

# Paws claws and judder things

Issue 3, Summer 2009/2010



**Gribbles**  
VETERINARY

## Interest Articles:

Testing for Bovine Viral  
Diarrhoea (BVD)  
virus 1

Investigating & clearing  
BVD from Lactating herd  
using PCR. 2

Finding a persistent  
infected (PI) BVD carrier  
with PCR 3

PCR technology – all  
you need to know 4

Christmas schedule 6

**We're on the Web!**

## In this issue.....

We are doing a special on Bovine Viral Diarrhoea (BVD) and PCR testing. Ensuring that cattle are BVD antigen free is an important step in controlling BVD. This newsletter profiles the approaches that should be taken when testing for BVD in a herd, but also includes the most frequently asked questions regarding PCR testing. We hope you find this useful.

*All the team here at Gribbles Veterinary Pathology want to wish you and your families a very happy Christmas and a safe and wonderful New Year.*

## TESTING FOR BOVINE VIRAL DIARRHOEA (BVD) VIRUS

by Fraser Hill, Michael Reichel and Richard McCoy

*Testing for Bovine Viral Diarrhoea (BVD) virus via polymerase chain reaction (PCR) in tank milks has been going strong now for 18 months. Utilising the 'bottom 10%' approach, where only the bottom 10% of producers in the milking herd with a positive tank milk PCR result are re-sampled in order to find the persistently infected (PI) animal in the herd, has been shown to be successful in over 90% of the cases (Hill et al, 2008).*

Another approach to finding PIs in milking herds is to use the re-validated milk BVD antibody ELISA to identify the 35% of herds in New Zealand (Voges 2008) with a high BVD antibody prevalence in the milkers. These herds also have evidence of elevated somatic cell counts (SCCs). These SCCs may be up to 30% higher than in BVD naïve herds early in the milking season (and still elevated by about 20% late in the season). Evidence-to-date suggests that 40% of those milking herds with a high antibody result will harbour a PI. We suggest a high BVD antibody ELISA result should be immediately followed by a PCR on a milk sample that includes all milking cows. A positive PCR on the tank milk should be followed by blood tests on the 'bottom 10%' of producers, in order to find the PI. After identification and elimination of the PI, a further check on the tank milk by PCR should now be undertaken to ensure the herd is clear.

A high antibody ELISA result indicates strong exposure to the virus, usually through the presence of a PI in the herd. Thus a BVD antibody ELISA positive result presents additional, valuable information about the herd even if the PCR test in step 2 is negative. If the PI is not amongst the milkers (PCR on milk is negative), a further check on dry stock classes (and any milker that wasn't included in the vat sample for whatever reason) is prudent before the herd can be given the BVD 'all clear'. These dry stock classes can also be approached with a two-pronged testing regime: initially a sample of 15 individuals of any age cohort can be tested by the antibody ELISA. If those results are all clear, there has not been any exposure to BVD virus in that class of stock. If any of those 15 samples comes back BVD antibody-positive, all animals in that group should be tested by a pooled PCR on blood samples. That protocol should also be followed in initially PCR-positive herds, after the elimination of the milking PI. All dry classes of stock not yet tested must be tested to be sure they are negative.

Identification and elimination of PIs is the first step to control BVD in the herd. Once accomplished, additional measures can be put in place to safeguard that investment (these may include vaccination, enhanced biosecurity etc.). BVD on the farm costs money – increased SCCs may push the milk into a zone where penalties apply, increased morbidity and mortality amongst PIs are also expensive, and with the increased prices for livestock these costs can easily exceed the costs of BVD recently modelled to be close to \$50,000 over ten years in BVD infected herds (Reichel et al, 2008).

**TESTING FOR BOVINE VIRAL DIARRHOEA (BVD) VIRUS Contd.**

**References**

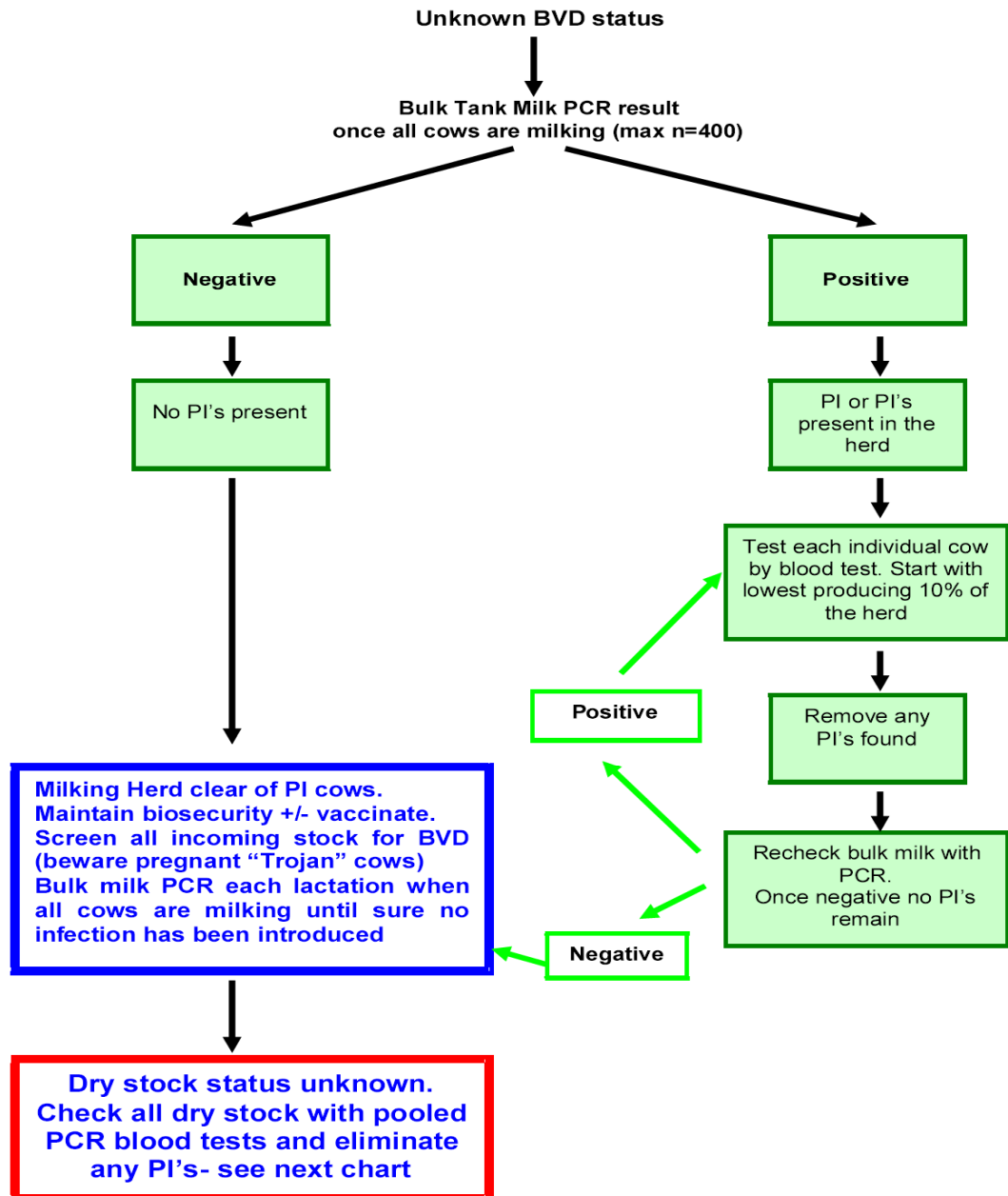
Hill FI, Reichel MP and Tisdall DJ (2008). Use of molecular and epidemiological information for the cost-effective diagnosis of BVD infection in New Zealand dairy cattle  
7<sup>th</sup> ESVV Pestivirus Symposium in Uppsala, Sweden, 16-19 Sept. 2008

Reichel MP, Hill FI and Voges H (2008)  
Does control of Bovine Viral Diarrhoea infection make economic sense?  
New Zealand Veterinary Journal **56**, 60-66

Voges H (2008). Herd BVDv exposure in NZ, Herd BVDv exposure & milk SCC.  
7<sup>th</sup> ESVV Pestivirus Symposium in Uppsala, Sweden, 16-19 Sept. 2008



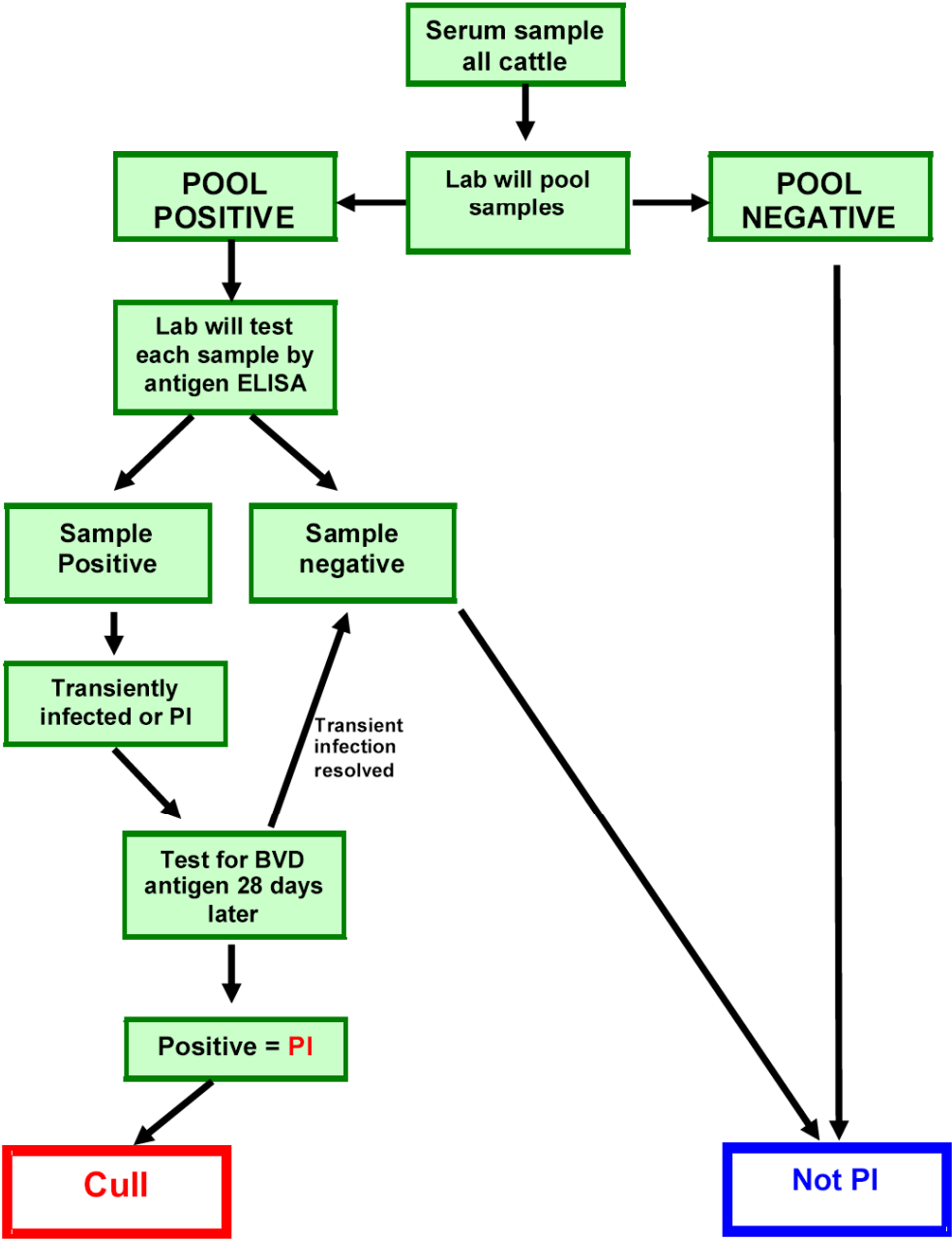
**Investigating and Clearing BVD from a Lactating Dairy Herd using PCR**



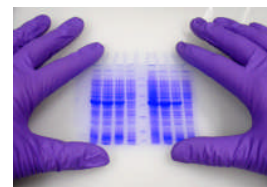


### Finding a persistently infected (PI) BVD carrier with PCR

First prevent reinfection.  
No introductions unless BVD antigen negative



## PCR Technology – all you need to know



### What does PCR mean?

PCR is short for polymerase chain reaction and is a technique for amplifying a specific region of an organism's DNA or RNA. The amplified region is defined by a set of two "primers" at which DNA synthesis is initiated by a thermostable DNA polymerase. Usually, at least a billion-fold ( $10^9$  fold) increase of a specific section of a DNA molecule can be realized. The PCR product can be detected either by agarose gel electrophoresis (conventional PCR) or in "real-time" by the use of fluorescently labelled probes.

### Will PCR replace conventional antibody diagnostic tests?

No, the PCR test is a direct test in that detects the genetic material of a target organism. The PCR test is independent of the host immune response. There are many situations where PCR is unable to effectively determine the presence of an infectious agent and antibody determination will remain the preferred diagnostic method. That said PCR is rapidly becoming more common as the method of choice for infectious diseases as it can more accurately identify if there is a current ongoing infection.

### What specific antigen testing will Gribbles be offering in the short, medium to longer terms?

In the short term Gribbles will be offering real-time PCR tests for BVDV, *Campylobacter fetus* subsp. *venerealis*, *Tritrichomonas fetus*, FIV and *Mycoplasma haemofelis*. In the longer-term tests for leptospirosis, polycystic kidney disease (PKD) and Neospora will be made available.

### What benefits does PCR bring to the diagnostic process?

PCR is highly sensitive and specific, has a rapid turnaround time and can be automated. Multiplexing of PCR tests is also possible allowing several tests to be performed on a single sample at the same time. PCR is especially useful in situations where isolation and identification of an organism presents difficulties as in the case of *Campylobacter fetus* subsp. *venerealis*.

### How sensitive is PCR testing?

PCR is extremely sensitive and has the potential to detect organisms at very low numbers in a sample. Theoretically, PCR is able to detect the presence of a single organism but more realistically the limit of detection would be in the range of 100 to 500 organisms. The sensitivity of PCR has allowed screening for BVDV in a herd using a single test on pooled serum samples or a bulk milk sample.

### Can serotypes be identified?

A PCR can be designed to distinguish between serotypes and strains of an organism where the nucleotide sequences of these are known. A PCR assay can also be designed to distinguish between vaccine and field strains of an organism.

### What types of samples can be used for PCR?

One of the great benefits of DNA testing is that there is no practical limit to the type of samples that can be analysed. For the detection of infectious diseases, an appropriate tissue sample taken from the site of infection can be tested for the presence of the agent. Whether that tissue is skin, liver lung or blood, the DNA/RNA can be effectively extracted and examined for the presence of the infectious agent. The PCR primers are specifically directed to the nucleic acid (DNA or RNA) of the target organism ignoring any host DNA that may be present. For genetic testing such as for polycystic kidney disease (PKD), any cellular material taken from the subject can be used (blood or buccal swabs are often the samples of choice for these tests).

### Are there any fish hooks we need to be aware of when taking samples for PCR tests?

PCR can be performed on DNA/RNA isolated from a wide range of sample types including paraffin embedded sections. It is important to select the sample type appropriate to the organism being tested and preferable to transport the samples chilled. In particular, RNA can be very difficult to work with and often requires samples to be fresh and stored refrigerated to get the best results.

### Is PCR more expensive or cheaper than conventional methods?

The cost of PCR is generally equivalent to the cost of a serological assay.

**Contact us:**

**Vic:**  
1868 Dandenong Road,  
Clayton, Vic 3168.  
**SA:**  
VETLAB, 33 Flemington St,  
Glenside, SA 5065.  
**NSW:**  
2 Leeds St,  
Rhodes, NSW 2138.

PHONE:  
1300 307 190

FAX:  
03 9538 6741

E-MAIL:  
[vets@gribbles.com.au](mailto:vets@gribbles.com.au)

**Our Team of Experienced & Dedicated Staff****Our BVD and PCR experts**

**Dr Mark Williamson, BVSc PhD MACVP** is a senior veterinary pathologist at Gribbles Veterinary. Mark graduated from the University of Melbourne in 1986 and worked in private practice, for universities and government laboratories in research and diagnostic testing of endemic and exotic diseases in Australia and the USA. He has worked as a veterinary pathologist since 1993. His area of interests is the diagnosis of infectious livestock disease by microbiological, molecular and serological testing.

**Dr Richard McCoy, BSc, MSc, PhD** completed Bachelor of Science with Honours in 1980 and Masters in 1983. He has worked at CSL in the Virology R&D and at CSIRO's Division of Animal Health working on the development of viral vector systems for use in veterinary vaccines and gene therapy. In 1995 completed a PhD based on using porcine adenovirus as a vaccine vector this work leading to a worldwide patent and on going development of the vector at CSIRO. Since 1997, Richard has worked in the biotech industry for human therapeutic and drug discovery companies before coming to Gribbles in 2003.

*If you have any questions or need advice regarding BVD or PCR testing please contact*

- Dr Mark Williamson: Phone: 03 9538 6735  
email: [mark.williamson@gribbles.com.au](mailto:mark.williamson@gribbles.com.au)
- Dr Richard McCoy: Phone: 03 9538 8841  
email: [rick.mccoy@gribbles.com.au](mailto:rick.mccoy@gribbles.com.au)

**BVD PCR Pricing**

<b>3798</b>	<b>Bovine viral diarrhoea (BVD) Pestivirus – single animal PCR</b> <i>Sample required:</i> Whole blood, tissue <i>Container:</i> EDTA, Yellow top	per head	<b>\$25.00</b>	<b>\$27.50</b>
<b>3778</b>	<b>Bovine viral diarrhoea (BVD) Pestivirus – pooled PCR</b> Up to 20 animals per pool Additional animals following the first 20 <i>Sample required:</i> Whole blood <i>Container:</i> EDTA	per pool	<b>\$170.00</b>	<b>\$187.00</b>
		per animal	<b>\$8.50</b>	<b>\$9.35</b>
<b>3794</b>	<b>Bovine viral diarrhoea (BVD) Pestivirus – Confirmatory PCR</b> <b>Part of pooled serum PCR screen (above)</b> Confirmatory PCR on positive pooled PCR samples. The positive animals in the pool will be identified. <i>Sample required:</i> Whole blood (note, samples submitted for initial pooled test will be used for the confirmatory test, no further sampling required). <i>Container:</i> EDTA			
<b>3799</b>	<b>Bovine viral diarrhoea (BVD) Pestivirus – PCR Single Vat Milk Sample</b> Up to 400 cows contributing to vat sample <i>Sample required:</i> Milk <i>Container:</i> 50 mL in sterile container		<b>\$120.00</b>	<b>\$132.00</b>

## Wishing you all the best for the Festive Season

**Our opening hours during the 2009 Christmas and New Year are as follows:**

LABORATORY	Wed 23/12/08	Thurs 24/12/08	Fri 25/12/08	Sat 26/12/08	Sun 27/12/08	Mon 28/12/08	Tues 29/12/08	Wed 30/12/08	Thurs 31/12/08	Fri 01/01/09	Sat 02/01/09	Sun 03/01/09
<b>Vet Help Desk</b>	Normal	Normal	Closed	Open 9am – 1pm	Open 9am – 7pm	Open 9am – 7pm	Normal	Normal	Normal	Closed	Normal	Normal
<b>Victoria:</b>												
Clayton	Normal	Normal	Closed	Closed	Normal Sunday service*	Normal Sunday service*	Normal	Normal	Normal	Closed	Normal	Normal
Seymour	Normal	Normal	On call 0419 397 848	On call 0419 397 848	On call 0419 397 848	On call 0419 397 848	Normal	Normal	Normal	On call 0419 397 848	On call 0419 397 848	On call 0419 397 848
Shepparton	Normal	Normal	Closed	Closed	Closed	Closed	Normal	Normal	Normal	Closed	Closed	Closed
Warnambool	Normal	Normal	Urgent samples only	Urgent samples only	Normal	Normal	Normal	Normal	Normal	Urgent samples only	Normal	Normal
<b>SA:</b>												
Glenside	Normal	Normal	Closed**	Closed**	Closed**	Closed**	Normal	Normal	Normal	Closed**	Normal	Normal
Gawler	Normal	Normal	Closed	Closed	Closed	Closed	Normal	Normal	Normal	Closed	Normal	Normal
Mt Gambier	Normal	Normal	On call 0401 143 095	Normal service	On call 0401 143 095	On call 0401 143 095	Normal	Normal	Normal	On call 0401 143 095	Normal	Normal
<b>NSW:</b>												
Rhodes	Normal	Normal	On call 0406 382 247	Open 1pm-5pm Otherwise On call 0406 382 247	Open 2pm-8pm Otherwise On call 0406 382 247	Open 1pm-5pm Otherwise On call 0406 382 247	Normal	Normal	Normal	Open 10am- 5pm Otherwise On call 0406 382 247	Normal	Normal

**Please Note:**

\* Holiday service for Clayton includes Haematologist 5.00pm to 9.00pm on 27<sup>th</sup> and no Haematologist on the 28<sup>th</sup>.

\*\* On call for Liver Fluke testing only.

