated for each respondent as previously reported (Lanyon et al., 2014), with a high score (on a scale of 1 to 7) representative of high self-perceived understanding of BVD or high interest in BVD, respectively. Similarly, a knowledge score was calculated on a scale of -16 to 16 (Lanyon et al., 2014), with a high score indicative of high demonstrated knowledge of BVD.

The median of each score was calculated. Any individual score greater than or equal to the median score was considered 'High'. Individual scores less than the median score were considered 'Low'.

Each of 31 dichotomous variables were tested for significant associations with high perceived understanding score, high knowledge score and high interest score, respectively, by calculation of an odds ratio (with 95% confidence interval) and p-value using MedCalc for Windows, Version 12.7.5.0 (MedCalc Software, Ostend, Belgium). A p-value less than 0.05 was considered indicative of a significant association. Odds ratios and p-values were also calculated

pair-wise to assess associations between perceived understanding score, knowledge score and interest score.

Results

In total, twenty-four variables (detailed in Table 1) were involved in significant interactions ($p < 0.05$). Eight management and demographic variables were associated with BVD-specific behaviours (Table 2). Three factors were positively associated with all three BVD-specific behaviours (seminar attendance, Pestigard use and BVD testing): being a stud producer, being a dairy producer or being concerned about BVD in the herd. Furthermore, being concerned about Johne's Disease in the herd and knowing who to report unexplained cattle deaths to were positively associated with BVD seminar attendance and BVD testing, but not with Pestigard use. In addition, being concerned about lice in the herd was negatively associated with BVD seminar attendance and Pestigard use.

Table 1. The questions and answer options from a postal questionnaire survey of 631 South Australian cattle farmers, along with the corresponding variable names of those variables that had a significant association with perceived understanding, knowledge and interest in bovine viral diarrhoea (BVD) and its control.

Variable Name	Question	Answer Options
Adequate Vaccination	How often do you administer [5in1 or 7in1 vaccine] to your cattle?	'NEVER' or 'SINGLE DOSE AS CALVES' recorded as 'NO'. 'DOUBLE DOSE AS CALVES' or 'ANNUALLY FOR LIFE' recorded as 'YES'.
Ag Related Occupation	Primary Occupation.	Free text. 'Agriculture related', as designated by authors, recorded 'YES'. 'Agriculture related' included farmer, grazier, livestock transport, fencing contractor and stock agent. 'Not Agriculture related', including teacher, doctor or tradesman was recorded 'No'.
Beef/Dairy	Are you involved in: Dairy/Beef? (Circle all that apply.)	Circled 'DAIRY' recorded as 'DAIRY'. Circled 'BEEF' recorded as 'BEEF'. Circled both 'BEEF' and 'DAIRY' recorded as 'BOTH'.
BVD Concern	What are your top three disease or parasite concerns with regard to your herd?	Free text. If 'BVD' or 'Pestivirus' listed, recorded 'YES'. If BVD not listed, recorded 'NO'.
BVD Seminar	Have you ever attended an educational session about or related to BVD?	'YES' or 'NO'.

BVD Ever	Testing Do you test cattle for Bovine Viral Diarrhoea (Pestivirus)?	‘YES’ or ‘NO’.
Commercial Breeder	What type of operation: Commercial Breeder	‘YES’ or ‘NO’.
Fertility Concern	What are your top three disease or parasite concerns with regard to your herd?	Free text. If Fertility listed, recorded ‘YES’. If Fertility not listed, recorded ‘NO’.
Gender	Gender (of respondent).	‘MALE’ or ‘FEMALE’.
Interest	Interest in BVD and its control	‘HIGH’ or ‘LOW’. See methods.
JD Concern	What are your top three disease or parasite concerns with regard to your herd?	Free text. If Johnes Disease listed, recorded ‘YES’. If Johnes Disease not listed, recorded ‘NO’.
Knowledge	Demonstrated knowledge of BVD	‘HIGH’ or ‘LOW’. See methods.
Lice Concern	What are your top three disease or parasite concerns with regard to your herd?	Free text. If Lice listed, recorded ‘YES’. If Lice not listed, recorded ‘NO’.
Mastitis Concern	What are your top three disease or parasite concerns with regard to your herd?	Free text. If Mastitis listed, recorded ‘YES’. If Mastitis not listed, recorded ‘NO’.
MN Involved	Are you currently involved in the Johnes Cattle MAP program?	‘YES’ or ‘NO’.
Perceived Understanding	Self-perceived understanding of BVD	‘HIGH’ or ‘LOW’. See methods.
Pestigard Ever	How often do you administer [Pestigard] to your cattle?	‘NEVER’ recorded as ‘NO’. ‘SINGLE DOSE AS CALVES’, ‘DOUBLE DOSE AS CALVES’ or ‘ANNUALLY FOR LIFE’ recorded as ‘YES’.
Quarantine	Do you use quarantine procedures when introducing new cattle or to isolate sick cattle?	‘YES’ or ‘NO’.
Report Don't Who	Reason: Know What would prevent you reporting unexplained cattle deaths: Don't know who to report to.	‘YES’ or ‘NO’.
Report Nothing	Reason: What would prevent you reporting unexplained cattle deaths: Other	Free text. If ‘Nothing’ listed, recorded ‘YES’. If ‘Nothing’ not listed, recorded ‘NO’.
Stud	What type of operation: Stud cattle.	‘YES’ or ‘NO’.
Trade/Fatten	What type of operation: Trade/Fatten Steers	‘YES’ or ‘NO’.
Vacc/Health Status New Stock	Do you ascertain the vaccination or health status of cattle entering your property?	‘YES’ or ‘NO’.
Worms Concern	What are your top three disease or parasite concerns with regard to your herd?	Free text. If Worms listed, recorded ‘YES’. If Worms not listed, recorded ‘NO’.

Table 2. The odds ratios and p-values of significant associations between demographic and management variables with BVD specific behaviours in a postal questionnaire survey of 631 South Australian cattle farmers. 1st group indicates group considered ‘positive’ for calculation of odds ratios, 2nd group indicates group considered negative. (ie. Odds ratio > 1 indicates 1st group is associated with positive action.)

Variable	1st Group	2nd Group	BVD Seminar		BVD Testing EVER		Pestigard	
			Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Stud	Y	N	2.7675	<0.0001	7.5502	<0.0001	3.0943	<0.0001
			1.6942 4.5208		4.4125 12.9192		1.8081 5.2957	
BVD Concern	Y	N	4.7353	<0.0001	4.9212	<0.0001	16.5789	<0.0001
			3.0103 7.4487		2.9092 8.3248		9.283 29.6091	
Beef/Dairy	Dairy/Both	Beef	4.4674	<0.0001	3.4369	0.0001	2.1105	0.0227
			2.5744 7.7523		1.8318 6.4485		1.11 4.0125	
JD Concern	Y	N	2.8378	0.0002	2.3273	0.0092	1.6158	0.1412
			1.639 4.9136		1.2326 4.3939		0.8527 3.0618	
Report Reason Don't Know Who	Y	N	0.3363	0.0036	0.38	0.0319	0.4825	0.0695
			0.1614 0.7006		0.157 0.9197		0.2197 1.0598	
Lice Concern	Y	N	0.5205	0.0041	0.7906	0.3677	0.4271	0.0013
			0.3333 0.8129		0.4743 1.3181		0.2548 0.7161	
Commercial Breeder	Y	N	0.8885	0.6884	0.4897	0.0226	0.6971	0.2542
			0.4984 1.5837		0.2651 0.9045		0.3749 1.2962	
Ag Related Occupation	Y	N	2.0545	0.0423	1.7822	0.1654	1.8483	0.1417
			1.0255 4.1159		0.7877 4.032		0.8146 4.1935	
BVD Seminar	Y	N			6.2378	<0.0001	4.5032	<0.0001
					3.7942 10.2552		2.7931 7.2604	
BVD Testing Ever	Y	N					5.6489	<0.0001
							3.2801 9.7284	

Sixteen variables were associated with general biosecurity behaviours: quarantine use, ascertaining the vaccination or health status of cattle before introduction, adequate vaccination against clostridial disease and involvement in the Johne's Disease Market Assurance Program (Table 3). All three BVD-specific behaviours were positively associated with general biosecurity behaviours, with BVD seminar attendance and Pestigard use associated with all four biosecurity behaviours, and BVD testing associated with ascertaining the health and vaccination status of stock prior to introduction, adequate vaccination with 5in1 or 7in1 vaccine and involvement in the Johne's Disease Market Assurance Program (CattleMAP), but not associated with quarantine practice. Being a stud producer, being a dairy producer or being concerned about BVD in the herd were positively associated with general biosecurity behaviours (in addition to BVD-specific behaviours), with all three associated with adequate vaccination and CattleMAP involvement; BVD concern and stud producer were also associated with vacc/health status before intro while stud producers were also associated with quarantine.

Finally, fourteen variables were associated with knowledge, perceived understanding and interest in BVD (Table 4). Four variables (concern about BVD in the herd, BVD testing, Pestigard use and quarantine) were positively associated with a perceived understanding of, demonstrated knowledge of and interest in BVD. Of the ten remaining variables displaying significant associations, nine were associated with both perceived understanding and demonstrated knowledge, including being a stud producer, being a dairy producer, knowing who to report unexplained cattle deaths to, attendance at a BVD seminar, ascertaining vaccination or health status of cattle before introduction, adequate vaccination with 5in1 or 7in1 and involvement in the Johne's Disease Market Assurance Program. Finally, having an agriculture related primary occupation was associated with demonstrated knowledge of BVD but not with perceived understanding.

Table 3. The odds ratios and p-values of significant associations between demographic and management variables and BVD specific behaviours with biosecurity behaviours in a postal questionnaire survey of 631 South Australian cattle farmers. 1st group indicates group considered ‘positive’ for calculation of odds ratios, 2nd group indicates group considered negative. (ie. Odds ratio > 1 indicates 1st group is associated with positive action.)

Variable	1st Group	2nd Group	Quarantine		Vacc/Health Status before		Adequate Vacc		MN Involved			
			Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value		
Pestigard Ever	Y	N	1.6969	0.0322	4.7263	<0.0001	6.5378	<0.0001	3.055	<0.0001		
			1.046 2.7528		2.3776 9.3952		3.9044 10.9475		1.8544 5.0329			
BVD Seminar	Y	N	1.676	0.0144	1.7159	0.0172	3.1413	<0.0001	3.6836	<0.0001		
			1.1084 2.5342		1.1004 2.6756		2.0708 4.7652		2.3459 5.7842			
Stud	Y	N	1.8303	0.0173	2.8862	0.0004	1.838	0.0118	16.7331	<0.0001		
			1.1125 3.0112		1.605 5.1901		1.1441 2.9528		9.8848 28.3258			
BVD Testing Ever	Y	N	1.2649	0.3513	3.1086	0.0004	3.75	<0.0001	7.1419	<0.0001		
			0.7717 2.0731		1.6663 5.7992		2.2555 6.2347		4.2742 11.9338			
BVD Concern	Y	N	1.161	0.4765	2.807	<0.0001	4.0753	<0.0001	2.0148	0.0041		
			0.7698 1.7509		1.7152 4.5938		2.6457 6.2772		1.2481 3.2526			
Commercial Breeder	Y	N	0.6268	0.0666	0.7965	0.3958	0.1335	<0.0001	0.3404	0.0001		
			0.3806 1.0325		0.4711 1.3467		0.0933 0.1909		0.2006 0.5775			
Beef/Dairy	Dairy/Both	Beef	0.8426	0.552	1.4348	0.2426	2.3543	0.0033	33.6667	<0.0001		
			0.4792 1.4817		0.7831 2.6289		1.3293 4.1696		16.9392 66.9125			
JD Concern	Y	N	1.3827	0.2377	1.7141	0.0777	1.651	0.0673	4.6529	<0.0001		
			0.8075 2.3678		0.9419 3.1192		0.965 2.8246		2.6723 8.1016			
Mastitis Concern	Y	N	1.1754	0.7331	1.2321	0.6749	1.4667	0.4261	6.7	<0.0001		
			0.4642 2.9761		0.4646 3.2678		0.5711 3.7666		2.7323 16.4295			
Worms Concern	Y	N	0.8803	0.496	0.8681	0.4701	1.14	0.4961	0.4173	0.0002		
			0.6099 1.2706		0.5913 1.2744		0.7817 1.6626		0.2633 0.6615			
Fertility Concern	Y	N	1.4239	0.6312	3.7258	0.2204	1.8274	0.3736	5.8477	0.0095		
			0.3364 6.0267		0.4545 30.5421		0.4842 6.8966		1.5388 22.222			
Trade/Fatten	Y	N	1.3309	0.3458	0.9553	0.8857	0.5496	0.0887	0.0921	0.0189		
			0.7346 2.4113		0.512 1.7824		0.2759 1.0948		0.0126 0.6746			
Gender	F	M	1.5922	0.0635	1.2362	0.4126	1.8193	0.0178	1.4884	0.1666		

				0.9742	2.6022		0.7444	2.0529		1.1088	2.9849		0.8498	2.6069
Report Nothing	Reason	Y	N	1.4661		0.109	2.03		0.008	0.8436		0.4946	0.8581	0.63
				0.9182	2.3407		1.203	3.4255		0.5179	1.3743		0.4604	1.5994
Ag Occupation	Related	Y	N	0.7616		0.2687	1.8393		0.0116	1.3591		0.2577	1.8693	0.0925
				0.4701	1.2339		1.1458	2.9524		0.799	2.3118		0.9019	3.8744
Report Don't Know Who	Reason	Y	N	0.795		0.3359	0.6211		0.0483	0.7542		0.28	0.5682	0.108
				0.4982	1.2686		0.3884	0.9964		0.4521	1.2582		0.2852	1.132
Quarantine		Y	N				2.9306		<0.0001	1.4002		0.0604	1.6806	0.0242
							2.0618	4.1654		0.9854	1.9895		1.0701	2.6395
Vacc/Health Status New Stock		Y	N							2.7415		<0.0001	3.5533	<0.0001
										1.8528	4.0566		2.0232	6.2404
Adequate Vaccination		Y	N										3.261	<0.0001
													2.0854	5.0993

Table 4. The odds ratios and p-values of significant associations between demographic and management variables, BVD specific and biosecurity behaviours with awareness, knowledge and perceived understanding of BVD in a postal questionnaire survey of 631 South Australian cattle farmers. 1st group indicates group considered ‘positive’ for calculation of odds ratios, 2nd group indicates group considered negative. (ie. Odds ratio > 1 indicates 1st group is associated with high awareness, knowledge or perceived understanding.)

Variable	1st Group	2nd Group	Perceived Understanding		Knowledge		Interest	
			Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
BVD Concern	YES	NO	9.7054	<0.0001	12.822	<0.0001	2.9274	0.0001
			4.6072 20.4449		6.8433 24.024		1.7195 4.9839	
BVD Testing Ever	YES	NO	22.3833	<0.0001	20.8024	<0.0001	3.9075	0.0002
			5.4387 92.1207		7.5023 57.6811		1.9078 8.0034	
Pestigard Ever	YES	NO	24.7217	<0.0001	12.1798	<0.0001	2.7304	0.0011
			6.0115 101.664		5.5186 26.8816		1.4972 4.9794	
Quarantine	YES	NO	2.2007	<0.0001	1.8794	0.0002	1.5454	0.0159
			1.5458 3.1331		1.3527 2.6111		1.085 2.2012	
Adequate Vaccination	YES	NO	2.3964	<0.0001	2.7131	<0.0001	1.3768	0.0969
			1.6097 3.5676		1.8933 3.8881		0.9438 2.0085	
BVD Seminar	YES	NO	9.1689	<0.0001	11.4085	<0.0001	1.4151	0.1283
			4.3808 19.1902		6.1329 21.2223		0.9046 2.2139	
MN Involved	YES	NO	3.4581	<0.0001	3.769	<0.0001	1.3543	0.2102
			1.9471 6.1417		2.3035 6.1667		0.8428 2.1763	
Vacc/Health Status New Stock	YES	NO	2.5933	<0.0001	2.1673	<0.0001	0.9443	0.7598
			1.8148 3.7058		1.5394 3.0514		0.6537 1.3639	
Report Reason: Don't Know Who	YES	NO	0.2818	<0.0001	0.4966	0.0032	1.3149	0.3172
			0.1742 0.456		0.3117 0.791		0.769 2.2485	
Beef/Dairy	DAIRY/ BOTH	BEEF	3.2761	0.0024	3.2365	0.0003	1.2786	0.432
			1.5249 7.0385		1.7108 6.123		0.6926 2.3605	
Stud	YES	NO	2.0583	0.0116	2.8578	0.0001	1.7115	0.0561
			1.1751 3.6053		1.6964 4.8145		0.9861 2.9703	
Gender	FEMALE	MALE	2.333	0.0047	1.6884	0.0352	1.1816	0.5316
			1.2972 4.1957		1.037 2.749		0.7005 1.9929	
JD Concern	YES	NO	2.3232	0.0148	2.2695	0.0051	1.1327	0.6789
			1.1792 4.5772		1.2789 4.0275		0.628 2.043	
Ag Related Occupation	YES	NO	1.0217	0.9334	1.6065	0.0492	0.7248	0.2428
			0.6173 1.691		1.0016 2.577		0.4223 1.2439	
Perceived Understanding	HIGH	LOW			13.7621	<0.0001	1.2016	0.3150
					8.9019 21.2757		0.8368 1.7384	
Knowledge	HIGH	LOW					1.617	0.0066
							1.1428 2.2879	

Discussion

There are many factors that may influence a farmer's attitudes and decision making process, including the physical and economic constraints of the farm, the farmer's demographics, education, experience and stage of life, the farm succession plan (Toma et al., 2013). The results of the present study revealed various associations between demographic and management characteristics, BVD-specific behaviours, biosecurity behaviours and attitudes towards BVD. Several variables were observed to have both direct and indirect associations with behavioural and attitude factors. For example, stud producers were significantly more likely to have attended a BVD seminar, significantly more likely to practice quarantine and significantly more likely to have high knowledge of BVD than non-stud producers. Stud producers were also indirectly associated with high BVD knowledge, due to associations between knowledge and BVD seminar attendance and between knowledge and quarantine. The demographic and management variables that were observed to be associated with a variety of behaviour and attitude variables may be factors that underlie a positive views towards disease control and biosecurity. These factors include: being a stud producer, being a dairy producer, having concerns about BVD and Johne's Disease in the herd, knowing who to report unexplained cattle deaths to and having a primary occupation that is related to agriculture. These characteristics may be indicative of the individual's experience in farming, which has previously been associated with strong biosecurity behaviour (Toma et al., 2013).

In the present study, dairy farmers were observed to be more likely to have a herd that is adequately vaccinated against clostridial disease. Dairy producers were also positively associated with BVD-specific behaviours, such as seminar attendance and vaccination with Pestigard, along with a high knowledge and understanding of BVD. Ridpath (2012) reported a similar finding, with US dairy producers four times as likely to have heard of BVD than beef producers and 34% more dairy farmers vaccinating against BVD than US beef cow/calf producers.

The positive associations observed with dairy farmers may also be indicative of an association with herd size, with the average South Australian dairy herd larger than the average beef herd.

Herd size has previously been noted as a potential factor contributing to farmer experience (Ridpath, 2012), with an individual more likely to experience adverse events when managing a larger herd. In that study, herd size was observed to be related to the likelihood of a farmer having heard of BVD, tested for BVD or vaccinated against BVD, with farmers managing a larger herd observed to be more knowledgeable about the disease (Ridpath, 2012).

The present study is the first of this nature undertaken in Australia. While direct comparison between Australian farmers and those in Britain and the US is difficult, similarities are certainly evident. Studies on the biosecurity practices of British cattle and sheep farmers have provided evidence that a variety of quarantine procedures are in place on many farms (Gunn et al., 2008, Toma et al., 2013). These procedures include: quarantine, disease testing, double fencing, prevention of nose to nose contact between livestock over boundary fences, limitation on access to farm buildings, use of disinfectants on trailers and equipment, disinfectant foot baths, use of artificial rather than natural breeding, control of vermin and wildlife, vaccination and health schemes, careful sourcing of stock from properties with good biosecurity and checking health, vaccination and testing records prior to purchase of stock (Gunn et al., 2008, Toma et al., 2013). Similarly, quarantine and vaccination have been reported to be in use in beef cow/calf herds in the US (Sanderson et al., 2000). Sanderson et al. (2000) reported that US beef breeders that quarantined introduced stock were more likely to vaccinate their herds and require cattle to be vaccinated prior to introduction, although these same producers were less likely to test incoming cattle for brucellosis. This suggests that producers may misunderstand the disease risks posed by incoming stock and choose to implement certain biosecurity procedures, rather than all protective measures available (Sanderson et al., 2000). The present study observed no relationship between quarantine and adequate vaccination against clostridial disease, which may indicate that a similar misconception of disease risk is present here, with producers not exhibiting a tendency to implement both measures simultaneously. Ridpath (2012) also raises concerns that the risk posed by pregnant animals is under-acknowledged by US farmers, as introduction of pregnant animals is a common practice. However, the authors of that paper acknowledge that this practice may be unavoidable for management reasons. Sanderson et al.

(2000) point out that the evaluation of biosecurity must not only focus on effectiveness and cost, but must relate to producer-specific factors such risk, risk aversion and potential disease losses. In a survey of British livestock veterinarians, while Gunn et al. (2008) revealed that veterinarians viewed farmers as unwilling, unable or lacking the interest or time to invest in biosecurity. These publications support the results of the present survey that suggest that knowledge and understanding of disease is associated with biosecurity and disease control behaviours.

In this study, the variable 'report reason: don't know who' was shown to be involved in several significant, negative interactions. This highlights the importance of the relationship between farmers and authorities including veterinarians and government departments: producers that feel they know who to report unexplained cattle deaths to are more likely to exhibit biosecurity behaviours, BVD-specific behaviours and high knowledge and understanding of BVD. Veterinarians, in particular, appear to be particularly valuable connections, with Gunn et al. (2008) and Barrett et al. (2011) identifying them as sources of advice that are positively viewed by British and Irish farmers, and noting that they have an important role in raising awareness and communicating information, along with the rural press, farm advisers, scientists and other farmers. In Britain, veterinarians have been observed to be most likely to provide farmers with the belief and motivation to comply with disease control recommendations (Gunn et al., 2008). This positive perception of veterinarians as a trusted information source affects the uptake of positive behaviours when recommended by the veterinarian (Toma et al., 2013). Meanwhile, government and media are perceived negatively by British farmers (Gunn et al., 2008), and their use in propagation of information may be most appropriately approached with care.

An interesting finding of this study is that farmers that are concerned with lice in their herd are less likely to have used Pestigard or attended a BVD seminar. This may reflect poor health management or poor awareness of disease associated with productivity losses, with more visible diseases (such as lice), taking priority. The observed association between concern with Johne's Disease and implementation of BVD-specific behaviours may also support this concept.

A study by Gunn et al. (2008) showed that British farmers have mixed perceptions of biosecurity, with farmers associating positively with increases in profitability gained through improved health a welfare and considering biosecurity to be a matter of personal pride and their own responsibility so as to secure a future in farming. However, these same farmers also associated biosecurity with decreased freedom, increased bureaucracy and rules, costly and as unlikely to achieve the desired outcome without the cooperation of all stakeholders. In general, the farmers in that study expressed positive views on biosecurity when self-referential and negative views when considering externally imposed biosecurity requirements. In the present study, high knowledge and perceived understanding of BVD was observed to be associated with positive biosecurity actions. This suggests that, when well informed, South Australian cattle farmers generally view biosecurity in a positive manner, resulting in positive action. This is supported by a similar finding of a very strong relationship between the knowledge and perceived importance of biosecurity and action observed by Toma et al. (2013). In that study, positive action was also associated with high perceived effect of disease outbreaks on farm profitability and to perceived usefulness of information sources.

When non-adoption of farm biosecurity is observed, the underlying cause may be a failure in knowledge transfer, with farmers that are unaware of the potential efficacy and economic benefits unlikely to implement biosecurity measures (Gunn et al., 2008). There is a need to identify communication gaps between researchers and veterinarians so as to educate veterinarians as the primary information source of farmers (Gunn et al., 2008). This may increase the access of farmers to relevant information and may help achieve behavioural change (Toma et al., 2013). In particular, arming veterinarians with evidence of efficacy and economic and health and welfare benefits may provide motivation (Gunn et al., 2008, Barrett et al., 2011, Ridpath, 2012) for veterinarians to identify their own roles in disease control and biosecurity (Gunn et al., 2008).

However, as veterinarians tend to charge for their time (Gunn et al., 2008), direct education of farmers may also be necessary to ensure farmers are aware of potential benefits from increased uptake of biosecurity (Toma et al., 2013). This has been highlighted as a particularly important

component of BVD control schemes (Lindberg and Alenius, 1999, Barrett et al., 2011). Farmer education has played a substantial role in many BVD control attempts around the world (Lindberg and Alenius, 1999, Barrett et al., 2011, Presi et al., 2011, Ridpath, 2012). It has been acknowledged that resource allocation for coordination of an education program delivering a simple, consistent message and agreed strategy is vital (More et al., 2010, Barrett et al., 2011).

The results of this study show that stud producers are associated with many positive behaviours, as well as with high knowledge, perceived understanding and interest of BVD. As such, these producers may be well placed to be trained to act as ‘champions’ for disease control to help implement positive change in their communities (Aoun et al., 2013a). Aoun et al. (2013a) showed that champions involved in a human health improvement scheme had positive experiences when taking on the leadership role, with success dependent upon the project being realistic and manageable, adequate training of the champions and development of a bond between champions and participants. The delivery of information by champions successfully increased awareness and motivation amongst participants, and resulted in positive health outcomes (Aoun et al., 2013b).

A greater effort may be needed to gain and maintain momentum in programs that aim to effect change (Aoun et al., 2013a). Large-scale farmer support is crucial to the success of BVD control, with such support potentially creating the necessary momentum and lending authenticity and authority to convince reluctant peers (Barrett et al., 2011). It is recommended that education targets an entire region at once in order to generate and capitalise on interest, but that a communication plan needs to be in place for the duration of a project, not only the commencement (Ridpath, 2012). Clear, visible progress in the early stages of a control scheme are vital to ensure ongoing interest and support (Barrett et al., 2011); this progress need not be entirely economical, but may also be a function of increased animal welfare and sociological wellbeing as a function of reduced stress, labour and complexity of management in the presence of reduced disease (More et al., 2010).

In conclusion, schemes for the control of BVD through implementation of biosecurity rely on the commitment and cooperation of farmer populations. This study revealed factors associated

with the uptake of such behaviours and supports the need for excellent education and awareness-raising programs in association with such projects. Dairy producers and stud producers are associated with higher knowledge and interest, as well as better general biosecurity and BVD-specific behaviours than beef producers and non-stud producers, respectively. These cohorts may represent ideal candidates to act as champions of BVD control. Veterinarians are also well-placed to support BVD education, with this study providing evidence that producers view veterinarians as a valuable, trustworthy information source. Improvements in knowledge of BVD could be related to improvements in other areas of animal health and biosecurity.

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7 Tools for Diagnosis of Bovine Viral Diarrhoea

Veterinarians, diagnosticians and stakeholders all benefit from a thorough understanding of the strengths and limitations of BVD diagnostic tests and methods. Furthermore, an appreciation of the pathogenesis of the disease allows informed decision-making regarding BVD diagnosis and the application of diagnostics for control purposes. This review paper, published in *The Veterinary Journal*, outlines the pathogenesis and diagnosis of BVD, and the relationship between these two crucial aspects of the disease.

Review: Bovine Viral Diarrhoea: Pathogenesis and Diagnosis

SR Lanyon, FI Hill, MP Reichel, J Brownlie (2014)

Bovine viral diarrhoea: pathogenesis and diagnosis

The Veterinary Journal

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7.1 *Testing of Bulk Milk and Pooled Serum to Reduce the Cost of Testing for Antibodies Specific to Bovine Viral Diarrhoea Virus in Milking and Non-Milking Cohorts, Respectively*

In control situations, herd-level antibody testing is often the first step towards establishing the infection status of any particular herd or cohort. However, individual testing can be prohibitively expensive. Testing of pooled samples has the potential to drastically reduce the cost of herd-level testing for antibodies specific to BVD. In milking cohorts, bulk milk samples are readily available, while in non-milking cattle (including young stock and beef cattle) pooled serum represents a diagnostic opportunity. The following two papers present the evaluation and validation of bulk milk and pooled serum, respectively, as diagnostic samples for the determination of within-herd seroprevalence and likelihood of the herd being actively infected.

Original Article: Milk as a Diagnostic Sample for the Identification of Bovine Viral Diarrhoea (BVD) Infected Dairy Herds Using a Commercially Available ELISA

SR Lanyon, R McCoy, E Bergman, MP Reichel (2014)

Milk as a Diagnostic Sample for the Identification of Bovine Viral Diarrhoea (BVD) Infected Dairy Herds Using a Commercially Available ELISA

Australian Veterinary Journal Vol. 92, Pp. 269 - 273

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Original Article: Pooling Serum to Identify Cohorts of Non-Milking Cattle Likely to be Currently Infected with Bovine Viral Diarrhoea Virus by Testing for Specific Antibodies

SR Lanyon, ML Anderson, MP Reichel (2014)

Pooling Serum to Identify Cohorts of Non-Milking Cattle Likely to be Currently Infected with Bovine Viral Diarrhoea Virus by Testing for Specific Antibodies

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7.2 An Observational Study of Diagnostic Opportunities in ('Trojan') Cows Carrying Fetuses Persistently Infected with Bovine Viral Diarrhoea Virus Following Acute Infection in Early Gestation

In efforts to control BVD, one major biosecurity risk still exists for which there is, as yet, no definitive solution: the so called 'Trojan' cow. That is, a pregnant, non-PI female carrying a PI fetus. The dam, having undergone acute infection in early gestation, is expected to test positive for BVDV specific antibodies but, generally, negative for antigen. In her uterus, however, she carries a PI calf that was produced after acute infection in the early stages of gestation. Introduction of such a female into a BVD-free population is, from a biosecurity perspective, equivalent to introduction of a PI individual. The consequences of the resultant outbreak may be dramatic.

The four manuscripts included in this section report the results of an experimental trial studying opportunities for the pre-natal diagnosis of fetal PI by detection of specific antibodies, antigen or virus in the dam during gestation.

7.2.1 Findings in pregnant females

The primary objective of the experimental trial reported in this chapter was to investigate opportunities for diagnosis of fetal PI by testing maternal samples. The following paper details the findings in the dams from this study during and immediately following their pregnancies.

Original Article: A longitudinal study of the antibody levels in serum from cows with differing gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) during early gestation

SR Lanyon, R McCoy, PD Cockcroft, MP Reichel (2014)

A longitudinal study of the antibody levels in serum from cows with differing gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) during early gestation

Veterinary Microbiology

Submitted Manuscript

Statement of Authorship

Title of Paper	Antibody Levels in Heifers with Differing Pregnancy Outcomes Following Acute Infection with Bovine Viral Diarrhoea Virus During Early Pregnancy
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Publication Details	SR Lanyon, R McCoy, PD Cockcroft, MP Reichel (2014) A longitudinal study of the antibody levels in serum from cows with differing gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) during early gestation Veterinary Microbiology, Submitted Manuscript

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A longitudinal study of the antibody levels in serum from cows with differing gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) during early gestation

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Abstract

Infection with bovine viral diarrhoea virus (BVDV) during early gestation can result in a variety of gestational outcomes including abortion, stillbirth and the birth of calves with neurological deficits or the production of immunotolerant, persistently infected (PI) calves. Seventeen seronegative pregnant heifers were infected with BVDV via exposure to a PI cow at approximately days 69-90 of gestation. Serum, nasal, saliva and vaginal swabs were collected weekly throughout gestation. Ear notch samples were collected every four weeks. The samples were analysed by antibody and antigen enzyme-linked immunosorbent assay (ELISA), agarose gel immunodiffusion (AGID) and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). All the heifers seroconverted within 28 days post-exposure, and sub-positive levels of BVDV antigen were detected in 5 (of 17) heifers on days 7, 9 and/or 14 post-exposure. No BVD virus or viral antigen was detected in any samples collected after seroconversion. AGID results of 3+ were achieved by all but one heifer, with 3+ results persisting until calving in some heifers. Heifers carrying PI fetuses (n=3) were observed to have significantly higher Ab ELISA results than heifers carrying non-PI calves consistently from day 77 post-exposure (days 146 to 167 of gestation) onwards. Heifers carrying calves with neurological deficits tended to exhibit lower Ab ELISA results than other heifers, but this difference was not significant. There is potential for the antibody difference between heifers carrying PI fetuses and those carrying non-

PI fetuses by Ab ELISA to be used as a method of pre-natal diagnosis, generally with diagnostic sensitivity of 100% and specificity of $\geq 70\%$ observed.

Keywords AGID; BVDV; ELISA; Pre-natal diagnosis; Serology

Introduction

Bovine viral diarrhoea virus (BVDV) infection has been shown to have significant economic impacts on infected herds. Losses may stem primarily from an increase in the incidence and severity of secondary disease due to an immunosuppressive effect (Brackenbury et al., 2003) and from reproductive losses, including reduced fertility, fetal death and subsequent abortion, stillbirth, or neurological deformations in the developing fetus (Grooms, 2004). There may also be calves that are immunotolerant to BVDV and as a consequence persistently infected (PI) with the virus (Brownlie et al., 1987). In general, PI calves are thought to result from infection prior to 120 days gestation (Brownlie et al., 1987), while infection after this time tends to produce calves that are seropositive at birth (Grooms, 2004). Neurological deformities commonly include hydrocephalus and cerebellar hypoplasia (Trautwein et al., 1986) and generally occur following infection between 90 to 150 days of gestation (Trautwein et al., 1986, Grooms, 2004). There is some variation in the timing at which a particular outcome will result as illustrated by a report of a PI calf born with a seropositive twin (Schoder et al., 2004). Persistently infected animals are epidemiologically important as a primary source of infection with a persistently high viral excretion rate. Pre-natal detection of a cow or heifer carrying a persistently infected calf would enable this potential source of infection to be removed from the herd or avoided at the point of entry. This would enhance biosecurity to protect naïve herds and assist in eliminating the infection from an already infected herd.

Brownlie et al. (1998), Lindberg et al. (2001) and Stokstad et al. (2003) have demonstrated that females carrying a PI fetus have higher levels of BVDV-specific antibodies than females carrying non-PI fetuses. Due to the highly infectious nature of PI calves, the ability to accurately differentiate females carrying a PI calf from those carrying a non-PI calf could have important

implications in BVDV control. The serological antibody profiles of females that experience abortion or stillbirth or deliver calves with neurological deficits following acute BVDV infection during gestation have not been reported. Therefore, the aims of this study were to investigate the serological antibody profile in heifers with varying gestational outcomes following natural BVDV infection under experimental conditions during early gestation, and to explore opportunities for pre-natal diagnosis of PI in the fetus by antibody, antigen and/or virus detection in the dam during gestation.

Methods

Animals

Twenty-three Angus and Angus cross heifers were oestrus synchronised and artificially inseminated (AI), then naturally mated for approximately three weeks (one cycle). Seventeen heifers were confirmed pregnant by per rectal ultrasound 90 days post-AI (approximately day 69 of gestation for heifers that conceived to natural mating). The heifers were confirmed naïve to BVDV by negative results in both antibody (Ab) and antigen (Ag) enzyme-linked immunosorbent assay (ELISA). These heifers formed the experimental group.

A two year old dairy cow, PI with BVDV type 1c was sourced locally (status confirmed by consecutive positive results on Ag ELISA and negative results of Ab ELISA). The BVDV strain was confirmed by sequencing. RNA was extracted from serum from the PI cow using Qiagen QIAmp Viral RNA Mini Kit (Qiagen Pty. Ltd., Chadstone, Victoria, Australia). Synthesis of cDNA was completed using Invitrogen High Capacity cDNA Reverse Transcription Kit (Life Technologies Australia Pty. Ltd., Mulgrave, Victoria, Australia) with primer specific to the 5' untranslated region (UTR) of the BVDV genome at an assay concentration of 1 mM (primer sequences: CTATCCTTCTCTGATTCTCTG). A 292 base pair fragment of the cDNA was amplified by PCR using primers specific to the 5' untranslated region (UTR) of the BVDV genome (primer sequences: CTAGCCATGCCCTTAGTAGGACTA and CAACTCCATGTGCCATGTACAGCA). The assay consisted of 1x MyTaq Reaction Buffer (Bioline Australia Pty. Ltd., Alexandria, New South Wales, Australia), 1 mM MgCl₂, 0.2 mM

dNTPs, 0.2 mM forward primer, 0.2 mM reverse primer, 0.2 µL MyTaq DNA polymerase (Bioline Australia Pty. Ltd., Alexandria, New South Wales, Australia) and 2 µL cDNA template in a total reaction volume of 50 µL. Reaction was incubated for 2 minutes at 94°C for initial denaturation, followed by 35 cycles of 95°C for 30 seconds, 57.7°C for 30 seconds and 72°C for 45 seconds. The reaction was completed by a final extension at 72°C for 2 minutes. The PCR products were purified using Qiagen MinElute PCR Purification Kit (Qiagen Pty. Ltd., Chadstone, Victoria, Australia) and submitted to Australian Genome Research Facility Ltd (Urrbrae, South Australia, Australia) for sequencing.

Infection with BVDV

The pregnant heifers were naturally infected with BVDV via exposure to and co-mingling with the PI cow from days 90 to 118 post-AI (day 90 post-AI = day 0 post-exposure). Co-mingling was conducted at a density of 24 m²/animal. On day 22 post-exposure, nasal mucous was transferred from the PI cow to the experimental heifers by nasal application of a rag to the PI cow and then to each experimental heifer, with reapplication to the PI cow in between each heifer to ensure all heifers became infected.

Gestational outcomes

The gestational outcome of each heifer was recorded as one of the following: abortion, neonatal calf death, live healthy calf, live PI calf, or live calf with neurological deficits. A heifer was considered to have aborted if: a) an abortion was observed (and fetus recovered), b) the heifer was found not pregnant by subsequent per rectal palpation, or c) the heifer failed to calve (and was subsequently found not pregnant). A neonatal calf death was recorded if the calf was found dead following parturition. A live calf was considered PI when: either, pre-colostral samples returned a positive result by Ag ELISA, or serum collected at 14 days of age returned a positive result by qRT-PCR; and, Ab ELISA returned a negative result on pre-colostral serum (where available). A live calf was considered to have a neurological deficit if clinical neurological signs were apparent. Remaining live calves were considered healthy.

Sample collection and storage

Serum samples, and nasal, vaginal and saliva swabs were collected from each heifer on days -7, 0, 5, 7, 9, 14, 21, 25 and 28, post-exposure, then weekly until six weeks post-calving. Swab samples were collected by application and repetitive stroking of a rayon tipped swab over the mucosal surface. In addition, ear notch samples were collected from each heifer every four weeks from day -7 post-exposure until six weeks post-calving using an Allflex Tissue Sampling Unit (Allflex Australia Pty Ltd. Capalaba, Queensland, www.allflex.com.au). All the samples were stored at -80°C until processing. At processing, all the ear notches were soaked in 250µL IDEXX ear notch tissue soaking buffer (IDEXX Laboratories Inc. Rydalmere, NSW) for 24 +/- 1 hours at room temperature. Nasal, vaginal and saliva swabs were processed by soaking of the swab tip in 1mL IDEXX ear notch tissue soaking buffer for 24 +/- 1 hours at room temperature. After soaking, ear notches and swab tips were removed from the supernatant and both supernatant and sample were stored separately at -80°C until testing.

Testing for BVDV specific antibodies

ELISA

Serum samples were tested for the presence of BVDV specific antibodies using commercially available Ab ELISA (IDEXX BVDV Total Ab ELISA, IDEXX Laboratories Inc. Rydalmere, NSW), performed according to manufacturer's instructions.

Avidity of antibodies was measured by the addition of an extra incubation and wash step following the 90 minute sample incubation and wash: one duplicate was incubated with approximately 300µL wash solution at room temperature for 5 minutes, while the corresponding duplicate was incubated with 100µL 8M urea at 37°C for 5 minutes. Both duplicates were washed, before continuing with the conjugate incubation specified by the standard ELISA

procedure. Avidity for a particular sample was calculated as: $\frac{S}{P} \text{ratio with urea treatment}}{\frac{S}{P} \text{ratio without urea treatment}} *$

100%.

AGID

Weekly serum samples from the first twelve weeks post-exposure, and fortnightly throughout the remainder of the study were also tested for the presence of Pestivirus specific antibodies by agarose gel immunodiffusion (AGID), using C24V BVDV reference strain as antigen. An AGID score of 1, 2, 3 or 3+ was considered positive. A 3+ result was recorded as 4 for statistical analysis and a negative result was recorded as 0. All AGID testing was performed by the New South Wales Department of Primary Industries (Elizabeth Macarthur Agricultural Institute, Menangle, NSW).

Testing for BVD virus and specific antigen

Selected serum samples, and swab and ear notch supernatants were tested for BVD virus by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), as previously described (Hill et al., 2007). In addition, all serum samples and selected ear notch supernatants were tested for BVDV specific antigen by commercially available Ag ELISA (IDEXX BVDV Serum/Ag Plus ELISA, IDEXX Laboratories Inc. Rydalmere, NSW), as per the manufacturer's instructions. Selected swab supernatant samples were also tested by Ag ELISA, with 50µL supernatant incubated with 50µL detection antibodies, as is the manufacturer's recommended protocol for ear notch supernatants. Results were expressed as corrected optical density (OD), with an OD > 0.3 considered positive.

Statistical analyses

Differences in antibody levels between heifers carrying PI calves and those carrying non-PI calves were assessed using a two-tailed student's t-test, with a p-value <0.05 considered significant, for both ELISA and AGID results.

The observed diagnostic sensitivity (DSe) $\left(\frac{\# \text{ heifers carrying PI calves that tested positive}}{\# \text{ heifers carrying PI calves}}\right)$, observed diagnostic specificity (DSp) $\left(\frac{\# \text{ heifers carrying non-PI calves that tested negative}}{\# \text{ heifers carrying non-PI calves}}\right)$ were calculated for diagnosis of calf PI status by Ab ELISA were calculated for eight different

positivity thresholds (0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 S/P ratio) at each time point using MedCalc for Windows version 12.7.7 (MedCalc Software, Ostend, Belgium). The Youden Statistic was also calculated at each combination of threshold and time point as: $DSe + DSp - 100\%$. The Youden Statistic, DSe and DSp for diagnosis of calf PI status by AGID were similarly calculated at a positivity threshold of >3 (ie. a 3+ result was considered positive). Approximate week of gestation of time points was back-calculated from day of calving, with heifers assumed to have calved at 40 weeks gestation. Heifers that aborted (n=4) were excluded from this analysis.

Ethics

This project was approved by the University of Adelaide Animal Ethics Committee (project number: S-2012-087).

Results

Gestational outcomes

One abortion was observed on day 253 post-AI, and the fetus recovered and found to be PI. Additionally, one heifer was found not pregnant by palpation at day 222 post-AI (following confirmation of pregnancy by palpation at day 133 post-AI) and two heifers failed to calve (despite confirmation of pregnancy by palpation at day 222 post-AI) and were subsequently found not pregnant. Of the heifers that carried their pregnancies to term, three delivered livecalves with neurological deficits, three delivered live PI calves, six delivered apparently healthy calves and there was one neonatal calf death. Gestational outcomes are summarised in Table 1. The calf which died in the neonatal period was observed to have superior brachygnathism (relative prognathism) with no other abnormalities revealed on post-mortem examination.

Table 1. Summary of the gestational outcomes of seventeen heifers following acute infection with bovine viral diarrhoea virus (BVDV) on day 69 – 90 of gestation.

Pregnancy outcome	Number of heifers	Percentage of heifers (95% CI)
Abortion, observed	1 ^a	23.5 (3.4 – 43.7)
Abortion, unobserved (found empty)	3	
Neonatal calf death	1	5.9 (0 – 17.1)
Live, calf with neurological deficit	3	17.6 (0 – 35.8)
Live, PI^b calf	3	17.6 (0 – 35.8)
Live, healthy calf	6	35.3 (12.6 – 58.0)

^afetus recovered and shown to be PI

^bPI = persistently BVDV infected

The calves with neurological deficits (n=3) exhibited a range of clinical signs. The most severely affected calf was laterally recumbent with muscular tremors and a weakened suck reflex, and was euthanized soon after birth. The two less severely affected calves were bright and alert, and were unable to stand at birth but were able to stand unaided at 24 hours and 10 days of age, respectively. As the ability of these calves to stand and walk developed, ataxia and wide-based stances became apparent. These calves were euthanized at approximately 4 months of age. Post-mortem examination revealed cephalic dysplasia, hydrocephalus, and cerebellar aplasia in all three clinically affected calves.

Seroconversion

By Ab ELISA, antibodies were detected in one (5.9%), eight (47.1%), 16 (94.1%) and 17 (100%) of heifers by days 14, 21, 25 and 28 post-exposure, respectively (Figure 1). By AGID, all heifers tested positive on day 21 post-exposure (Figure 2).

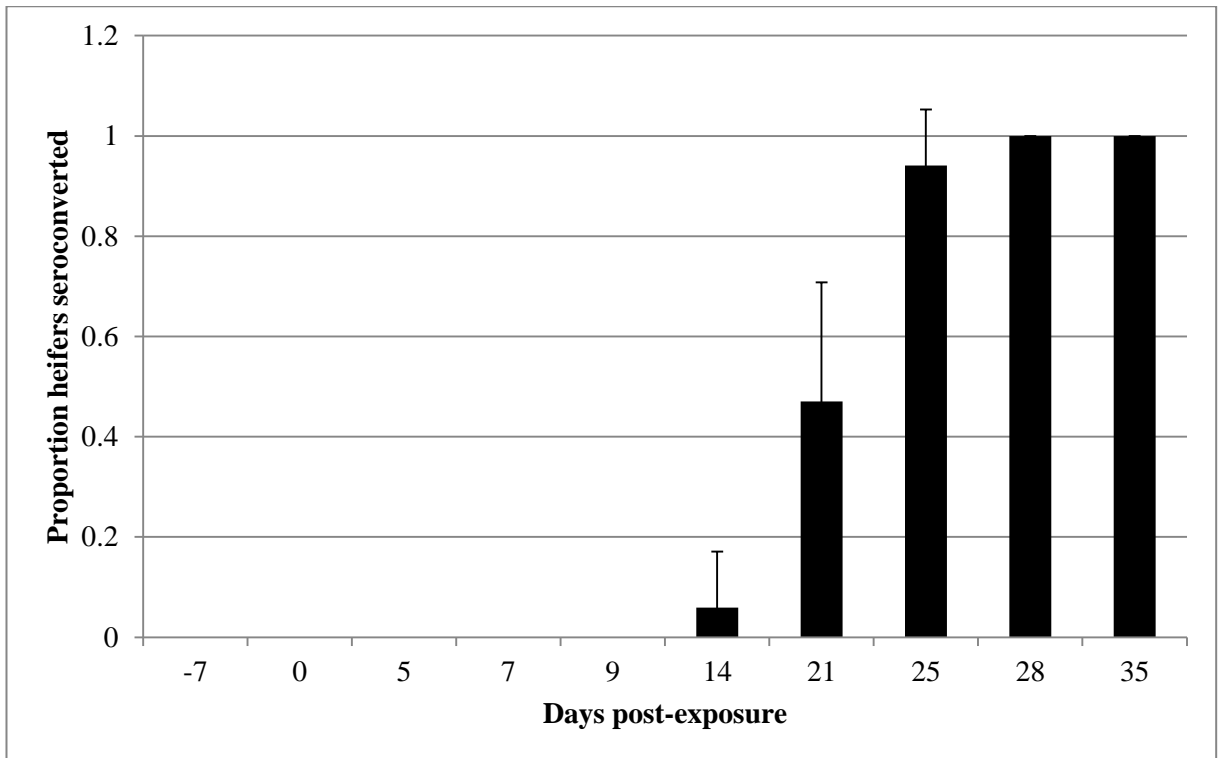


Figure 1. Timing of seroconversion of seventeen heifers following exposure to bovine viral diarrhoea virus (BVDV) by co-mingling with a persistently BVDV infected cow from days 0 to 28 post-exposure at a density of 24 m²/animal. Error bars show 95% confidence intervals.

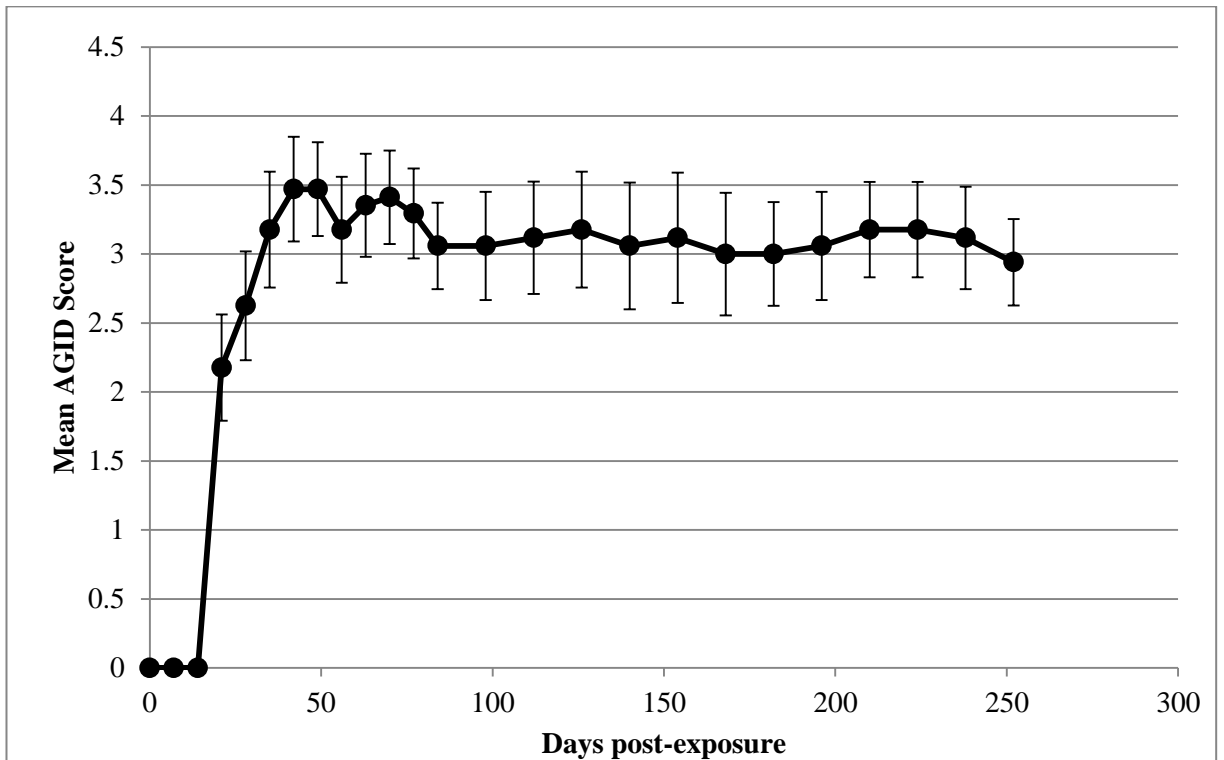


Figure 2. The mean agarose gel immunodiffusion (AGID) score in seventeen heifers following acute infection with bovine viral diarrhoea virus (BVDV). Error bars show 95% confidence intervals.

Levels of BVDV specific antibodies in heifers following acute infection in early gestation

ELISA

By ELISA, antibody levels (S/P ratio) continued to rise in all heifers throughout gestation, until day 175 post-exposure (the last sampling time point before the birth of the first calf) (Figure 3). Antibody levels appeared to begin to decline at around the time of calving, with a steeper decline observed in heifers that had delivered PI calves than those that delivered non-PI calves (Figure 4). A rapid decline in antibody levels post-calving was observed in heifers that delivered a calf with neurological deficits (Figure 4), however, this decline did not result in those heifers exhibiting significantly lower antibody levels than heifers that delivered healthy calves.

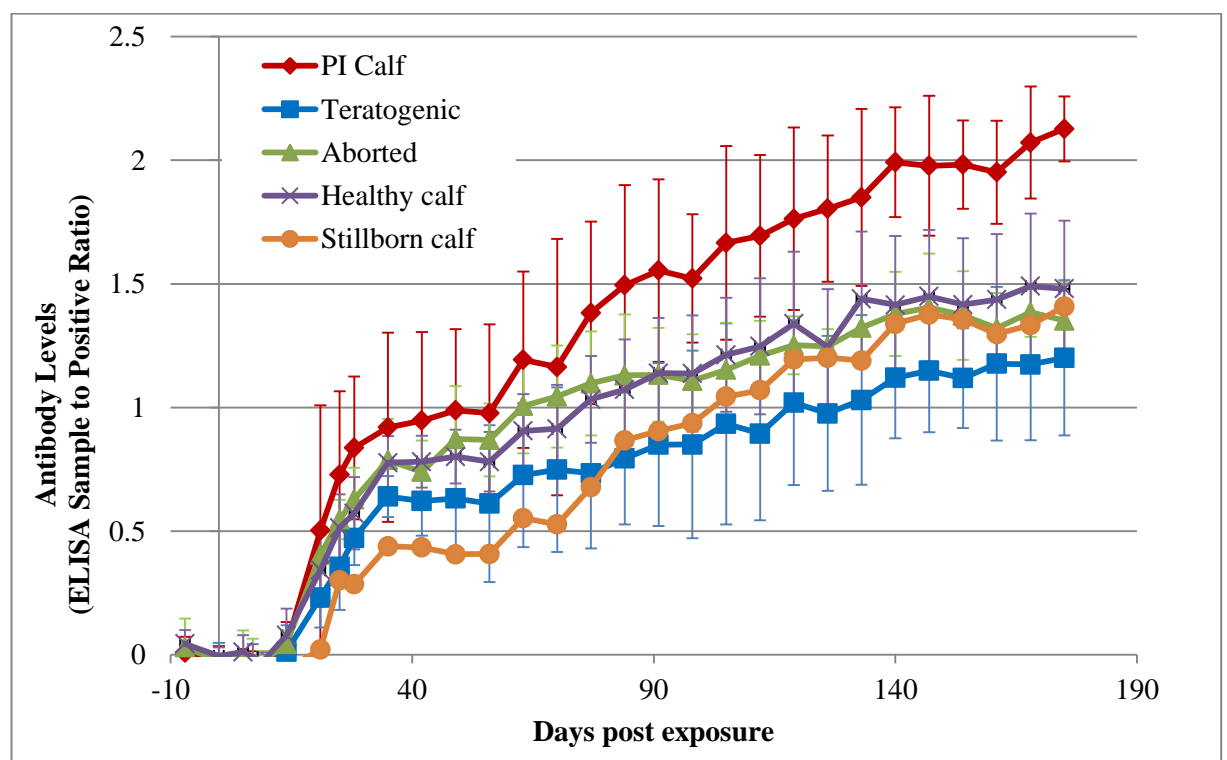


Figure 3. The antibody levels over gestation in seventeen heifers with varying gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) on day 69 – 90 of gestation (day 0 post-exposure). Gestational outcomes were classified as: abortion (n=4), neonatal calf death (n=1), live calf with neurological deficit (n=3), live persistently BVDV infected (PI) calf (n=3), healthy calf (n=6), with the first live calf born on day 179 post-exposure. Antibody levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA), and expressed as a sample to positive (S/P) ratio. Error bars show 95% confidence intervals.

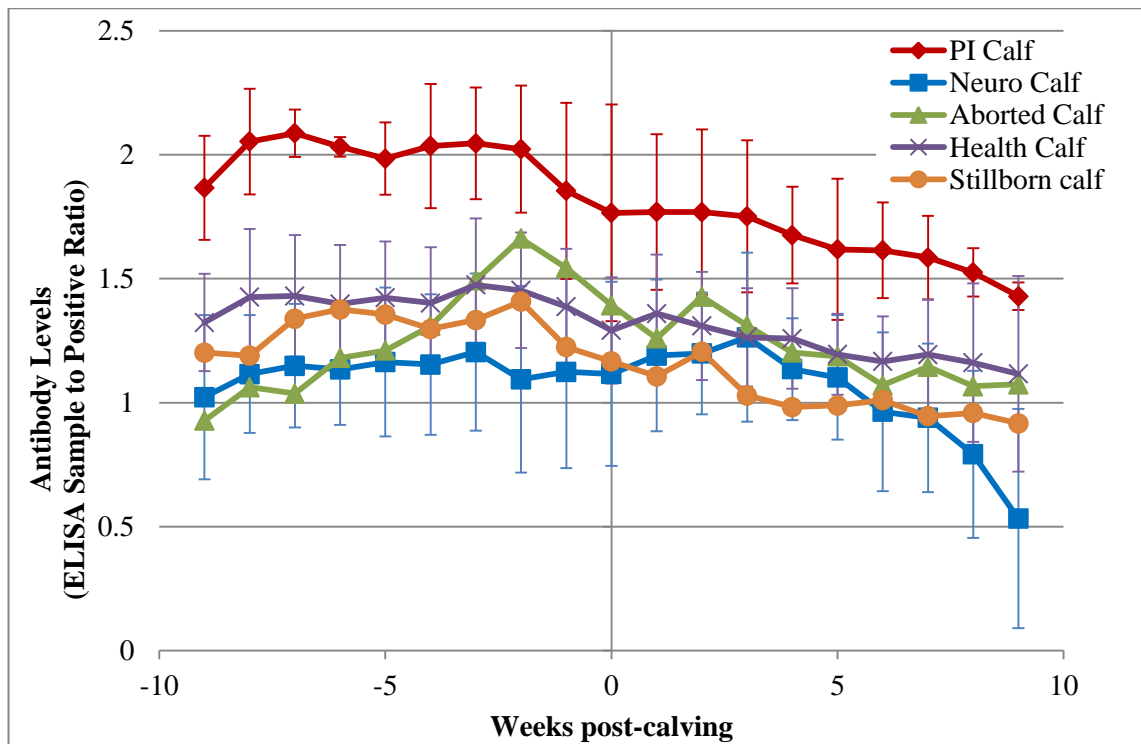


Figure 4. The antibody levels nine week pre- and post-calving in seventeen heifers with varying gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) on day 69 – 90 of gestation (day 0 post-exposure). Gestational outcomes were classified as: observed abortion (n=1), neonatal calf death (n=1), live calf with neurological deficit (n=3), live persistently BVDV infected (PI) calf (n=3), healthy calf (n=6). Antibody levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA), and expressed as a sample to positive (S/P) ratio. Error bars show 95% confidence intervals. (Note: unobserved abortions could not be included as day of abortion is unknown.)

The mean Ab ELISA results were significantly higher in heifers carrying a PI calf, than those carrying non-PI calves at days 28 and 63 post-exposure, and consistently from day 77 post-exposure throughout gestation, and until seven weeks post-calving ($p < 0.05$). By eight weeks post-calving, antibody levels in cows that carried PI calves had returned to levels that were not significantly different from cows that carried non-PI calves.

The avidity of BVDV specific antibodies increased steadily from days 28 to 168 post-exposure, with no significant differences between heifers with different gestational outcomes (Figure 5).

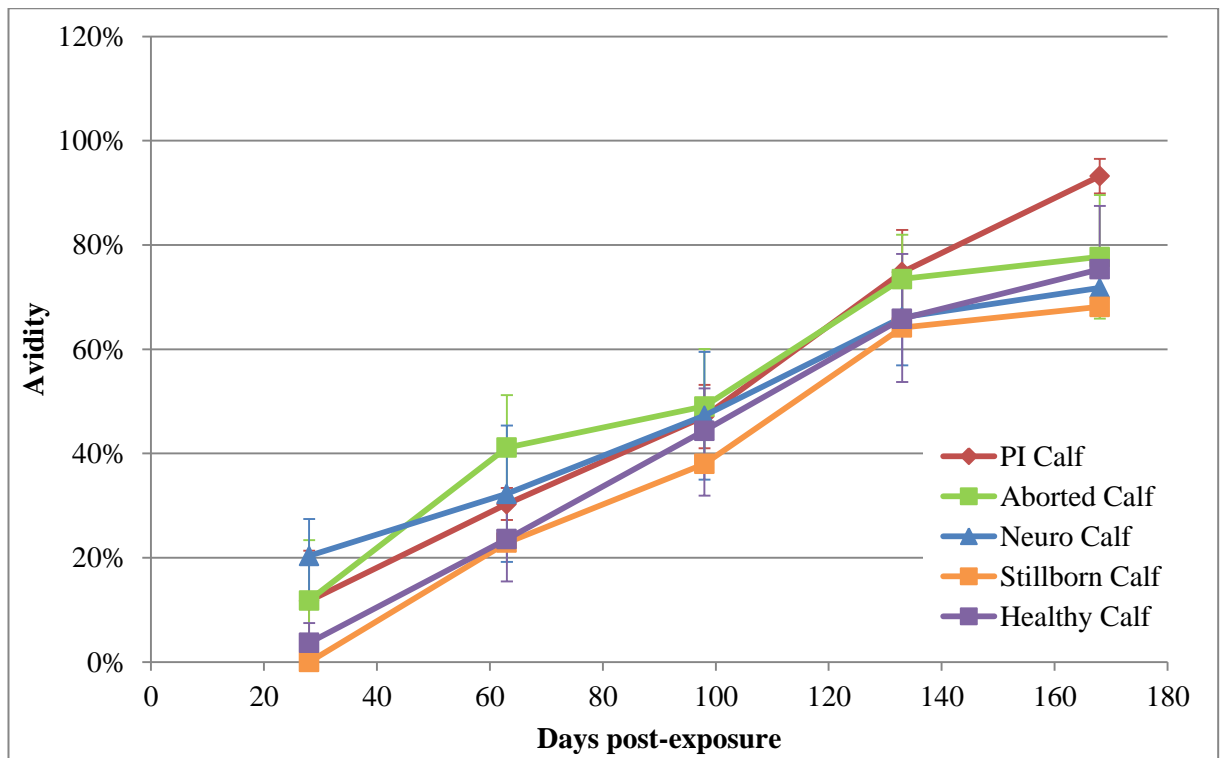


Figure 5. The avidity of antibodies in seventeen heifers with varying gestational outcomes following acute infection with bovine viral diarrhoea virus (BVDV) on day 69 – 90 of gestation (day 0 post-exposure). Gestational outcomes were classified as: abortion (n=4), neonatal calf death (n=1), live calf with neurological deficits (n=3), live persistently BVDV infected (PI) calf (n=3), healthy calf (n=6). Antibody avidity was measured by calculating the ratio (x100%) of the results (sample to positive (S/P) ratios) of urea treated to non-urea treated replicates tested by enzyme-linked immunosorbent assay (ELISA). Error bars show 95% confidence intervals.

AGID

When tested by AGID, the mean result of all heifers continued to rise from 2.17 (95% CI: 1.79 – 2.56) at day 21 post-exposure (the first time-point in which positive results were observed) to 3.47 (95% CI: 3.09 – 3.85) at 42 and 49 days post-exposure. The mean AGID score of all heifers then declined until day 77 post-exposure, before remaining between 2.9 and 3.2 for the duration of the trial (that is, until day 252 post-exposure). No significant differences were observed between mean AGID score of heifers that delivered a PI calf and heifers that delivered a non-PI calf.

Detection of BVD virus and specific antigen in heifers following acute infection in early gestation

Five heifers showed sub-positive peaks in Ag ELISA results on days 7, 9 or 14 post-exposure, with one heifer returning a weak positive result on day 9 post-exposure (Figure 6). Two of the heifers exhibiting antigen peaks delivered live PI calves, while an additional two aborted (one of which was observed to abort a PI fetus). The fifth heifer exhibiting an antigen peak delivered a healthy calf. A serum pool containing contributions from all seventeen heifers of day 9 post-exposure returned a positive qRT-PCR result.

No positive qRT-PCR or Ag ELISA results were observed for any sample from any heifer after day 21 post-exposure.

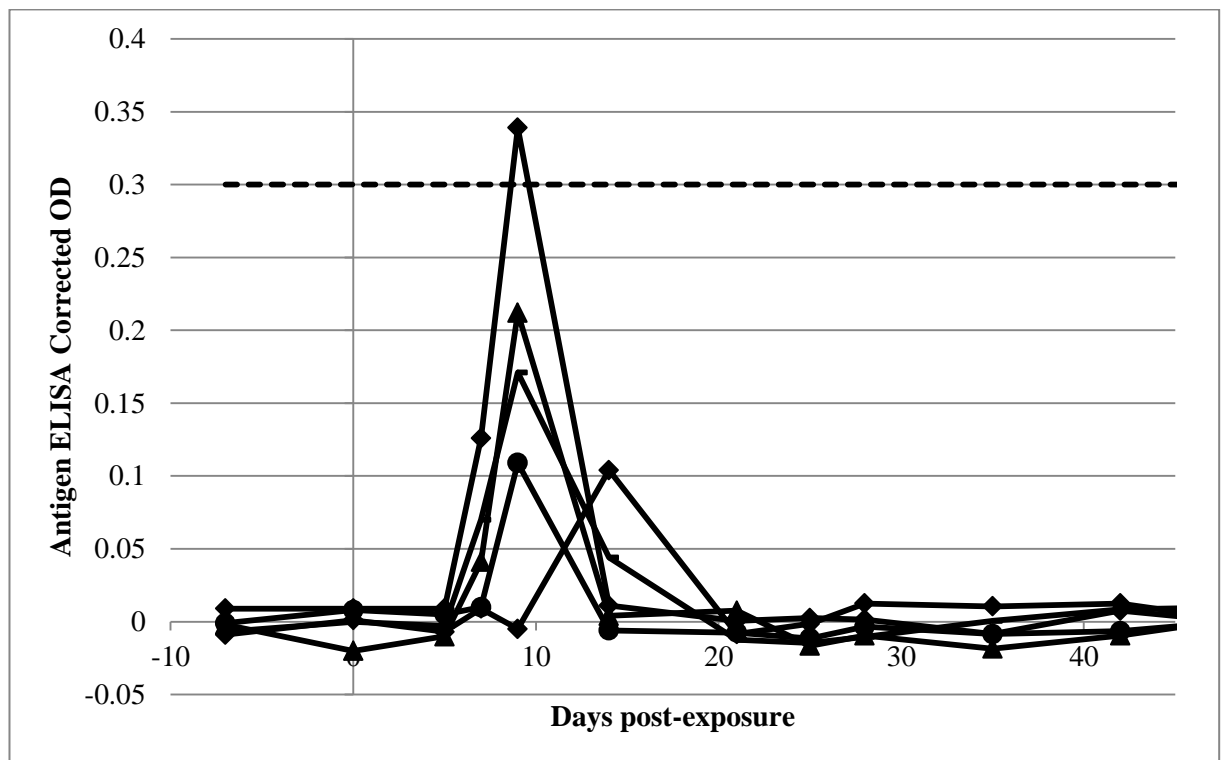


Figure 6. The antigen levels in five heifers that exhibited an antigen peak following acute infection with bovine viral diarrhoea virus (BVDV) on day 69 – 90 of gestation (day 0 post-exposure). Antigen levels were measured by commercially available enzyme-linked immunosorbent assay (ELISA) and expressed as corrected optical density (OD). Dotted line represents the manufacturer’s recommended threshold for positivity.

Application of antibody levels for pre-natal diagnosis of persistent infection

ELISA

When using antibody ELISA results for pre-natal diagnosis of fetal PI, 100% (95% CI: 30.5 – 100.0%) DSe for the detection of heifers carrying PI calves was observed for approximately the last 24 weeks of gestation when the threshold for positivity (that is, the threshold at which an Ab ELISA result is considered positive) was set at 0.6 S/P ratio. Conversely, 100% (95% CI: 69.0 – 100.0%) DSp was observed at 28 of the 29 timepoints prior to calving when the positivity threshold was set at 2.0 S/P ratio. Declining antibody levels observed in the two weeks prior to calving resulted in a decrease in DSe at these timepoints when thresholds ≥ 1.6 S/P ratio were applied. In general, DSe and DSp $\geq 80\%$ were observed simultaneously for limited time periods at any given threshold, the longest period being approximately 11 weeks (from weeks 28 to 38 of gestation, inclusive) at a threshold of 1.6 S/P ratio (Table 2). The corresponding Youden statistics are shown in Table 3. A Youden statistic of 100%, signifying simultaneous 100% DSe and 100% DSp, was achieved on only five occasions.

Table 2. The diagnostic sensitivity (DSe) and specificity (DSp) for diagnosis of fetal persistent bovine viral diarrhoea virus (BVDV) infection (PI) by detection of specific antibodies by enzyme linked immunosorbent assay (ELISA) in the dam (n=3 carrying PI, n=10 carrying non-PI) from 24 weeks before calving, at each of eight sample-to-positive (S/P) ratio thresholds for positivity. Grey shading: DSe or DSp = 100%.

		Weeks before calving																								
		-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
S/P Ratio Threshold	DSe (%)*	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	0.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	0.8	67	67	33	67	67	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1.0	33	67	33	67	67	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1.2	0	33	33	33	33	67	67	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1.4	0	0	0	0	0	33	33	33	67	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	1.6	0	0	0	0	0	0	33	33	33	33	33	33	100	100	100	100	100	100	100	100	100	100	100	67	67
	1.8	0	0	0	0	0	0	0	0	33	33	0	33	33	33	67	33	100	100	100	100	67	100	67	67	33
2.0	0	0	0	0	0	0	0	0	0	0	0	33	33	33	33	33	67	100	100	33	67	67	67	33	33	
DSp (%)**	0.6	80	70	30	20	30	20	20	20	20	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.8	80	80	70	70	60	50	50	40	40	20	30	20	20	20	20	20	10	0	0	0	0	0	10	10	10
	1.0	100	100	100	90	90	90	70	60	60	40	40	40	30	20	30	20	20	20	20	20	20	20	20	20	20
	1.2	100	100	100	100	100	90	90	100	70	90	70	60	50	40	40	30	40	20	30	20	20	20	20	20	50
	1.4	100	100	100	100	100	100	100	100	100	90	90	90	80	80	70	60	50	60	70	70	80	60	60	70	90
	1.6	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	90	100	90	80	90	80	80	80	80	90
	1.8	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	100	90	90	90	90	100	90	90	90	90
	2.0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	100	100	100
*95% confidence intervals on DSe:														**95% confidence intervals on DSp		DSp = 50.0%		95% CI = 18.9 – 81.1%								
DSe = 0		95% CI = 0 – 69.5%;												DSp = 0		95% CI = 0 – 31.0%		DSp = 60.0%		95% CI = 26.4 – 87.6%						
DSe = 33.3%		95% CI = 5.5 – 88.5%;												DSp = 10.0%		95% CI = 1.7 – 44.5%		DSp = 70.0%		95% CI = 34.8 – 93.0%						
DSe = 66.7%		95% CI = 11.6 – 94.5%;												DSp = 20.0%		95% CI = 3.1 – 55.6%		DSp = 80.0%		95% CI = 44.4 – 96.9%						
DSe = 100.0%		95% CI = 30.5 – 100.0%												DSp = 30.0%		95% CI = 7.0 – 65.2%		DSp = 90.0%		95% CI = 55.5 – 98.3%						
														DSp = 40.0%		95% CI = 12.4 – 73.6%		DSp = 100.0%		95% CI = 69.0 – 100.0%						

Table 3. The Youden Statistic (%) ($J = DSe + DSe - 100\%$) for diagnosis of fetal persistent bovine viral diarrhoea virus (BVDV) infection (PI) by detection of specific antibodies by enzyme linked immunosorbent assay (ELISA) in the dam (n=3 carrying PI, n=10 carrying non-PI) from 24 weeks before calving, at each of eight sample-to-positive (S/P) ratio thresholds for positivity. Grey shading: highest in column.

Youden Statistic (%)		Weeks before calving																								
		-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
S/P Ratio Threshold	0.6	80	70	30	20	30	20	20	20	20	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.8	47	47	33	37	27	50	50	40	40	20	30	20	20	20	20	20	10	0	0	0	0	0	10	10	10
	1.0	33	67	33	57	57	90	70	60	60	40	40	40	30	20	30	20	20	20	20	20	20	20	20	20	20
	1.2	0	33	33	33	33	57	57	100	70	90	70	60	50	40	40	30	40	20	30	20	20	20	20	20	50
	1.4	0	0	0	0	0	33	33	33	67	90	90	90	80	80	70	60	50	60	70	70	80	60	60	70	90
	1.6	0	0	0	0	0	0	33	33	33	33	33	33	100	90	90	100	90	80	90	80	80	80	80	80	57
	1.8	0	0	0	0	0	0	0	0	33	33	0	33	33	33	57	33	90	90	90	90	67	90	57	57	23
	2.0	0	0	0	0	0	0	0	0	0	0	0	33	33	33	33	33	67	100	100	33	67	57	67	33	33

Figure 7 shows the highest threshold at which 100% (95% CI: 30.5 – 100.0%) DSe was observed for each time point from approximately 16 to 38 weeks gestation, and the corresponding DS_p. The highest threshold at which 100% (95% CI: 30.5 – 100.0%) DSe was achieved was observed to increase as gestation progressed. The DS_p observed at these thresholds was variable (20% - 80%) in earlier gestation (< 21 weeks). After approximately 21 weeks gestation, DS_p ≥ 70% was observed and maintained for the remainder of gestation.

Heifers which aborted were excluded from this analysis. However, an observed abortion was recorded in one heifer, and the fetus recovered and found to be PI. This heifer, prior to the abortion event at approximately week 36 of gestation, returned positive results at the thresholds presented in Figure 7 at 4 (19.0%) out of 21 sampling time points (weeks 18, 19, 20 and 34 of gestation).

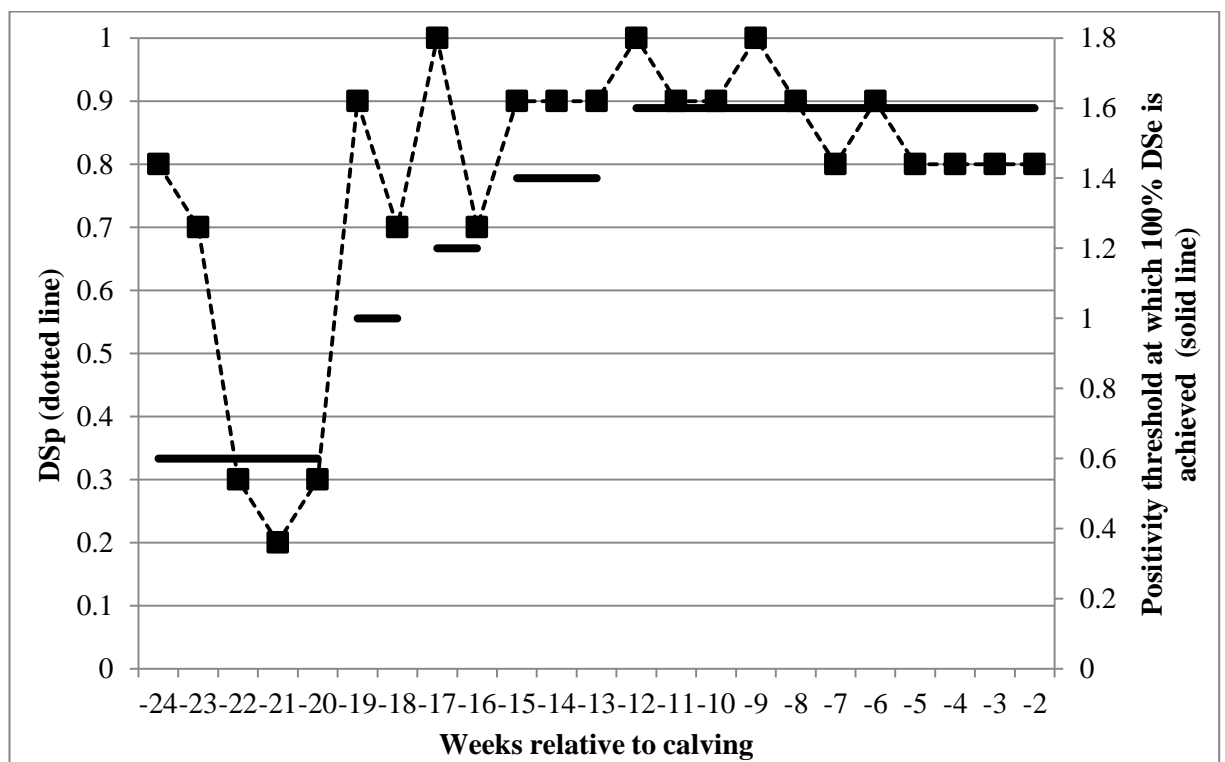


Figure 7. The maximal positivity thresholds (solid line) at which 100% diagnostic sensitivity (DSe) is achieved for diagnosis of persistent bovine viral diarrhoea virus (BVDV) infection in a fetus by testing serum from the dam by commercially available antibody enzyme linked immunosorbent assay (ELISA) at a particular stage of gestation, when the result (expressed as a sample-to-positive (S/P) ratio) is deemed positive when it exceeds the threshold, and; the corresponding diagnostic specificity (DS_p)(dotted line) observed at that threshold.

AGID

When considering an AGID score of 3+ to be indicative of a heifer carrying a PI fetus, DSe was lower than that observed using the ELISA, with one (of three) heifers returning a positive (3+) result on only one occasion. The remaining two heifers returned positive results consistently from week 23 of gestation onwards. Similarly, DSp was lower when using the AGID than when using Ab ELISA, as nine of ten heifers returned a positive (3+) result at at least one timepoint and several maintained 3+ results until the late stages (up until week 38) of gestation, producing false positive results.

Discussion

In this trial, acute BVDV infection was established in the experimental heifer group following exposure to a PI cow at day 90 post-AI, with positive antigen results observed in some (n=5) heifers, and seroconversion evident in all (n=17). The timing of the (albeit small) antigen peak at between days 7 and 14 (majority at day 9) post-exposure is consistent with previous literature (Raizman et al., 2011). Similarly, seroconversion was observed between 14 and 28 days post-exposure by ELISA and at day 21 by AGID, which is consistent with the timing of seroconversion observed by Raizman et al. (2011) and Tsuboi et al. (2013). The majority of the heifers in this trial (15 out of 17) seroconverted within five days of each other by ELISA (days 21 to 25 post-exposure), and all seroconverted at the same timepoint (day 21 post-exposure) when measured by AGID. This may suggest that the heifers contracted the infection within a tight time frame (potentially within a few days following initial exposure). This is not surprising, given the high BVDV challenge from the PI animal, and the high stocking density during the co-mingling. Incidence of infection has previously been reported to be as high as 96% in 6 months in herds of barn housed cattle containing a PI individual (Houe et al., 1993).

The results of the present study are consistent with the findings of previous reports (Brownlie et al., 1998, Lindberg et al., 2001, Stokstad et al., 2003) that females carrying a PI fetus have significantly higher levels of BVDV-specific antibodies when measured by ELISA compared to females carrying a non-PI fetus. In this study, the difference between the heifers carrying PI

fetuses and those carrying non-PI fetuses was statistically significant from 77 days post-exposure (146 to 167 days gestation) onwards. This agrees with results observed by Stokstad et al. (2003) where a statistically significant difference in antibody levels was observed from day 135 gestation (approximately day 54 to 61 days post-infection) onwards. The AGID results did not replicate this finding, perhaps due to a higher variability within cohorts of heifers with the same gestational outcome (particularly those that delivered PI calves) than the variability observed in the Ab ELISA.

In contrast to previous studies, the antibody levels of non-PI carrying heifers also continued to rise throughout gestation when measured by Ab ELISA. Stokstad et al. (2003) observed consistent, low antibody levels in females that were carrying non-PI fetuses, while the present study demonstrated a continual rise in Ab ELISA results in all heifers until calving, with those carrying PI fetuses rising faster than those carrying non-PI fetuses. The results of the present study are more in line with those observed in that study when the antibody response was measured by AGID, with the mean AGID result observed to remain consistently high, but not increasing throughout gestation. That study showed only a small increase in antibody levels at the time of seroconversion (within one month following experimental inoculation) and no further increase in antibody levels, despite seropositive status in the resulting neonatal calves evidencing the induction of acute infection. However, the present study showed a rapid rise in antibody levels immediately following seroconversion when measured by both Ab ELISA and AGID, with the ELISA results remaining at that high level and the AGID results decreasing slightly after the acute period. Lindberg et al. (2001) also failed to demonstrate a continuing rise in Ab ELISA levels over the course of gestation in females carrying non-PI fetuses, although the time since acute infection in those females was unknown. The continuing increase in Ab ELISA results following acute infection in those heifers carrying non-PI fetuses observed in the present study is more in line with titres rising for at least 10 to 12 weeks post-infection (Brownlie et al., 1987) to reach high antibody levels consistent with serological evidence of 'recent infection' (Lanyon et al., 2013). It is important to note that the heifers in this trial that were carrying non-PI fetuses represent the cohort from which it would be most difficult to

distinguish dams carrying a PI fetus: the highest antibody levels in an animal (other than when carrying a PI fetus) are expected to occur shortly after acute infection (Brownlie et al., 1987). It is at this time that an individual could generate sufficiently high antibody readings to produce a false positive result with regards to identifying a heifer carrying a PI fetus. In particular, a recently seroconverted female carrying a non-PI fetus may be indistinguishable from a female in early- to mid-gestation carrying a PI fetus. Indeed, this was observed to be the case when diagnosis using AGID was attempted with several heifers returning 3+ results several months after seroconversion (and well into their pregnancies) despite carrying non-PI calves. In fact, the results of the present study suggest 3+ AGID results may persist much further than the 1-3 months previously suggested (McGowan and Kirkland, 1991, Kirkland and MacKintosh, 2006). By Ab ELISA, the lower thresholds utilised to achieve DSe in early- to mid-gestation are likely to result in lower DS_p than that observed in the current study. This agrees with observations by Lindberg et al. (2001) that only poor DS_p was achieved earlier in gestation (likely due to this very reason). As such, a high threshold (for example, 1.6 S/P ratio) combined with testing only in the last 12 weeks of gestation may be the most practical application. Any pregnant female returning an antibody result over 1.6 S/P ratio should be treated with suspicion.

In the current study, heifers carrying fetuses with neurological deficits (n=3) tended to have lower Ab ELISA results than heifers carrying non-affected calves, however, this difference was not statistically significant; this is the first report of such a finding and further research in a study with a larger number of animals may add clarity to this finding. It may be hypothesised that the induction of cellular apoptosis in the developing fetus (in turn resulting in neurological deformation) may accelerate the clearance of the virus from the dam-fetus unit. This might reduce the effective duration of viraemia and, in turn, the magnitude of the immune response of the heifer to infection. It should be noted that the heifer in this study that was carrying the fetus with the most severe neurological deficit also had the lowest antibody levels throughout gestation, and could have resulted in an artificially low mean antibody results for this group.

For other diseases of cattle, such as *Neospora caninum*, measurement of antibody avidity has allowed differentiation of recent and chronic exposure to the pathogen (Bjorkman et al., 1999).

A female carrying a PI fetus may be subject to continued immunogenic stimulus due to the excretion of BVDV by the fetus. Hypothetically, this ongoing stimulus could mimic the condition of chronic exposure and, hence, high avidity of specific antibodies. However, this study has shown this not to be the case, with no differences in antibody avidity observed between heifers with different gestational outcomes. Antibody avidity was observed to increase over time, consistent with the immune response maturing following infection (Bjorkman et al., 1999).

Non-PI females carrying PI fetuses, such as those induced in this study, have the potential to introduce BVDV infection into BVDV-free herds. Although the dam herself poses no infectious risk, the birth of the PI calf in a naïve herd could result in an epidemic BVD outbreak with significant financial impact. As such, the simple, accurate detection of females carrying PI fetuses could have important implications for BVDV control and prevention. The results of this study demonstrate that serological antibody levels in the dam as measured by Ab ELISA can be used for the diagnosis of fetal PI, while AGID testing is less successful. For the Ab ELISA, positivity thresholds at different stages of gestation were set such that 100% (95% CI: 30.5 – 100.0%) DSe was achieved (that is, all n=3 heifers carrying PI fetuses returned results above the positivity threshold), as maximum (100%) DSe is crucial to ensure PI fetuses do not go undetected. At the set thresholds, DS_p was maintained at $\geq 70\%$ from approximately week 21 of gestation onwards. By comparison, Stokstad et al. (2003) reported DSe with a 95% confidence interval of 79 – 100% from day 204 (approximately week 29) of gestation onwards, but did not report DS_p. Lindberg et al. (2001) achieved DSe $\geq 90\%$ during the 7th to 9th months (approximately 28 weeks onwards) of gestation, with a maximum corresponding DS_p of 67% (and minimum of 37%). Finally, Brownlie et al. (1998) applied a positivity threshold determined under experimental conditions to a field BVDV outbreak at approximately 180 days (approximately 25 weeks) gestation and achieved an observed DSe and DS_p of 73% and 82%, respectively. The present study applied a threshold for diagnosis earlier in gestation (21 weeks) and achieved higher DS_p than previous studies, without compromising 100% (95% CI: 30.5 – 100.0%) DSe.

In order to maximise DSp, without compromising DSe, the positivity threshold applied in this study increases as gestation progresses. However, the application of a variable threshold is reliant on accurate knowledge of fetal age. This may not always be feasible in practical, on-farm scenarios. A single threshold throughout gestation may be more achievable. A low threshold will maintain DSe but is likely to compromise DSp, particularly in later gestation, while a high threshold may decrease DSe in earlier gestation.

An apparent limitation, in this study, is the failure to reliably detect a heifer that aborted a PI fetus. Adjustment of the positivity thresholds to ensure detection of this heifer would drastically compromise DSp. However, as the non-viable calf aborted by this heifer was PI, it poses an, albeit lessened, infectious risk. Such infectious material may be sufficient to induce acute infection in animals that are in contact with it (and, in turn, produce a live PI calf).

Therefore, while antibody levels may be a valuable indicator of calf status, it would be preferable to utilise a combination of antibody and antigen or virus detection methods for accurate diagnosis of fetal PI. Unfortunately, this study provided no evidence that BVD virus or specific antigen can be detected in maternal serum, swab supernatants or ear notch supernatants by either Ag ELISA or qRT-PCR. If the virus excreted by the PI fetus does pass the placenta and cross into maternal circulation, the present study suggests it may do so at such low levels that it is undetectable by Ag ELISA and qRT-PCR. Fux and Wolf (2013) demonstrated, not only that antigen detection by ELISA suffers from interference by colostrum-derived BVDV-specific antibodies in young PI calves, but that qRT-PCR also suffers a drop in signal in the presence of colostrum-derived antibodies. As such, it may not be entirely unexpected that any low levels of virus in maternal circulation would be masked from detection by the presence of the high levels of BVDV-specific antibodies that have been demonstrated in our heifers. The samples collected in this study were selected with industry relevance (including ease and safety of sample collection, ease of preparation and cost) in mind. Historically, peripheral blood lymphocyte preparations have been utilised for antigen detection by ELISA (Gottschalk et al., 1992) with recent advancements allowing serum to become the preferred sample. Similarly, transport and storage media can be utilised (Fulton et al., 2009) and may help to preserve viral

RNA on swab samples. These methods, while originally ruled out for ease of preparation and cost reasons may present more viable options for antigen detection in future studies on cattle carrying BVDV PI fetuses.

In conclusion, levels of BVDV-specific antibodies are significantly higher in heifers carrying a PI fetus than those carrying a non-PI fetus. This antibody difference may be used to gain an indication of the likelihood of a heifer carrying a PI pre-natally, however, this method has some practical limitations.

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7.2.2 Findings in calves

A secondary outcome of the experimental trial reported in this chapter was the observation of calves born following fetal BVDV infection. The first paper in this section presents the clinical and pathological findings in three calves born during this trial that exhibited varying degrees of neurological deformation. The two papers presented in Chapter 7.3.3.2 inform on the issue of diagnosis of PI in very young calves.

Manuscript: Neurological Deformations in Three Calves Following Fetal Infection with Bovine Viral Diarrhoea Virus at Day 90 of Gestation: Clinical Signs and Post-Mortem Findings

SR Lanyon, PD Cockcroft, MM McAllister, MP Reichel (2014)

Neurological Deformations in Calves Following Fetal Infection with Bovine Viral Diarrhoea Virus at Day 90 of Gestation: Clinical Signs and Post-Mortem Findings

Manuscript in Publication Format

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Neurological deformations in three calves following fetal infection with bovine viral diarrhoea virus (BVDV) at day 90 gestation: clinical signs and post-mortem findings

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Abstract

Three calves were born displaying varying degrees of clinical neurological signs following fetal infection with bovine viral diarrhoea virus (BVDV) at day 90 gestation as part of a larger research trial. All three calves were seropositive for BVDV-specific antibodies at birth (prior to colostrum ingestion) indicative of immunocompetence prior to fetal infection. Clinical signs included recumbency, ataxia, wide-based stance, vision impairment, inability to stand or maintain standing position, weaving motions of the head and a tendency to low head carriage. Post-mortem examination revealed hydrocephalus and cerebellar hypoplasia, consistent with previous reports of congenital BVDV infection. Video footage is included as supplementary files to this report.

Keywords Hydrocephalus; Cerebellar atrophy; Ataxia; *in utero* infection

Introduction

Bovine viral diarrhoea (BVD), caused by a Pestivirus (BVDV) of the family Flaviviridae (Collett et al., 1988), is one of the world's most prevalent and economically important diseases of cattle. While the majority of losses originate from immunosuppression (Brackenbury et al., 2003) and reproductive disease (Grooms, 2004), neurological deformations in newborn calves

are among the most dramatic aspects of the disease's clinical manifestations. Neurological deformities, characterised by hydrocephalus and cerebellar hypoplasia and resulting in ataxia, recumbency and/or blindness, are well characterised (Allen, 1977, Trautwein et al., 1986). These deformities are generally accepted to occur as a result of fetal infection between days 80 and 150 days of gestation (Lanyon et al., 2013b). When the fetal infection occurs prior to the development of immunocompetence (approximately 120 days gestation), the calf may be born persistently infected (PI) with BVDV. However, more commonly, these infections occur after fetal immunocompetence develops and the neurologically affected calves are seropositive for BVDV specific antibodies prior to the ingestion of colostrum (Grooms, 2004).

This paper reports the clinical and pathological observations of three calves with varying degrees of neurological deformation, born during a larger research trial in which the dams were naturally infected with BVDV Type 1c under experimental conditions by exposure to a PI individual between days 90 and 118 of gestation. Included supplementary to this report is video footage of two of these affected calves.

Methods

Three neurologically affected calves were born following natural infection of their dams by exposure to a BVDV PI cow between days 90 and 118 of gestation as part of a larger research trial. Exposure was conducted under experimental conditions by co-housing of the dams and the PI cow at a density of 24 m²/animals, with single shared feed and water sources. Serum was collected from each calf within 8 hours of birth, prior to colostrum ingestion. The most severely neurologically affected calf (No Tag) was euthanased by intravenous barbiturate on the day of birth, while calves #06 and #09 were euthanased at completion of the trial at approximately four months of age.

Serum from each calf was tested for antibodies specific to BVDV by antibody ELISA (IDEXX BVDV Total Ab ELISA, IDEXX Laboratories Inc. Rydalmere, NSW), following the manufacturer's recommended serum testing protocol. Results were expressed as a sample-to-

positive (S/P) ratio, with an S/P ratio >0.3 considered positive as per manufacturer's recommendation and previous validation (Lanyon et al., 2013a).

Sera were also tested by ELISA for BVDV antigen (IDEXX BVDV Serum/Ag Plus ELISA, IDEXX Laboratories Inc. Rydalmere, NSW), following the manufacturer's protocol. Results were expressed as a corrected optical density (OD) with a corrected OD >0.3 considered positive, as per manufacturer's recommendation.

Results and Discussion

All three calves tested positive for BVDV-specific antibodies and negative for specific antigen by ELISA on serum collected prior to colostrum ingestion.

The most severely affected (No Tag) was laterally recumbent at birth (Figure 1). At intervals, the calf displayed with ataxic movement of the head towards the shoulder and paddling movement of the forelimbs. The calf displayed visible fasciculation, a severely weakened suckle reflex, and an ability to bellow weakly (see video in supplementary material).

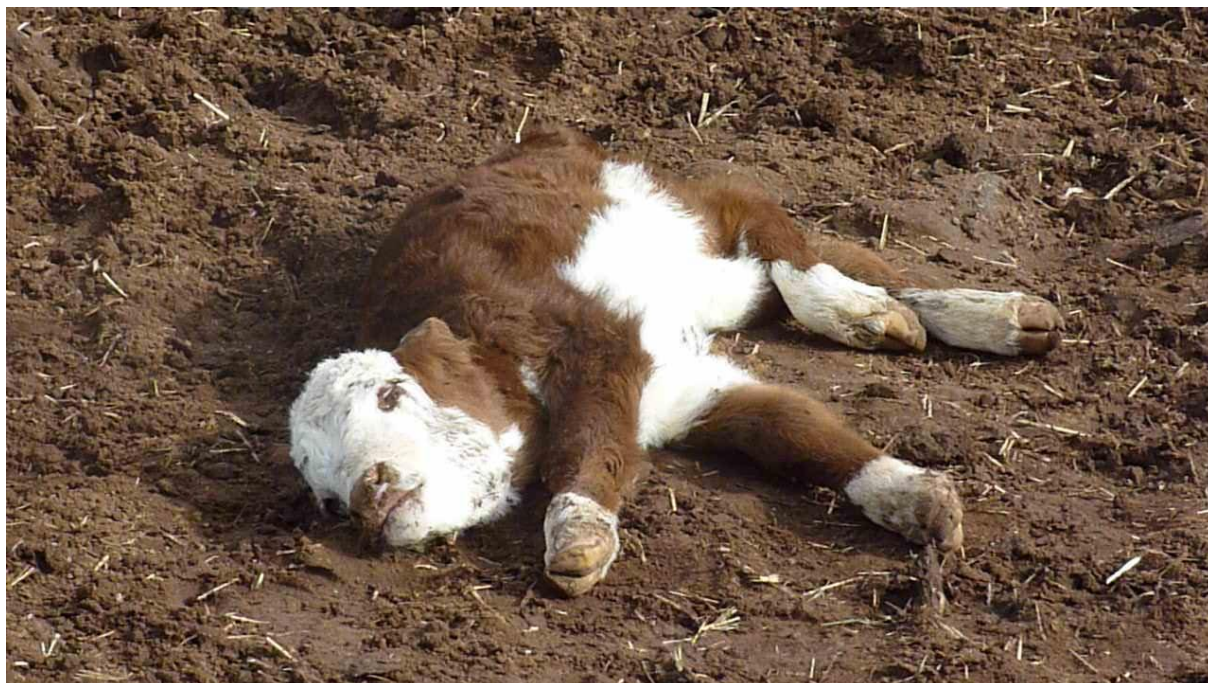


Figure 1. Laterally recumbent calf (No Tag), born with severe clinical neurological signs following fetal infection with bovine viral diarrhoea virus (BVDV) at day 90 gestation. Calf was euthanized on day of birth.

The next most severely affected calf (#06) was in a sitting position and appeared bright and alert at birth. This calf did not make independent attempts to rise but would attempt to rise with human assistance. Despite human assistance the calf was unable to achieve a standing position. The dam was milked and the calf was bottle fed colostrum within 6 hours of birth. The calf remained unable to rise despite human intervention for several days over which time it was bottle fed commercial milk replacer. Over this time, the calf generally sat with its head resting on the ground, intermittently lifting and weaving the head from side to side (often in response to stimuli), before letting the head fall and pound on the floor. With human aid, the calf succeeded in learning to rise and walk independently by 14 days of age and subsequently suckled from the dam, with supplementary milk replacer. Ataxia and wide-based stance was evident throughout life (see video in supplementary material). The calf tended towards low head carriage and horizontal weaving movements of the head. This calf was also apparently substantially vision impaired with a green reflective appearance to the eyes under sunlight. The calf was apparently unable to see obstacles. The calf was never observed to attempt to 'play' as the non-affected calves in the cohort would, but would follow humans around the paddock at a fast walking pace, apparently able to focus visually on (and 'lock on' to) the human form, particularly when moving. When pressed to run, the calf would generally fall into lateral recumbency.

The least severely affected calf (#09) also appeared bright and alert at birth and was sitting upright, but was unable to rise independently. The calf could briefly independently maintain an upright position, after rising with human assistance. The calf suckled within eight hours of birth with human support and was independently mobile within 24 hours of birth. Ataxia and wide based stance was evident throughout life, along with a tendency toward low head carriage (Figure 2). At >3 months of age, this calf was observed to attempt to 'play': the calf would run and buck with the non-affected calves in the paddock, although failing to maintain the speed and coordination of the other calves. Frequently, attempts at bucking would end in the calf falling laterally, but rising quickly. The calf was able to maintain a run without falling.



Figure 2. Calf #09, born with clinical neurological signs following fetal infection with bovine viral diarrhoea virus (BVDV) at 90 days gestation, showing low head carriage and wide-based stance at approximately 2 months of age.

Upon post-mortem, all three calves showed severe neurological malformations characterised by hydrocephalus and cerebellar hypoplasia (or atrophy). The most severely affected calf (No Tag) that was euthanased at birth was observed to have multiple abnormalities (Figure 3): the occipital lobes of the cerebral cortex were absent; the lateral ventricles were dilated and opened caudally into the cranial vault; the remaining cerebral cortex was thinned; and, the cerebellum was completely absent. The resultant diagnosis was: severe congenital cephalic dysplasia, characterised by bilateral hydranencephaly of the occipital cortices, hypoplasia of the remaining cerebral cortex, and cerebellar aplasia (or complete atrophy). Calf #06 was observed to have a hypoplastic left testicle located in the inguinal region, hydrocephalus with markedly dilated ventricles and cerebellar hypoplasia (Figure 4). Finally, calf #09 was observed to have hydrocephalus with bilateral ventricular dilation resulting in collapse of above tissue, and marked cerebellar hypoplasia (Figure 5).

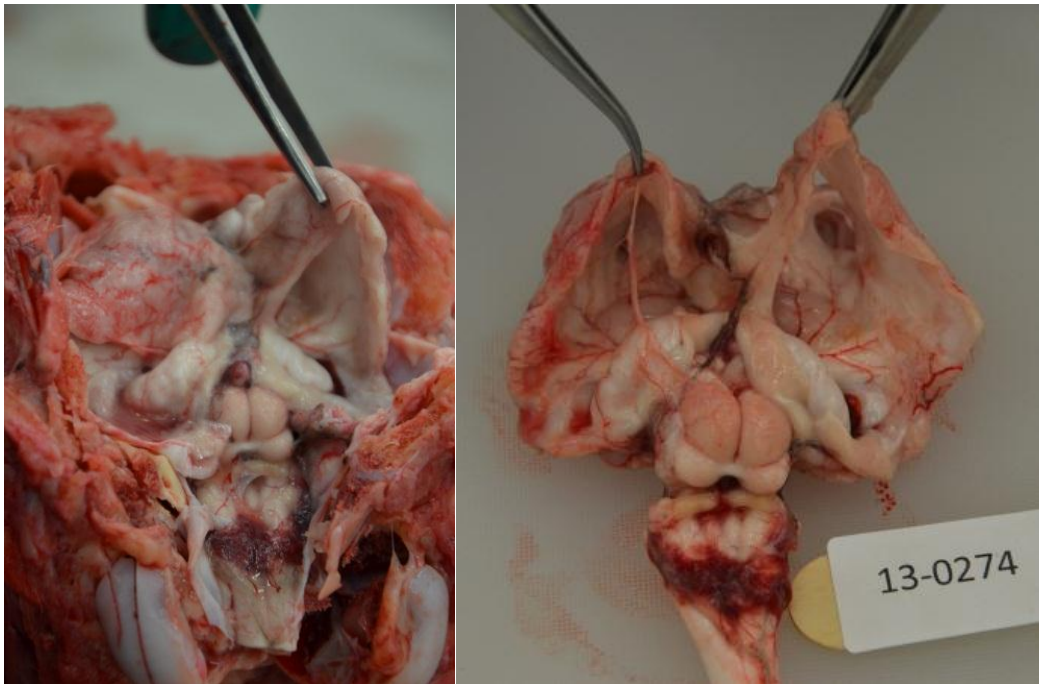


Figure 3. Cerebellar hypoplasia and hydrocephalus in calf (No Tag) following foetal infection with bovine viral diarrhoea virus (BVDV) at approximately 90 days gestation. Calf was laterally recumbent with ataxic movement in head and forelimbs, tremors and weakened suckling reflex; euthanased on day of birth.



Figure 4. Cerebellar hypoplasia and hydrocephalus in calf #06 following foetal infection with bovine viral diarrhoea virus (BVDV) at approximately 90 days gestation. Calf was bright and alert at birth but unable to rise despite human intervention, began walking at approximately 10 days of age and displayed ataxia and wide-based stance.

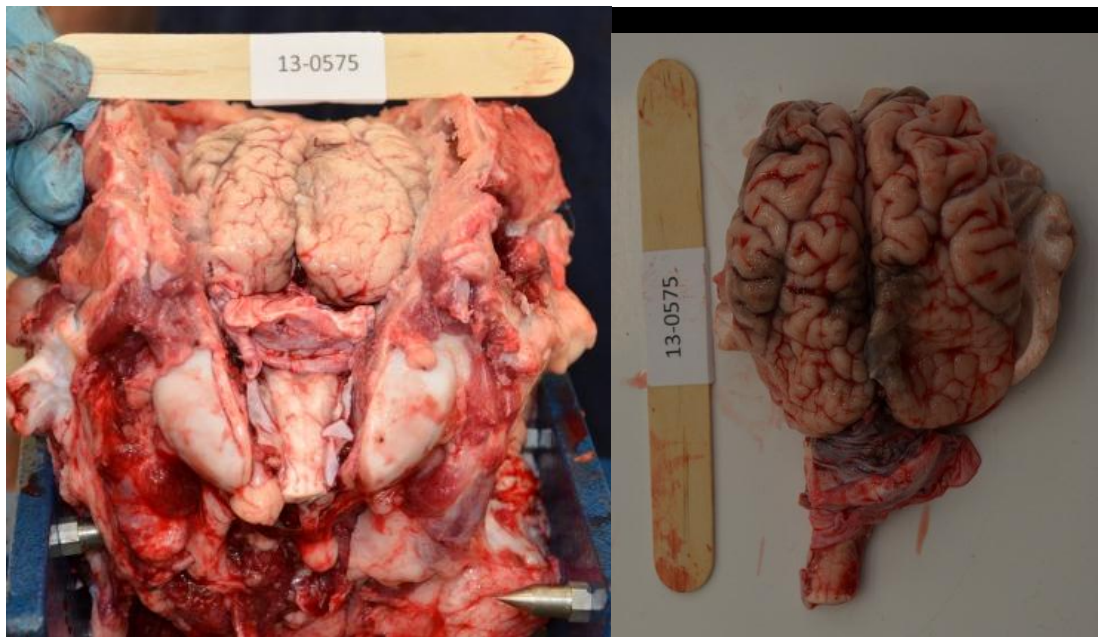


Figure 5. Cerebellar hypoplasia and hydrocephalus in calf #09 following foetal infection with bovine viral diarrhoea virus (BVDV) at approximately 90 days gestation. Calf was bright and alert at birth but unable to rise without human intervention. Calf suckled with human assistance and was independently mobile within 24 hours of birth, displaying ataxia and wide-based stance.

The clinical signs observed in the affected calves in this case are consistent with previous reports of neurological deformations following foetal infection with BVDV (Allen, 1977, Trautwein et al., 1986, Otter et al., 2009). All the signs observed here have been previously reported and associated with BVDV infection, including: recumbency, blindness, ataxia (Allen, 1977, Trautwein et al., 1986, Otter et al., 2009), wide-based stance, rhythmic, weaving movements of the head, inability to stand (Allen, 1977, Trautwein et al., 1986) and inability to maintain standing position (Trautwein et al., 1986), tremor (Otter et al., 2009), low head carriage, loss of balance and falling when pressed to run, an ability to bellow, and thrashing, paddling movement of the legs and throwing of the head across the body in recumbent calves (Allen, 1977). The calves can otherwise be conscious, bright and alert (Trautwein et al., 1986, Otter et al., 2009). As is observed here, cases are rarely consistent, with clinical signs varying from calf to calf, within and between studies.

The clinical signs observed correspond to neurological malformations. Hydrocephalus, hydranencephaly and cerebellar hypoplasia, in particular, are known to be associated with fetal BVDV infection and clinical neurological signs (Allen, 1977, Trautwein et al., 1986). The

timing of infection in the present case (day 90) was consistent with the infection in Trautwein et al.'s (1986) study (days 90 to 118) that observed similar neurological outcomes. In that study, virus was isolated from one of five clinically affected calves, and two pathologically but not clinically affected calves. This is in agreement with the observation in the present study that all three calves appeared to be serologically free of BVDV antigen and positive for antibody, suggesting that clinically neurologically affected calves are likely to result from infection following the development of immunocompetence, and hence be seropositive, not PI. However, virus was isolated or viral antigen demonstrated in the majority (23 of 31) clinically affected calves tested by Otter et al. (2009). The clinical signs in several of the calves reported in that study were more mild than those observed here and by Trautwein et al. (1986), with tremor being the predominant clinical observation. As the time of fetal infection was unknown in that study, those calves could have been subject to fetal infection earlier in the period of central nervous system organogenesis than the calves in the present study at that of Trautwein et al. (1986) (and therefore, prior to immunocompetence), resulting in PI calves with more mild neurological abnormalities, rather than seropositive calves with severe neurological presentation.

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Trautwein, G, Hewicker, M, Liess, B, Orban, S & Grunert, E 1986. Studies on transplacental transmissibility of a bovine virus diarrhoea (BVD) vaccine virus in cattle. iii. Occurrence of central nervous system malformations in calves born from vaccinated cows. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*, 33, 260-268.

7.2.2.a Characterising and overcoming the effect of interference by colostrum-derived immunoglobulins on diagnosis of persistent infection in young, colostrum-fed calves

The two papers presented in this section present data collected from ten calves that were born during the experimental trial reported in this chapter. The first paper reports the findings in serum, ear notch and swab samples collected from the calves over the first twelve weeks of life, and details the observed interference with antigen detection following colostrum ingestion. The second manuscript reports a new methodology for treating serum samples from young calves prior to antigen testing with the aim of removing colostrum-derived antibodies from the sample and improving the diagnostic sensitivity of PI diagnosis. This manuscript has been prepared ready for submission to The Veterinary Journal and is awaiting submission of a provisional patent prior to submission.

Original Article: Bovine Viral Diarrhoea Virus (BVDV) Detection in Persistently Infected (PI) Calves and their Non-PI Herdmates: Findings in Serum, Ear Notch and Swab Supernatants

SR Lanyon, S Manning, PD Cockcroft, MP Reichel (2014)

Bovine Viral Diarrhoea Virus (BVDV) Detection in Persistently Infected (PI) Calves and their Non-PI Herdmates: Findings in Serum, Ear Notch and Swab Supernatants

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By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Original Article: Pre-treatment of serum samples to reduce interference of colostrum-derived specific antibodies with detection of bovine viral diarrhoea virus (BVDV) antigen by ELISA in young calves

SR Lanyon, MP Reichel (2014)

Pre-treatment of serum samples to reduce interference of colostrum-derived specific antibodies with detection of bovine viral diarrhoea virus (BVDV) antigen by ELISA in young calves

The Veterinary Journal

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Contribution to the Paper	Helped interpret data and draft and edit manuscript.		
Signature		Date	27/8/14

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Pre-treatment of serum samples to reduce interference of colostrum-derived specific antibodies with detection of bovine viral diarrhoea virus antigen by ELISA in young calves

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Abstract

The antigen ELISA is the preferred method for the diagnosis of persistently bovine viral diarrhoea virus infected (BVDV PI) individuals, however, colostrum-derived antibodies may interfere with antigen detection in young PI calves. This study aimed to assess serum pre-treatment methods for reducing such interference. Dilution series showed that antibody levels equivalent to those observed in colostrum-fed calves were able to eliminate all antigen signals in a serum sample. When serum was treated with EDTA at pHs 4.5, 5.5, 6.5 and 7.5, boiled, centrifuged and the supernatant recovered, BVD antibody was undetectable. Antigen signal recovery in excess of 90% was achieved when pH was 5 (+/- 0.5). When applied to samples from three PI calves (which were negative in the antigen-capture ELISA without treatment), the antigen signal improved and gave a positive result in each case. This may provide a major improvement in the diagnosis of young PI calves.

Keywords Colostrum-derived antibody; Diagnostic gap; Heat; Treatment; Pestivirus

Body Text

Accurate diagnostic testing allows for control and mitigation of losses associated with bovine viral diarrhoea virus (BVDV) through the identification and eradication of persistently infected (PI) cattle. The widely used antigen ELISA has one limitation: colostrum-derived specific antibodies may interfere with antigen detection in very young calves (Fux and Wolf, 2012, Lanyon et al., 2014). Reduction of this interference (and elimination of the ‘diagnostic gap’) would be of benefit to control and mitigation efforts. Therefore, this study aimed to assess serum pre-treatment methods to increase the signal in the antigen ELISA in young, colostrum-fed calves.

Serum collected from a cow previously confirmed as PI with BVDV was serially diluted in either sample diluent (IDEXX Laboratories Inc.) or pooled serum from 17 antibody positive cows, resulting in samples ranging from neat PI serum to neat diluent or pooled antibody positive serum. All dilutions were tested for the presence of BVDV-specific antigen and antibody by ELISA, according to the manufacturer’s instructions (BVDV Total Ab ELISA; BVDV Serum/Ag Plus ELISA; IDEXX Laboratories Inc.) with results expressed as sample-to-positive (S/P) ratios or corrected optical densities (OD), respectively.

When diluted in sample diluent, the PI serum was diluted 1:127 (<1% PI serum) before producing a negative antigen result (Fig 1), while dilution in pooled antibody positive serum resulted in a negative corrected OD from a dilution of 3:1 (75% PI serum) onwards. This dilution series clearly demonstrates that the presence of BVDV-specific antibodies in a serum sample can eliminate the antigen detection signal when the same sample is tested by antigen ELISA. Antibody titres up to $10^{4.8}$ have been observed in young PI calves following colostrum ingestion (Fux and Wolf, 2012), which can produce antibody ELISA results as high as an S/P ratio of two in the first week of life (Lanyon et al., 2014). Equivalent antibody levels are shown here to be sufficient to eliminate a corrected OD > 3. Very low antibody levels (S/P ratio 0.3 to 0.5) were observed to produce a three-fold decrease in antigen signal. From the dilution series, a sample consisting of 50% pooled antibody positive cow serum and 50% PI cow serum was

identified as producing test results that mimic those observed in young, colostrum-fed PI calves and, therefore, this 50/50 mixture was subsequently used as the experimental sample (Table 1).

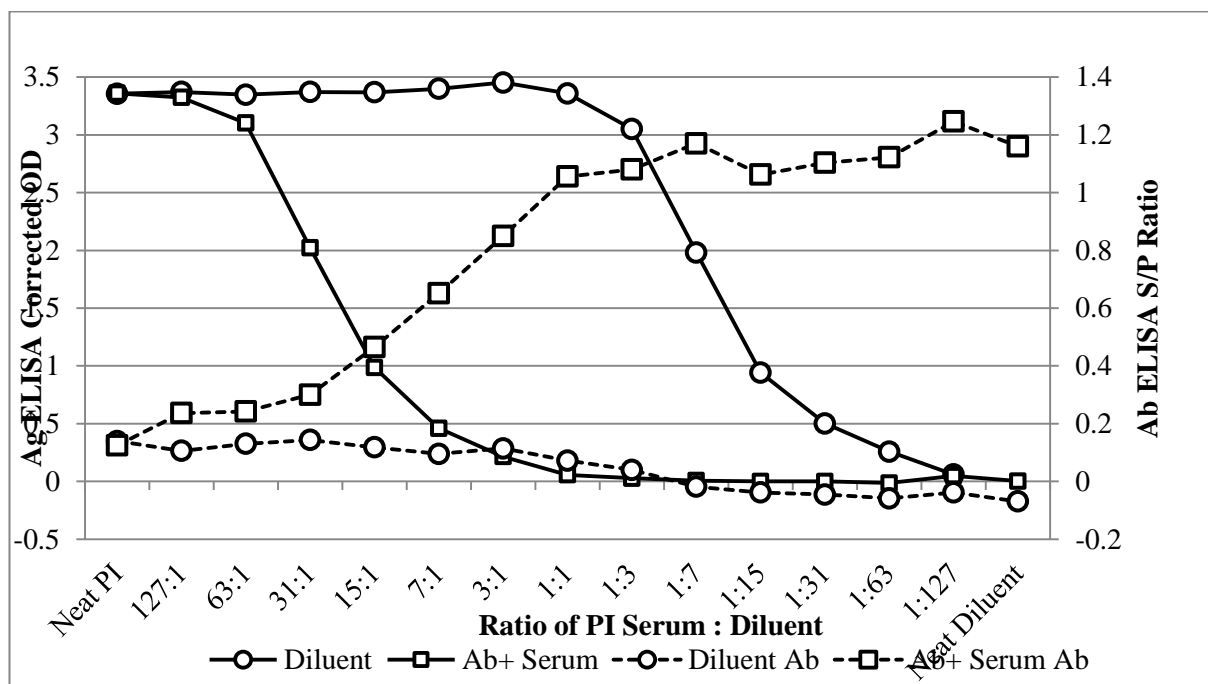


Figure 1. The levels of bovine viral diarrhoea virus (BVDV) specific antibodies (Ab)(dotted lines) and antigen (Ag)(solid lines) as measured by ELISA (IDEXX BVDV Total Ab ELISA; IDEXX BVDV Serum/Ag Plus ELISA; IDEXX Laboratories Inc., Rydalmere, NSW) in serum from a persistently BVDV infected (PI) cow when serially diluted in either sample diluent (IDEXX Laboratories Inc., Rydalmere, NSW) (circles) or pooled serum from seventeen antibody positive cows previously infected with BVDV under experimental conditions (squares). Ag ELISA results are expressed as corrected optical density (OD). Ab ELISA results are expressed as sample-to-positive (S/P) ratio.

Table 1. Composition of three samples experimentally created to mimic the bovine viral diarrhoea virus (BVDV) specific antigen and antibody content of serum collected under varying biological situations.

Sample ID	Biological Equivalent	Sample Composition	Antibody Content	Antigen Content
Negative Control	Non-PI individual	100% Pooled Antibody Positive Serum	Positive	Negative
Experimental Sample	PI calf following colostrum ingestion	50% PI Serum; 50% Pooled Antibody Positive Serum	Positive	Positive
Positive Control	Adult PI individual	50% PI Serum; 50% Sample Diluent	Negative	Positive

Sample treatment methods were adapted from More and Copeman (1991). An aliquot of 100 μ L of each sample was treated by: a) boiling for 7 min, or; b) addition of an equal volume (100 μ L) of 0.1 M Na₂EDTA (Sigma-Aldrich Co.), pH 4.5, 5.5, 6.5 and 7.5 (+/- 0.1) followed by boiling for 7 min. All treated samples were then centrifuged at 16,000 g for 7 min and supernatant recovered. All supernatants, along with the untreated samples, were tested for BVDV specific-antigen by ELISA (IDEXX BVDV Ag/Serum Plus; IDEXX Laboratories Inc.). The treated and untreated negative control was also tested for BVDV specific-antibodies by ELISA (IDEXX BVDV Total Ab; IDEXX Laboratories Inc.). Signal recovery was calculated as:

$$\frac{\text{Signal recovered by treatment}}{\text{Signal lost by addition of antibodies}} = \frac{(\text{Corrected OD treated experimental sample}) - (\text{Corrected OD untreated experimental sample})}{(\text{Corrected OD untreated positive control}) - (\text{Corrected OD untreated experimental sample})}$$

All sample treatments produced an increase in antigen signal in the experimental sample and a decrease in signal in the positive control (Fig 2). The negative control sample tested negative for antigen (corrected OD <0.1) regardless of treatment, and tested positive for antibodies when untreated (S/P ratio = 1.4) but negative for antibodies (S/P ratio <0.1) after any treatment suggesting that the specific antibodies were successfully removed from the samples. Treatment with 0.1 M Na₂EDTA pH 4.5 or 5.5 resulted in the highest signal recovery of 95% and 93% respectively, while resulting in only small decreases in the signal of the positive control. As such, treatment with EDTA pH 5 (+/- 0.5) was applied to serum samples collected from three PI and seven non-PI calves every 2 d from the day of birth until 14 d of age, and weekly until 5 weeks of age as part of a previous research trial (Lanyon et al., 2014; University of Adelaide Animal Ethics Committee project S-2012-087, approved 4 July 2012).

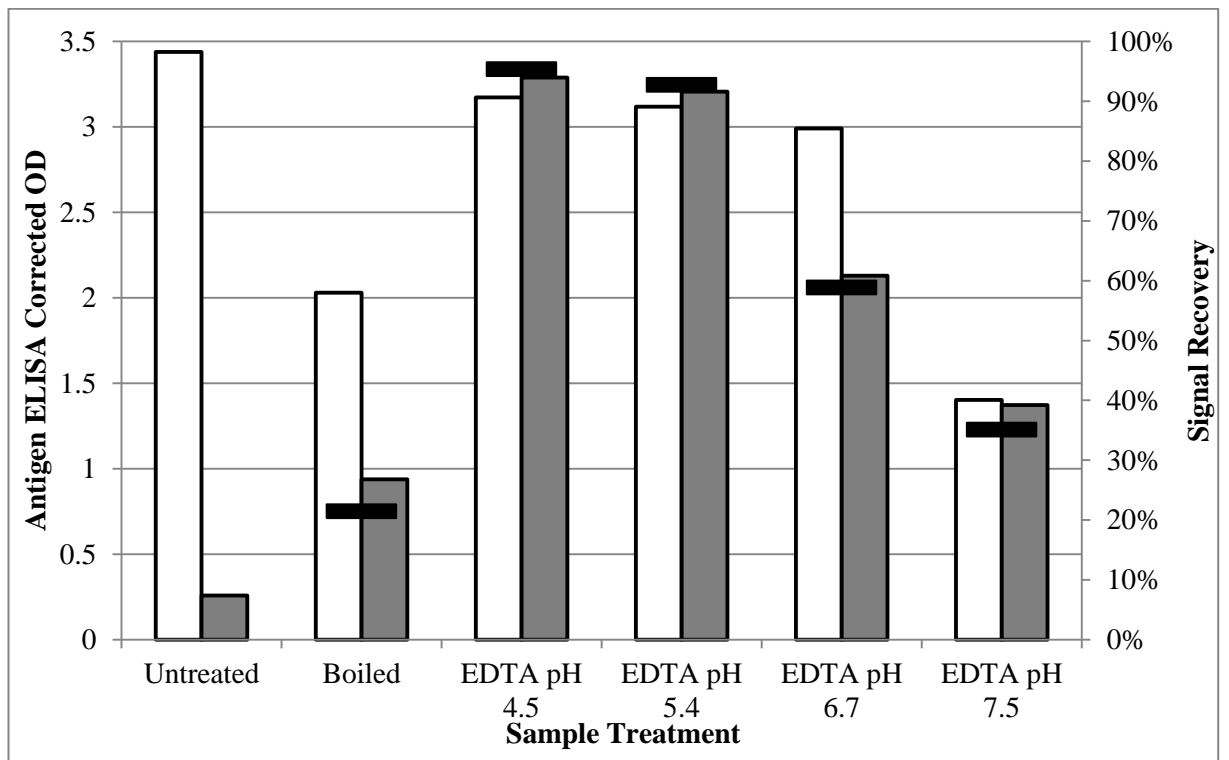


Figure 2. The detectable levels of bovine viral diarrhoea virus (BVDV) specific antigen (Ag) as measured by ELISA (IDEXX BVDV Serum/Ag Plus ELISA; IDEXX Laboratories Inc., Rydalmere, NSW) and expressed as a corrected optical density (OD) in positive control (white) and experimental sample (grey), and the signal recovery (black) when treated: a) by boiling for 7 minutes, or b) by addition of an equal volume of 0.1M Na₂EDTA (Sigma-Aldrich Co., Castle Hill, NSW) at pH of 4.5, 5.5, 6.5 or 7.5 prior to boiling for 7 minutes. All samples were centrifuged and supernatant recovered and tested.

On untreated serum samples from PI calves, the antigen ELISA returned a negative result until between 6 d and 4 weeks of age (Fig 3). Following treatment, all three PI calves returned a strong positive antigen ELISA result (corrected OD > 1.8) at all timepoints, providing evidence that pre-treatment of serum samples can eliminate interference by colostrum-derived antibodies and improve the sensitivity of detection of PI calves.

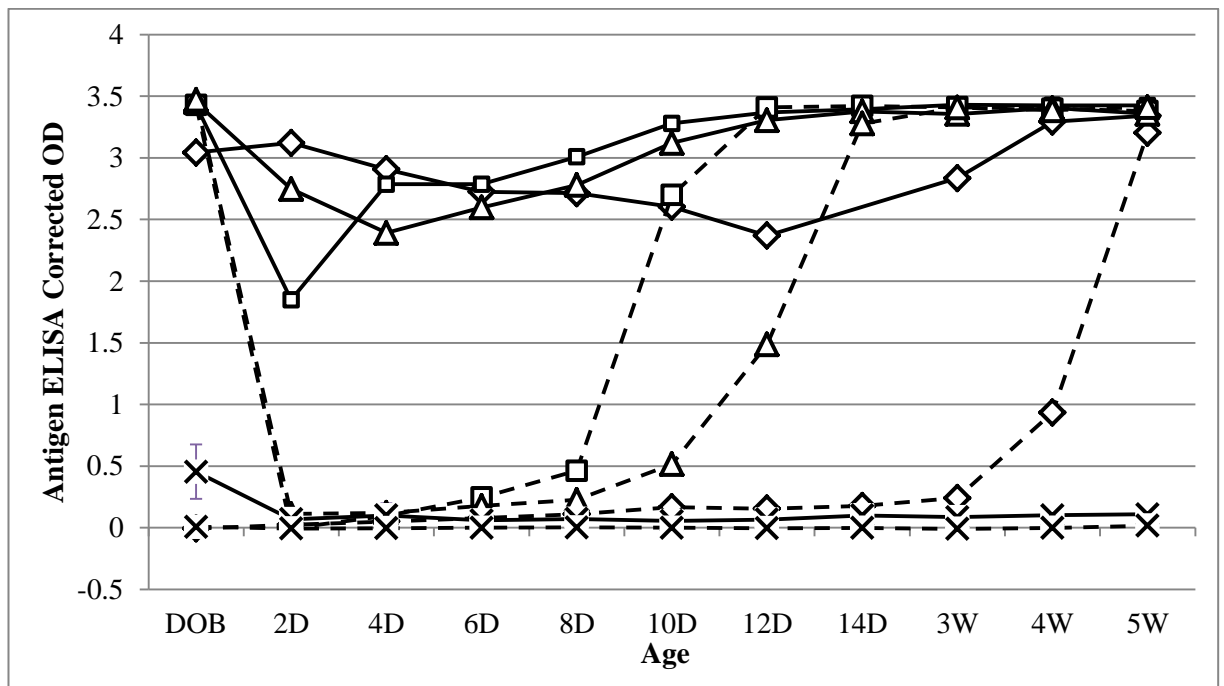


Figure 3. The detectable levels of bovine viral diarrhoea virus (BVDV) specific antigen as measured by ELISA (IDEXX BVDV Serum/Ag Plus ELISA; IDEXX Laboratories Inc., Rydalmere, NSW) in serum from three colostrum-fed persistently BVDV infected (PI) calves (#03 ◇, #07 □ and #11 △) and the mean levels in seven colostrum-fed non-PI calves (X) from day of birth (DOB) to 5 weeks of age (5W) when untreated (dotted lines) or treated by addition of an equal volume of 0.1M Na₂EDTA (Sigma-Aldrich Co., Castle Hill, NSW) at pH 5 (+/- 0.5) prior to boiling for 7 minutes, centrifugation at 16,000 RCF for 7 minutes and recovery of the supernatant for testing (solid lines). Error bars indicate 95% confidence intervals.

When untreated, all ($n=7$) non-PI calves returned negative antigen ELISA results at all timepoints (Fig 3). When treated, 69/70 (98.6%) of samples from non-PI calves returned negative antigen ELISA results at 2 d or more of age. On the day of birth, 4/7 non-PI calves returned positive antigen results (corrected OD >0.3) on the day of birth with the corrected OD ranging from 0.37 to 0.91. This may be associated with the in utero BVDV infection that these particular calves underwent. An adjustment in the positivity threshold may be appropriate for use on treated samples.

In conclusion, the antibody interference with the antigen ELISA in serum from colostrum fed PI calves can be substantially reduced by pre-treating the serum sample using the method described. This method requires further validation but may provide an additional and important

tool in the early diagnosis of PI calves using serum sample and represents a significant advance in the field of BVDV diagnosis.

Acknowledgements

The authors acknowledge Meat and Livestock Australia (MLA) for funding the larger trial from which the calf serum samples utilised in this project were sourced. Thanks also to IDEXX Laboratories Inc. for providing all ELISA kits used in this project. Neither MLA nor IDEXX had any role in the study design, the collection, analysis and interpretation of data, the writing of the manuscript or the decision to submit the manuscript for publication. Preliminary results were presented as an abstract at the XXVIII World Buiatrics Congress, Cairns, 27th July to 1st August 2014.

Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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8 Discussion and Conclusions

The papers presented in this thesis demonstrate that while BVD has significant impacts in affected cattle populations, the tools exist to reduce the prevalence of infection. Indeed, control programs in several European countries have had good success in reaching this goal and have been observed to be economically beneficial.

The prevalence of BVD in Australia is still apparently high, with evidence of exposure present in all states and in the majority of cattle herds. Some of the unique aspects of Australia's cattle industries, including scale, uncontrolled mating and extensive management practices may pose challenges to effective BVD control in Australia. However, Australia also has the necessary expertise, infrastructure and access to the necessary tools to overcome such challenges. As identified in other countries, systematic control efforts require high levels of stakeholder awareness and compliance to ensure success.

Education is acknowledged as a crucial component of any control scheme, and a control program in Australia would likely require a hand-in-hand education program. However, before an education program could be implemented, an understanding of the current awareness and attitudes of BVD amongst stakeholders would be beneficial. The results of a postal survey, presented in Chapter 6.2, revealed that while overall knowledge of BVD is low, interest in the disease is high amongst South Australian cattle farmers. The survey also identified demographics of farmers which would most benefit from participation in an education program, and would allow such a program to be tailored and targeted with these individuals in mind. Similar surveys of cattle farmers in other states and of other stakeholders, such as veterinarians and livestock carriers, would be of benefit to provide a broader picture of the current attitudes and awareness within the cattle industry as a whole.

Generally, the tests available for the diagnosis of BVD are quite accurate, and an appropriate understanding of the pathogenesis of the disease allows the selection of the right test for a particular diagnostic goal. However, a few diagnostic challenges do still remain in the approach to BVD:

the need for cost-efficiency is an ongoing challenge, particularly in light of the large number of animals that may need to be tested during a control scheme. For this reason, the exploration, validation and interpretation of bulk testing methods presented in Chapter 7.2 is a vital contribution to the feasibility of BVD control. Furthermore, the challenge of diagnosis of unborn and very young PI individuals adds an unnecessary cost and logistical difficulty to BVD control. At present, control programs rely on follow-up testing around 12 months after initial testing to identify PI calves that were in utero at the time of initial testing. In addition, special testing protocols are necessary to identify colostrum-fed PI calves, with the favoured antigen ELISA subject to interference from colostrum-derived BVDV-specific antibodies. The experimental trial presented in Chapter 7.3 informed on these diagnostic issues. While the results showed that antibody levels in a female during pregnancy may provide some indication of the BVDV status of the fetus, the accuracy of diagnosis using antibody levels still leaves some room for improvement. Further studies may focus on more invasive, expensive and/or preparation intensive samples and techniques in the hope of detecting BVD virus circulating in the ‘Trojan’ dam. Previous limitations of the ELISA for detection of BVDV antigen in serum from young, colostrum-fed PI calves were also observed in the present study (Chapter 7.3.3.2). However, these limitations were overcome by treatment of the serum samples prior to ELISA testing. While wider validation of this method will be necessary prior to commercial uptake, the simple, rapid and inexpensive nature of this sample treatment represents a significant advance in BVD diagnosis. Finally, the report in Chapter 7.3.3.1 of three calves with neurological deficits demonstrates more severe lesions than those generally associated with BVDV, and is a timely reminder that the effects of BVD infection can be dramatic and unexpected. While the birth of calves showing severe clinical neurological signs is one of the more noticeable aspects of BVD, it represents only a small component of the disease’s true impact. In conclusion, the research presented in this thesis contributes towards the feasibility of BVD control in Australia and around the world, providing validation of bulk testing methods, a novel method for testing of PI calves and evidence of the high level of interest in BVD within the South Australian cattle industry.

9 Appendix 1: Supporting Publications – Published Papers

The following publications support the main body of work presented in this thesis. The first paper, by Nasir et al., reports the findings of a serological survey of South Australian cattle which, although having been undertaken during the course of the PhD, does not relate directly to the main body of work. The second paper, published in Australian Veterinary Journal, although published during the timeframe of the PhD, is the product of work undertaken prior to the PhD and submitted for award of an Honours degree. It appears here as a supporting publication.

Original Article: Seroprevalence of Neospora caninum and Besnoitia besnoiti in South Australian Beef and Dairy Cattle

A Nasir, SR Lanyon, G Schares, ML Anderson, MP Reichel (2012)

Seroprevalence of Neospora caninum and Besnoitia besnoiti in South Australian Beef and Dairy Cattle

Veterinary Parasitology

Vol. 186, Pp. 480 - 485

Nasir, A., Lanyon, S.R., Schares, G., Anderson, M.L. & Reichel, M.P. (2012) Sero-prevalence of *Neospora caninum* and *Besnoitia besnoiti* in South Australian beef and dairy cattle. *Veterinary Parasitology*, v. 186(3-4), pp. 480-485

NOTE:

This publication is included on pages 169-174 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://doi.org/10.1016/j.vetpar.2011.11.032>

Original Article: Validation and Evaluation of a Commercially Available ELISA for the Detection of Antibodies Specific to Bovine Viral Diarrhoea Virus (Bovine Pestivirus)

SR Lanyon, ML Anderson, E Bergman, MP Reichel (2013)

Validation and Evaluation of a Commercially Available ELISA for the Detection of Antibodies Specific to Bovine Viral Diarrhoea (Bovine Pestivirus)

Australian Veterinary Journal Vol. 91, Pp. 52 - 56

Lanyon, S.R., Anderson, M.L., Bergman, E. & Reichel, M.P. (2013) Validation and evaluation of a commercially available ELISA for the detection of antibodies specific to bovine viral diarrhoea virus (bovine pestivirus).

Australian Veterinary Journal, v. 91(1-2), pp. 52-56

NOTE:

This publication is included on pages 176-180 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://doi.org/10.1111/j.1751-0813.2012.01010.x>

10 Appendix 2: Supporting Publications – Conference Papers

The following supporting publications are conference contributions presenting work undertaken during the PhD or the preceding Honours degree (marked by an asterisk) at a variety of both international and domestic scientific meetings. These meetings include the 26th, 27th and 28th World Buiatrics Congresses in Santiago, Chile, 2010 and Lisbon, Portugal, 2012 and Cairns, Australia 2014, respectively, and the World Association of Veterinary Laboratory Diagnosticians Symposium, Berlin, Germany, 2013. Domestically, contributions to the Australian Veterinary Association Annual Conferences in Adelaide, 2011 and Canberra, 2012 along with papers presented at the Australian Association of Veterinary Laboratory Diagnosticians Meetings in Brisbane, 2010 and Geelong, 2013 are listed here.

10.1 Oral Conference Presentations

SR Lanyon, MP Reichel (2014) Bovine viral diarrhoea (“Bovine Pestivirus”) in Australia: To control or not to control? SA Rural Veterinary Practitioners Seminar Robe, South Australia September 20 – 21 2014

SR Lanyon, PD Cockcroft, MP Reichel (2014) Diagnostic opportunities in the ‘Trojan cow’ and her persistently bovine viral diarrhoea virus (BVDV) infected calf XXVIII World Buiatric Congress Cairns, Australia July 27 – August 1 2014

CA Evans, SR Lanyon, SK Manning, MP Reichel (2014) Reproductive performance in pregnant ewes experimentally infected with BVDV and transmission rates in sheep co-mingled with BVDV PI calves XXVIII World Buiatric Congress Cairns, Australia July 27 – August 1 2014

SR Lanyon, ML Anderson, MP Reichel (2014) Identifying Champions: farmer attitudes to endemic disease management in South Australia, with a focus on Bovine Viral Diarrhoea (Bovine Pestivirus) Australian and New Zealand College of Veterinary Scientists Science Week Gold Coast, Queensland July 10 – 12 2014

SR Lanyon, PD Cockcroft, MP Reichel (2013) Antibody levels in heifers following acute infection with bovine viral diarrhoea virus (BVDV) during early gestation Ninth Annual Meeting of the Australian Association of Veterinary Laboratory Diagnosticians Geelong, Victoria November 28-29, 2013

CA Evans, SR Lanyon, MP Reichel (2013) Bovine viral diarrhoea virus in Australian sheep flocks: effect on reproductive performance and potential for cross-species transmission Ninth Annual Meeting of the Australian Association of Veterinary Laboratory Diagnosticians Geelong, Victoria November 28-29, 2013

SR Lanyon, PD Cockcroft, MP Reichel (2013) Diagnosing persistently Bovine Viral Diarrhoea Virus (BVDV) infected foeti by detection of BVD virus or viral antigen in the (“Trojan”) dam World Association of Veterinary Laboratory Diagnosticians Symposium Berlin, Germany June 5-8, 2013

*SR Lanyon, J Rogers, A Kessell, MP Reichel (2012) Economic analysis of an acute outbreak of bovine viral diarrhoea virus (BVDV) in a South Australian dairy herd - a case study XXVII World Buiatrics Congress Lisbon, Portugal June 3-7, 2012

SR Lanyon, ML Anderson, MP Reichel (2012) Farmer attitudes towards preventative health care - a South Australian perspective Australian Veterinary Association Annual Conference Canberra, Australian Capital Territory May 20-25, 2012

MP Reichel, A Nasir, SR Lanyon, ML Anderson (2011) Neospora caninum and Besnoitia besnoiti infection in South Australian cattle 23rd International Conference of the World Association for the Advancement of Veterinary Parasitology Buenos Aires, Argentina August 21 - 25, 2011

*SR Lanyon, ML Anderson, G Gilbert, MP Reichel (2011) An update of bovine viral diarrhoea ("Bovine Pestivirus") in South Australia Australian Veterinary Association Annual Conference – Peer reviewed session. Adelaide, South Australia May 15-20, 2011

*SR Lanyon, FI Hill, ML Anderson, R McCoy, MP Reichel (2011) New approaches to testing for bovine viral diarrhoea virus ("Bovine Pestivirus") Australian Veterinary Association Annual Conference - Peer reviewed session. Adelaide, South Australia May 15-20, 2011

*MP Reichel, SR Lanyon (2011) Pooling of samples for BVD ("Pestivirus") testing Australian Cattle and Reproduction Veterinarians Annual Conference Launceston, Tasmania April 13 - 16, 2011

*SR Lanyon, ML Anderson, MP Reichel (2010) Comparison of AGID versus ELISA in the diagnosis of bovine viral diarrhoea virus (BVDv) infection in cattle Sixth Annual Meeting of the Australian Association of Veterinary Laboratory Diagnosticians Brisbane, Queensland November 25-26, 2010

*SR Lanyon, FI Hill, R McCoy, ML Anderson, MP Reichel (2010) Reducing the cost of testing for bovine viral diarrhoea through pooled serological testing XXVI World Buiatrics Congress Santiago, Chile November 14–16, 2010

10.2 Poster Conference Presentations

SR Lanyon, ML Anderson, MP Reichel (2014) Identifying Champions: farmer attitudes to endemic disease management in South Australia, with a focus on Bovine Viral Diarrhoea (Bovine Pestivirus) XXVIII World Buiatric Congress Cairns, Australia July 27 – August 1 2014

CJ Jenvey, MP Reichel, SR Lanyon, PD Cockcroft (2014) Investigation of the diagnostic value of colostrum BVDV antibody concentrations in identifying PI calves following experimental infection of beef heifers XXVIII World Buiatric Congress Cairns, Australia July 27 – August 1 2014

SR Lanyon, PD Cockcroft, MP Reichel (2013) Detection of viral antigen in saliva, urine and nasal mucous from a cow persistently infected with Bovine Viral Diarrhoea Virus (BVDV) using a commercially available antigen enzyme-linked immunosorbent assay (ELISA) World Association of Veterinary Laboratory Diagnosticians Symposium Berlin, Germany June 5-8, 2013

SR Lanyon, PD Cockcroft, MP Reichel (2013) Assessment of the diagnostic gap for the detection of newborn, colostrum fed calves that are persistently infected (PI) with Bovine Viral Diarrhoea Virus (BVDV) World Association of Veterinary Laboratory Diagnosticians Symposium Berlin, Germany June 5-8, 2013

SR Lanyon, D Keevers, M Anderson, MP Reichel (2012) A survey of farmer attitudes and awareness to Bovine Viral Diarrhoea in South Australia XXVII World Buiatrics Congress Lisbon, Portugal June 3-7, 2012

*MP Reichel, SR Lanyon, FI Hill, R McCoy, ML Anderson (2011) Establishing herd exposure and PI probability from pooled serum samples The 8th European Society for Veterinary Virology Pestivirus Symposium Hanover, Germany September 25 – 28, 2011

*SR Lanyon, FI Hill, MP Reichel (2010) Reducing the cost of testing for bovine viral diarrhoea through pooled serological testing European College of Veterinary Public Health Annual Meeting and Conference Nottwil, Switzerland October 7 - 8, 2010

11 Appendix 3: Supporting Publications – Other Papers

The following two supporting publications are non-refereed contributions. The first paper was published in the Australian Cattle Veterinarian, targeting an audience of veterinary clinicians working with in the cattle industries. That paper reports a case study, with an economic analysis of an acute BVD outbreak in a cattle herd. The second publication was presented in the University of Adelaide's s-Science magazine, produced for secondary school students and teachers. Both these publications aimed to generate awareness of BVD in Australia, an area that was identified as a priority in Chapter 6 of this thesis.

*Non-Refereed Publication: Economic Analysis of an Acute Outbreak of Bovine Viral
Diarrhoea Virus (BVDV) in a South Australian Dairy Herd – A Case Study*

SR Lanyon, J Rogers, A Kessell, MP Reichel (2012)

Economic analysis of an acute outbreak of bovine viral diarrhoea virus (BVDV) in a South
Australian dairy herd – a case study

The Australian Cattle Veterinarian Vol. 63, Pp. 14-17

Lanyon, S.R., Rogers, J., Kessell, A & Reichel, M.P. (2013) Economic analysis of an acute outbreak of bovine viral diarrhoea virus (BVDV) in a South Australian dairy herd - a case study.

The Australian Cattle Veterinarian, v. 63, pp. 14-17

NOTE:

This publication is included on pages 187-190 in the print copy of the thesis held in the University of Adelaide Library.

E-Science Magazine Publications: Identifying a 'Trojan Cow'

SR Lanyon, MP Reichel (2013)

Identifying a 'Trojan cow'

e-Science magazine The University of Adelaide.

Issue 7, Pp. 18-19

Identifying a ‘Trojan cow’

The beef and dairy cattle industries contribute AUD\$11.8 billion to the Australian economy every year. These industries are at their most productive and profitable when the animals are healthy and free from disease. However, several diseases of cattle are present and causing financial losses in the Australian cattle population. One of these diseases is Bovine Viral Diarrhoea Virus (BVDV), more commonly known to farmers as Pestivirus.

Infection with BVDV can be so mild that most farmers would not notice any abnormalities in infected, non-pregnant cattle. Despite this, BVDV infection still impacts the health and productivity of these animals; the virus may cause temporary drops in milk production or growth, and it makes cattle more susceptible to other diseases such as mastitis or respiratory infections (both of which have significant impacts on profitability). More dramatic effects are seen when the infection occurs in reproductively active animals. Infection with BVDV can reduce conception rates or can cause a cow to abort or deliver a stillborn or neurologically deformed calf.

When a BVDV infection occurs during the first trimester of the cow’s pregnancy, the virus can establish a ‘persistent infection’ (PI) in the developing calf. During this early stage of pregnancy, the calf’s immune system is still developing and is learning what normal, healthy particles look like and what foreign, dangerous particles (that need to be attacked) look like. When the virus is present

during this stage, the calf’s immune system incorrectly learns that BVDV is a normal, healthy particle. Because of this, when the calf is born, it is unable to attack and clear the virus; instead, the virus replicates and the calf is highly infectious and can transmit the infection to almost every animal it comes in contact with.

One of the most effective ways to reduce the impact that BVDV infection has on the productivity and profitability of cattle herds is to use diagnostic tests to identify all the PI cattle and remove them from the herd. When no PI animals are present, the infection dies out and the reproductive loss and increased susceptibility to other diseases is minimised. However, at present, we cannot identify PI calves until after they have been born. This means that, although we can remove all the PI cattle in a herd at once, we must then follow this up by testing every calf born for at least nine months to ensure no more PI calves are born.

A research project has recently followed a group of cows that were carrying PI calves through their pregnancy and tracked the levels of antibodies against BVDV that were present in their blood. The results showed that the cows carrying PI calves had much higher antibody levels than cows carrying uninfected calves – so much so that the PI calves (and their mothers) could be identified before the calf was even born. However, the antibody levels of the cow alone did not provide completely accurate identification of which cows were carrying



A BVDV affected calf.

Caitlin Jenvey

PI calves, so further investigations are being carried out in the hope of increasing this accuracy. If this is successful, it will be possible to remove all the PI animals – born and unborn – from a cattle herd at once, thereby eradicating BVDV and improving the productivity of the herd.

This research was performed by Sasha Lanyon and Michael P Reichel from the School of Animal and Veterinary Sciences, University of Adelaide with the support of IDEXX Laboratories Inc.

This research will be published in due course.

12 Appendix 4: Media Coverage

The articles listed in this final appendix are a sample of the media attention that the research contained within this thesis has attracted. Primarily, these articles were published following a press release on 12th June 2013 discussing the experimental trial detailed in Chapter 7.3. The trial afforded an opportunity to stimulate, via distribution of a media release, discussion of BVD in the non-scientific communities. Like those non-refereed publications listed in Appendix 3, these articles aimed to generate further awareness of BVD in Australia. These articles represent national coverage with an audience of well over half a million Australians.

C Miller (2014) Sasha hot on pestivirus trail. The Stock Journal. 23 January 2014, South Australian Beef Field Day Book Liftout Pp. 9

V Edwards (2013) Test for Killer Virus will Banish ‘Trojan Cow’. The Australian. 17 June 2013, Pp.6

B Neindorf (2013) Finding a Test to Detect Carrier Calves. ABC Rural Online. 18 June 2013.
URL: <http://www.abc.net.au/news/2013-06-18/nrn-carrier-calves/4762112>

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