Infectious reproductive disease pathogens in dairy herd bulls

AS Hancock, a,b*, PJ Younis, b DS Beggs, a,c PD Mansella a and MF Pymana

Objective Investigate the presence of infectious reproductive disease pathogens in dairy herd bulls in south-west Victoria, Australia, using a cross-sectional study.

Methods Dairy herd bulls from 32 herds were sampled for bovine viral diarrhoea virus (BVDV: 256 bulls, 32 herds) prior to the natural mating period, bovine herpes virus-1 prior to (10 bulls, 5 herds) and after (118 bulls, 19 herds) the natural mating period, and for Campylobacter fetus spp. and Tritrichomonas foetus after the natural mating period (61 bulls, 7 herds). BVDV was detected from an ear-notch sample using a commercially available rapid assay ELISA, bovine herpes virus-1 and T. foetus were screened for by PCR from a penile swab and preputial sample respectively, and C. fetus spp. were screened for by culture of preputial samples.

Results None of the bulls tested positive for BVDV antigen. Campylobacter fetus venerealis (or C. fetus) was cultured in 6.6% (4/61) of bulls, representing 2 of the 7 (28.6%) farms that were not vaccinating bulls against bovine genital campylobacteriosis. Bovine herpes virus-1 was identified in 7.8% (10/128) bulls sampled; T. foetus was not identified in any samples.

Conclusion Bovine genital campylobacteriosis is present in south-western Victoria, despite longstanding recommendations to vaccinate bulls. Screening bulls for persistent infection with BVDV is probably justified, despite the absence of persistently infected bulls in this study. Further research is warranted to investigate the potential reproductive implications of BHV-1, and the presence of T. foetus.

Keywords bovine herpes virus; bovine viral diarrhoea virus; Campylobacter fetus; dairy bulls; Tritrichomonas foetus; venereal disease

Abbreviations AI, artificial insemination; BVDV, bovine viral diarrhoea virus; BHV-1, bovine herpes virus-1; BGC, bovine genital campylobacteriosis; CFV, Campylobacter fetus venerealis; CFF, Campylobacter fetus fetus; IPB, infectious pustular balanoposthitis; IPV, infectious pustular vulvovaginitis; PI, persistently infected

A selection of bulls were tested for the presence of some common infectious reproductive pathogens including bovine viral diarrhoea virus (BVDV), Campylobacter fetus spp., bovine herpes virus-1 (BHV-1) and Tritrichomonas foetus, as part of a larger study examining management practices and breeding soundness of dairy herd bulls in south-west Victoria, Australia.

BVDV is a pestivirus with a complex pathophysiology and epidemiology. Infection can result in infertility, early embryonic death, abortion, congenital defects and the birth of persistently infected (PI) animals.1 International estimates of BVDV prevalence suggest that in endemic populations the seroprevalence of BVDV ranges from 60% to 85%, with 1–2% of animals being PI.2 Australian data support these findings, with BVDV seroprevalence levels of 77%3 and 61%4 reported. No recently published data specific to Australian dairy herds or the prevalence of PI bulls in these herds are available. Various strategies for managing the risk of BVDV associated with bulls are used in south-west Victoria, including vaccination and the testing of bulls, to ensure that they are not PI prior to breeding.

Bovine genital campylobacteriosis (BGC), or vibriosis, is a venereal disease that does not cause clinical signs in infected bulls, but infection of females can result in early fetal losses.5 The pathogens involved are two subspecies of Campylobacter fetus (subspecies venerealis (CFV) and fetus (CFF)), with the former being the major cause of reproductive losses.5 BGC was reported as a major cause of bovine abortion in Victoria in the 1980s,6 with CFV isolated from 77.5% and CFF isolated from 22.5% of abortions due to C. fetus.5 An effective vaccine is commercially available5 and veterinarians have been recommending vaccination of bulls for many years. There are no recently published data reporting the prevalence of either the BGC pathogens or outbreaks of abortions caused by BGC in Victorian dairy herds.

Infection with BHV-1 can result in several manifestations of disease, depending on the subtype of virus present. BHV-1 includes the subtypes BHV-1.1, BHV-1.2a and BHV-1.2b, with the first subtype generally associated with respiratory disease and abortions, and BHV-1.2 subtypes being isolated from genital organs.5 BHV-1.2 is the cause of infectious pustular balanoposthitis (IPB) and infectious pustular vulvovaginitis (IPV).15 Exposure to BHV-1 is common in Australian cattle, with estimates of seroprevalence varying from 15%11 to 30%.4 Several studies have reported that the only subtype of BHV-1 isolated from genital and respiratory samples in Australia is 1.2b,11–14 suggesting that Australia is possibly free of the 1.1 and 1.2a subtypes. A vaccine for the respiratory forms of BHV-1.2 exists, but there are no routine tests or control programs for genital BHV in Victorian dairy herds.

Trichomonosis is a venereal protozoal disease that is nearly identical in its epidemiology to vibriosis. It is caused by the flagellate protozoan T. foetus and, similarly to CFV, the bull is a carrier, with infection resulting in early fetal losses.5 Surveys in cattle in north-eastern Australia found T. foetus infection in 30.2% of slaughtered bulls15 and 17.7% of all cattle surveyed.16 Anecdotally, it is commonly presumed that because of the widespread use of artificial insemination (AI),
T. foetus is not present in Victorian dairy herds. There are no recently published reports of trichomonosis in dairy herds in Australia.

We submitted laboratory samples from a selection of dairy herd bulls that were being evaluated for bull breeding soundness to determine whether these pathogens were present.

Materials and methods

Herds and bulls
Paddock bulls from 32 dairy herds were sampled between May 2013 and February 2014. Herds were chosen on the basis that they were seasonal or split-calving dairy herds located within the client base of The Vet Group, from a region that includes a large area of south-west Victoria. For inclusion in the study, managers of herds must have been planning to use natural service bulls for a mating period that immediately followed an AI mating period and had all agreed to be involved in a separate project that involved a complete bull breeding soundness evaluation.

Farmers completed a questionnaire that included questions regarding their use of testing and control practices for BVDV and BGC.

Bovine viral diarrhoea virus
Ear-notch samples (skin biopsies) were taken prior to the bull mating period from 256 bulls on 32 farms. The samples were tested using a commercially available antigen–ELISA rapid detection kit (Idexx, Westbrook, ME, USA) to determine whether any bulls were PI with BVDV prior to mating. Results were reported by the test kit as positive or negative. Any positive samples were to be rested 1 month later to rule out transient infection. This test has a reported sensitivity of 100% and specificity of 98%.17

Bovine genital campylobacteriosis
In the seven herds that had not vaccinated for BGC in the previous 12 months, all bulls (n = 61) were sampled for BGC at a post-mating visit using a specialised device for taking preputial scrapings from bulls (Tricamper™, Department of Primary Industries, QLD, Aust). Preputial scrapings were collected using two devices inserted past the tip of the penis and into the prepuce. The devices were agitated back and forth to scrape the preputial mucosa and the surface of the penis for a period of 30 s to obtain a diagnostic sample. The devices were then placed in Thomann transport and enrichment media and sent immediately to a laboratory (R & D Veterinary Services, Karana Downs, QLD, Aust) for culture. Cultures were performed according to the OIE standards for diagnosis of BGC.18 This test has a reported sensitivity of 38% and specificity of 100%.19 As well as CFV and CFF, all other campylobacter-like species that were cultured were also reported.

Bovine herpes virus-1
Prior to the mating period, all bulls showing signs of balanoposthitis (n = 10, 5 farms) from the 32 farms were sampled for BHV-1. It was planned to test all bulls after the mating period, but logistical problems resulted in bulls from only 19 herds being tested (n = 118 bulls). The post-mating samples were taken from bulls with and without balanoposthitis. A sample of the surface of the penis was taken using a dry swab and submitted immediately to a laboratory (Gribbles, Clayton, VIC, Aust) for real-time PCR assay, looking for the presence of BHV-1. The PCR was performed according to OIE standards for diagnosis of BHV-1.18 This test has a reported sensitivity of 83% and specificity of 94%.20

Trichomonosis
The preputial samples taken from the 61 bulls for BGC culture were also used for PCR identification of T. foetus. To reduce costs, individual bull samples were pooled for each farm, up to a maximum of 10 bulls per pool. This resulted in one or two pooled samples per farm, with a total of 10 samples. The PCR was performed according to OIE standards for diagnosis of trichomonosis,18 which has a reported sensitivity of 66% and specificity of 98%.21

Results

Herd, bull and management data
Of the 32 herds in the study, 15 (47%) had a split calving period and 17 (53%) were strictly seasonal calving herds. The average number of cows in the participating herds was 425 (154–910) and of those an average of 328 (100–825) cows were participating in the mating period being studied. Of the 256 bulls in the study, 154 (60%) were purchased off-farm and 102 (40%) were reared on-farm. There were 70 (27%) bulls that had previously not been used for mating at any time prior to the study and 186 (73%) were experienced. There were 171 (67%) Holstein bulls, 54 (21%) Jersey bulls and 31 (12%) bulls of other breeds. The average age of the bulls in this study was 3.2 years (1.2–8.2).

Of the 32 dairy farmers, in the past 12 months 78% had vaccinated their bulls for BGC (vibriosis) and 6% had vaccinated for BVDV; only 3% had previously ear-notched bulls to test for BVDV (Table 1).

Testing results
Of the 256 bulls tested, none tested positive for BVDV antigen.

In assessing bovine genital campylobacteriosis (vibriosis), CFV, CFF, non-pathogenic Campylobacter and campylobacter-like species were isolated. Of the 61 bulls, 29 (47.5%) either had no growth, or fungal overgrowth (Table 2.)

Of all 128 samples tested by BHV-1 PCR, 10 (7.8%) were positive (Table 3).

PCR testing for trichomonosis was negative in all samples.

<table>
<thead>
<tr>
<th>Vaccinated bulls for BGC (vibriosis) in last 12 months</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated bulls for BVDV in last 12 months</td>
<td>2 (6%)</td>
<td>30 (94%)</td>
</tr>
<tr>
<td>Previously ear-notched bulls to test for BVDV</td>
<td>1 (3%)</td>
<td>31 (97%)</td>
</tr>
</tbody>
</table>

Table 1. Questionnaire responses from dairy farmers in south-west Victoria, Australia, regarding BVDV and BGC vaccination, and BVDV screening
Table 2. Results of Campylobacter cultures of post-mating preputial scrapings from dairy herd bulls in south-west Victoria, Australia

<table>
<thead>
<tr>
<th>Farm no.</th>
<th>C. fetus veneralis</th>
<th>C. fetus fetus</th>
<th>C. hyointestinalis</th>
<th>C. sputorum mucosalis</th>
<th>C. sputorum bubulus</th>
<th>C. laridis</th>
<th>Arcobacter spp.</th>
<th>No growth/no CLO/fungal overgrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>3</td>
<td>1</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>13</td>
</tr>
</tbody>
</table>

*Some bulls had more than one organism isolated.

CLO, campylobacter-like organisms.

Table 3. Sampling information and results of BHV-1 PCR of samples from dairy herd bulls in south-west Victoria, Australia

<table>
<thead>
<tr>
<th>Sample type</th>
<th>% PCR positive (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples (pre- and post-mating)</td>
<td>7.8 (10/128)</td>
</tr>
<tr>
<td>Bulls with lesions pre-mating</td>
<td>40.0 (4/10)</td>
</tr>
<tr>
<td>Bulls with lesions post-mating</td>
<td>9.1 (1/11)</td>
</tr>
<tr>
<td>Bulls without lesions post-mating</td>
<td>5.6 (6/107)</td>
</tr>
</tbody>
</table>

BHV-1, bovine herpes virus-1.

Discussion

Bulls that are PI with BVDV are at risk of having lower conception rates and are able to infect susceptible animals, resulting in reproductive problems and birth of PI animals. The absence of PI bulls in our study is not an unexpected finding, based on the internationally reported low prevalence of PI animals and our small sample size. This prevalence may be further reduced because PI bulls are at higher risk of dying or being slaughtered for ill thrift as yearlings, consequently not being selected as breeding animals. It could be postulated that the absence of PI bulls detected in the present study was related to a heightened awareness of BVDV among the herd owners and subsequently high levels of testing and removal of PI bulls. The results of the management questionnaire (Table 1) do not support this theory, as a very low proportion of herd owners tested for or vaccinated against BVDV in their bulls. Despite the fact that no viraemic bulls were detected during the course of this study, screening for PI bulls in dairy herds is important, because although the probability of finding one may be low, the potential impact may be high.

The use of ELISA antigen testing of bulls to screen for BVDV may not be sufficient to detect infected bulls. It has been demonstrated that bulls can be non-viraemic and still be shedding BVDV in semen months after exposure to BVDV and subsequent seroconversion. To identify such bulls, a PCR test to detect BVDV antigen in semen must be performed. As the prevalence of bulls with persistent testicular infections is at this stage unknown, we suggest that ear-notching of bulls should remain the main method of routine screening for BVDV in dairy herd bulls for the time being. Further studies should be conducted to determine the prevalence and significance of the shedding of BVDV in semen.

There are no clinical signs associated with BGC in bulls. The disease is often only suspected after the mating period has ended and poor reproductive performance is observed, which may be weeks or months after the infection occurred. The detection of this pathogen in bulls in non-vaccinated herds is an important finding. Even though there has been widespread use of AI to reduce the spread of venereal disease, in conjunction with widespread vaccination of bulls in the study area (Table 1), these results demonstrate that BGC is still present in non-vaccinated, natural-service bulls. Despite the small number of culture-positive bulls, the clinical significance of these positives is undeniable, because of the high specificity of bacterial culture methods for the detection of CFV (100%). Ironically, it is possible that the use of AI contributes to the maintenance and propagation of BGC infection in herds also using natural service. In these infected herds, cows that have previously conceived to AI could potentially add to the pool of naive (susceptible) cows presented to the herd bulls each mating. Consequently, these cows are able to be infected by, and infect herd bulls. This could be investigated further by assessing the pre- and post-mating infection status of dairy cows.

Vaccination is a necessary and effective measure for the control of BGC. It has been demonstrated that vaccination of bulls for BGC can result in a long duration (up to 2 years) of immunity and can also help to clear a bull of CFV infection. Further work in herds that use BGC vaccine would be necessary to determine whether BGC is present in vaccinated herds. Based on our results, we conclude that BGC is probably endemic in south-west Victoria and we recommend that all dairy herd bulls should continue to be vaccinated for BGC.

The presence of BHV-1.2 infection may not directly affect conception rates, but bulls with severe IPB lesions (possibly with secondary infections) may display unwillingness or inability to mate, and may have reduced semen quality caused by viraemia. Cows that have IPV...
from BHV-1.2 infection may develop endometritis and therefore may have reduced fertility.28 Our results indicate the presence of genital BHV-1 infection in bulls in this study. Additionally, some bulls without IPB lesions were PCR positive and some bulls with lesions were PCR negative. The PCR results only confirmed the presence of BHV-1 in these bulls and not the subtype. It is reasonable to infer from past studies that any PCR-positive bulls with IPB lesions (exemplified by one of the bulls examined pre-mating: Figure 1) are most likely related to BHV-1.2 infection.10 It is not possible to say from the present results whether PCR-positive bulls without lesions have BHV-1.1 or –1.2 infection. Virus from some of the PCR-positive samples in this study has been isolated and archived for use in a larger study and the results of these isolations will add to the understanding of BHV.

The discrepancies seen between the presence of lesions and a positive BHV-1 PCR test may also be related to the lesions seen in PCR-negative bulls being caused by other pathogens. The presence of an emerging genital infection that can cause balanoposthitis, Ureaplasma diversum, has recently been demonstrated in Australian cattle.29 Given that U. diversum was found in the Upper Murray and Riverina regions of Victoria and New South Wales, it is possible that this pathogen is present in south-west Victoria. Another explanation for PCR-negative bulls with penile lesions is the presence of convalescing lesions in animals no longer shedding virus genetically. The presence of PCR-positive bulls without lesions may be explained by the presence of latent infection. This is a common occurrence in BHV infection, where viral DNA is present without replication, and is often not accompanied by clinical recrudescence of IPB.10 Discrepancies in our results may also be explained by false-positives and -negatives in the PCR process, as although the real-time PCR method we used may be more sensitive than virus isolation, the moderate specificity may allow for some false-positive results.30

The clinical findings presented here warrant further research into genital BHV infection in Australian dairy herd bulls. Although we were unable to do so in this study, it would have been useful to assess the pre- and post-mating BHV-1 antibody status of the bulls. Paired serology could clarify the ambiguity surrounding the presence of penile lesions in PCR negative bulls. Trichomonosis and BGC have similar epidemiological patterns. As CFV was present it is not unreasonable to believe that T. foetus may also be present in the population. The negative results need to be interpreted with care because of the small sample size. Further testing of dairy herd bulls to investigate the presence of T. foetus is required.

Conclusions

There is scope for improvement in the management of reproductive diseases in dairy bulls. The awareness by farmers of the importance of BVDV prevention in the herds studied was limited. Screening bulls for persistent infection with BVDV may be justified. Despite the fact that no PI bulls were found in this study, the introduction of a PI bull could have a major effect on fertility and health, particularly in a naive herd. In addition, C. fetus is present in south-western Victoria despite a longstanding recommendation to vaccinate bulls. A strong recommendation of this study is that farmers should continue vaccination of bulls to control vibriosis. Further research is warranted to investigate the potential reproductive implications of BHV-1 and the presence of T. foetus.

Acknowledgments

Funding for the laboratory testing was provided by Zoetis, Idexx and Dairy Australia. The authors acknowledge the expert assistance provided by Dr Sharon de Wet at R & D Veterinary Services in the processing of the Campylobacter spp. cultures and T. foetus PCR.

References


(Accepted for publication 7 May 2015)