

Campylobacter and Tritrichomonas within Southern Australian Cattle

Introduction

Infertility or poor reproductive performance in cattle, can occur as the result of infectious agents. These agents can be responsible for decreased ovulation rates, poor fertilization rates and low embryonic survival (Givens 2006).

Infected herds can have a substantial amount of reproductive wastage, representing a large financial loss for producers. Within the first year of a venereal infection such a campylobacteriosis, gross margins may be reduced by as much as 66%, decreasing to 36% once the disease becomes established within the herd (Hum, S, Hornitzky and Berg 2009). For many properties infection may remain undetected and could be responsible for continued production losses (Hum, S, Quinn and Kennedy 1994).

This report considers two common venereal diseases and the impact that they could be having in Southern Australian Cattle. This will be done through examination of prevalence data, diagnostic methods and management principles for two diseases, *Campylobacter fetus venerealis* and *Tritrichomonas foetus*.

Aetiology and epidemiology

Two classic venereal agents of disease associated with poor reproductive performances in cattle are *Campylobacter fetus ssp. venerealis* (CFV) and *Tritrichomonas foetus*. Although these agents are phylogenetically different, they have nearly identical ecology, epidemiology and pathology (BonDurant 2005).

CFV is a highly motile gram negative spiralled bacterium rod, which induces Campylobacteriosis in cattle, historically termed vibriosis (Michi et al. 2016; Quinn et al. 2011). Vibriosis is associated with lowered fertility, embryo mortality and abortion. Fertility investigations require distinguishing CFV from a closely related subspecies *C fetus fetus* (CFF), a commensal of the gastrointestinal tract which occasionally causes sporadic abortion due to ascending infection (BonDurant 2005; Michi et al. 2016).

Tritrichomonas foetus (*T foetus*) is a flagellate protozoan and obligate parasite of the bovine reproductive tract that causes trichomoniasis (Mueller et al. 2015). The parasite inhabits the prepuce of bulls with no symptoms but is spread to females where it leads to abortion (1-8 months gestation), infertility and occasionally pyometra (Grahn et al. 2005).

Both the parasite and bacterium are transmitted via coitus with asymptomatic infected bulls, and have highest prevalence where natural breeding is widely practiced. Both survive within raw and contaminated bull semen, making them transmissible via artificial insemination (AI) if poor hygiene is practiced (Michi et al. 2016). Infected females can transmit the disease back to males, allowing for spread within the herd. Transmission from bull to bull mounting is rare, as both pathogens are susceptible to desiccation and ultraviolet light, limiting their survival to 6 hours under normal atmospheric conditions (BonDurant 2005; Hum, S, Hornitzky and Berg 2009).

Heterosexual sexually transmitted disease transmission rates are reported to be that of 30-70% for infected bulls breeding with susceptible females, however this depends the individual bull's age, libido and ranking in the dominance hierarchy of the herd (BonDurant 2005; Christensen and Clark

1979). Age is a notable factor, with aged bulls more susceptible to both pathogens, due to the increased development of microscopic invaginations of penile and preputial epithelium termed crypts (Ondrak 2016).

Clinical signs

In bulls, both vibriosis and trichomoniasis are clinically asymptomatic, with inapparent chronic infections, which if not detected allows for the disease to perpetuate within the herd (Ondrak 2016). There are some subtle histological changes with both diseases, with an increased accumulation of neutrophils deep to the non-keratinised stratified squamous epithelium of the prepuce and glans. There is also an infiltrate of lymphocytes and plasma cells penetrating the epithelium and coalescing to form lymphoid nodules within the subepithelium (BonDurant 2005). In the female, both organisms induce inflammation within the reproductive tract, with a neutrophilic and eosinophilic infiltrate. Like in the male, subepithelial and periglandular lymphoid nodules develop.

Within 7-10 weeks of infection, the increasing inflammatory changes and possibly the trophoblast cause enough damage to kill the conceptus within pregnant females. A small number of cows which abort will also develop a 'post coital' pyometra, suspected due to bacterial contamination at the time of foetal loss. (BonDurant 2005).

CFV and *T foetus* in herds, is characterised by temporary infertility, late embryo/early foetal (30-70 days) abortions or sporadic abortions later in pregnancy, as a result of subacute diffuse mucopurulent cervicitis, endometritis and salpingitis (Hum, S, Hornitzky and Berg 2009). Females may present with late return to oestrus, irregular oestrus, small calf crops and prolonged calving intervals (Givens 2006). Abortions are usually sporadic and generally occur in carriers around the third trimester of pregnancy (Hum, S, Hornitzky and Berg 2009). Shortly after infection, vaginal discharge may develop due to subclinical endometritis, cervicitis and vaginitis.

Diagnosis

Both vibriosis and trichomoniasis are usually diagnosed using cell culture and/or polymerase chain reaction (PCR) (Michi et al. 2016). The best sample for demonstrating these organisms is preputial smegma obtained via scraping, however, in the case of *Campylobacter*, vaginal mucus aspirations can also be utilised (BonDurant 2005). The most efficient method of sampling vaginal and preputial secretions is via the insertion of an insemination or infusion pipette into the vaginal fornix or preputial cavity and aspirating the secretions (Michi et al. 2016)

Tritrichomonas foetus

T foetus is often diagnosed by cultivation of live organisms from reproductive secretions, in selected media such as Diamond's or InPouch (Grahm et al. 2005). The culture is incubated at 37° for several days with light microscopy observations. The protozoan is identified based on its morphology and characteristic motility, this is prone to false positives due to low specificity (Sp) and identification of other Tricomonads such as *Tetratrichomonas* spp (Michi et al. 2016; Mukhufhi et al. 2003). Culturing is time consuming, has low sensitive (Se) in field conditions and is prone to contamination with other trichomonadid, and as such a better test is essential (Mutto, Giambiaggi and Angel 2006; Grahm et al. 2005) .

Diagnostic testing employing both culture and PCR for *T foetus* yields a higher Se and improved Sp, which may be the most cost-effective and practical approach to assessing bulls prior to the breeding season (Michi et al. 2016). Amplification of DNA material by PCR is a potential highly sensitive and specific test that can allow for minute sections of genomic material to be amplified and then detected using specific primers TFR3/TFR4 (Mukhufhi et al. 2003). PCR is time sensitive (Se 90% at 6 hours to 31% at 5 days), which can result in the failure of detection of trichomonas's if there is any delay in testing after sampling (Mukhufhi et al. 2003; Cobo et al. 2007).

Campylobacter fetus venerealis

CFV can be isolated from the genital tract of cattle via preputial smegma and vaginal mucus, or from the internal organs of aborted fetuses (OIE 2008). The organism can be identified when cultured at 37° for at least 3 days micro-aerobically in a transport rich media (Givens 2006). Culture of preputial and vaginal washings for vibriosis has relatively poor sensitivity (Truyers et al. 2014), and should be combined with PCR or serological testing to reduce false negatives. Differentiating between CFV and CFF is difficult and requires biochemical typing, ELISA, direct immunofluorescence or molecular based assays such as PCR (McGoldrick et al. 2013).

An enzyme linked immunosorbent assay (ELISA) test has been developed, which detects IgA antibodies present in the vaginal mucus (Hum, S, Quinn and Kennedy 1994). This has a sensitivity of 98.5%, and displays the results as positive, inconclusive, suspect (low positive) and negative, providing an estimate of risk of vibriosis within the herd (McGowan et al. 2014; Tuyers et al. 2014).

Due to the recent publication of the complete genome of CFV, improved diagnostics such as real time PCR have been developed allowing for detection of *C fetus venerealis* directly from clinical samples, limiting the need to perform prior culturing (Hum, S, Hornitzky and Berg 2009). McGoldrick et al. (2013) successfully developed two real-time assays that have a reported sensitivity of 98.7% and specificity for 98.7% for the detection of CFV.

Treatment

There is no current effective legal or standardised treatment for Trichomoniasis in cattle. There has been documented success using nitroimidazole drugs, although these are not registered for use in cattle (BonDurant 2005). In the absence of effective treatment options, bovine sexually transmitted diseases need to be controlled via diagnostic testing and the culling of infected animals (Michi et al. 2016). Males remain carriers for >3 years, though females can clear *T foetus* within 3-5 heat cycles post-abortion. If they are still with bulls, they may conceive and calve normally, however re-infection can occur due to short lived immunity if they are re-exposed (Walker and McKinnon 2011). Thus sexual rest is recommended, until infection is cleared and a non-infected bull is available (BonDurant 2005).

For bulls of extreme value infected with vibriosis, individual treatment can be conducted, however this is only recommended in bulls less than 3 years old (Truyers et al. 2014). CFV specific antibiotics can be flushed into the sheath of the affected bull's preputial cavity and/or systemic antibiotic therapy may be administered. Young bulls should be given 6-9 months of sexual rest, vaccinated and tested clear prior to reintroduction into the breeding herd (State of Queensland 2016). Streptomycin has been discussed in the literature as an effective drug for the treatment of vibriosis, and can be used off-label for cattle, with a permit issued to veterinarians (BonDurant 2005; Chemical Review

Section 1999). The treatment of infected females is not recommended due to poor results and their development of protective immunity (Truysers et al. 2014).

There is some evidence that immunization of infected bulls with an appropriately adjuvanted antigen can clear campylobacteriosis, thus limiting dependence on restricted antibiotics. Although this is not successful for all bulls, it was found to sufficiently reduce the number of CFV organisms, limiting the bulls ability to transmit an infectious dose to susceptible females (BonDurant 2005).

Prevention

With proper hygiene, artificial breeding can be used to prevent the spread of both organisms, and has been successful in dairy herds and small farm beef herds. Unfortunately it may be impractical for many extensively managed beef properties (Hoffer 1981). Therefore strict adherence to prevention and control strategies, in particular vaccination and the management of non-productive females and non-virgin bulls is key to effective control of both diseases. (Ondrak 2016).

Vibriosis can be controlled with good herd management and regular vaccination of breeding of bulls (Walker 2005). Eradication of CFV can be achieved in infected herds in a cost-effective manner, using vaccination. A vaccination, consisting of two injections, 4-6 weeks apart followed by annual boosters renders animals highly resistant to infection, and is recommended to be done 4 weeks prior to joining (Hum, Steven 2004). In herds with confirmed infection, annual vaccination of the replacement heifers and all bulls is indicated, with vaccination of cows suggested for severely affected properties. Once infection is under control, annual bull vaccination should be continued but vaccination of heifers and cows can cease (State of Queensland 2016).

There have been several studies where the systemic immunization of bulls with *T foetus* antigens have prevented or cleared genital infections, however there is no commercial vaccine available in Australia (Michi et al. 2016; Walker and McKinnon 2011). Trichomoniasis can be eradicated by the culling of infected bulls and ensuring that cows have at least 3 months sexual rest (Walker 2005).

In the most herds, the best strategy for control is the culling of infected bulls and sexual rest or culling of infected females. In large herds, this may prove too costly to replace all breeding bulls and it may be necessary to adopt a two-herd system; splitting the herd into separate mating groups with virgin bulls only for one group. The gradual replacement of older or infected bulls with virgin or bulls less than 3 years of age, should take place to reduce the risk of disease transmission (BonDurant 2005). Any new bulls brought into the breeding herd should be virgin, vaccinated and/or tested and cleared of both diseases (with repeated culture and/or PCR).

Pregnancy testing at 2 and 6 months after the bulls are removed is highly recommended, with the culling of all empty cows under high suspicion of infection with either CFV or *T foetus*. Any cows that present with vaginal discharge, or have aborted should be individually investigated for the presence of disease (Walker and McKinnon 2011). This will allow for the removal of any carrier females and prevent the spread to any of the breeding bulls.

Export Implications

Bovine genital campylobacteriosis is present in all Australian States and Territories, and is included in the Office International des Épizootics (OIE) category B list of diseases. The disease is considered an

important international trade barrier, with many countries requiring certification of negative disease status for animals and animal products (Hum, S, Hornitzky and Berg 2009; Chaban et al. 2012).

Currently Australia exports most of their live cattle for breeding to Russia, Kazakhstan, China, Turkey and Indonesia (Rickards and Nicol 2012). Health information certificates need to be issued for most of these countries for the export of semen, many of which having requirements for originating from administrative territories and AI centres officially free of contagious diseases such as campylobacteriosis and trichomoniasis for the last 12 months. Many donor bulls are required to be kept in quarantine conditions for a month prior to semen collection, and are subject to test with negative results for campylobacteriosis and trichomoniasis in accordance with OIE guidelines.

Eradication of these two diseases will ensure Tasmanian breeding cattle have a reputation for disease freedom, thus allowing for greater access into all types of live export and reproductive material markets will be maintained. Confirmed disease freedom can have benefits for individual farms, allowing them to expand their business into export, and with eventual eradication in certain regions may reduce the need for required laboratory testing, easing financial burdens. Thus, there are huge export implications for the confirmed presence and the control of CFV and *T foetus* for Tasmania cattle.

Southern Australian Prevalence

Based on Northern Australian Data

A large north Australian study by McGowan *et al.* (2014) reported 4-14% of the northern Australian herds had a high prevalence of CFV vaginal mucus antibody present within breeding cows. Current lax quarantine for breeding bulls, means it is easy for properties in Southern Australia to acquire bulls from more Northern parts of the country where both vibriosis and trichomoniasis are endemic (Walker and McKinnon 2011). There is little to no published data available for current prevalence of either CFV or *T foetus* within Southern states, although anecdotal evidence suggests prevalence may not be as low as perceived (Lane et al. 2015).

Victoria

Historically, Victoria has identified Campylobacteriosis to be a major cause of abortions and decreased fertility. In the 1980's, a study of 265 bovine abortions found 11% to be due to Campylobacter fetus (77.5% due to CFV, 22.5% due to CFF) (Jerrett et al. 1984).

Recent studies relating to CFV prevalence in southern Australia include a cross-sectional study investigating the presence of infectious reproductive disease pathogens in dairy herd bulls in South-West Victoria. A survey study of paddock bulls from 32 dairy herds, identified a Campylobacter species within 6.6% of the bulls tested, representing 28.6% of the farms not vaccinating their bulls against bovine genital campylobacteriosis (Hancock et al. 2015).

Trichomoniasis is currently a notifiable disease in Victoria (State of Victoria 2015), with no recently reported cases. Although it is not identify as cause of infertility in Victoria, this may be due to inadequacy of procedures used in investigations rather than a true lack of prevalence and significance (Loveridge and Gardner 1993).

Tasmania

Vibrio was a major problem in Tasmania, up until the middle of the last century, when a cattle reproduction unit was set up by the then Department of Agriculture. Dairy farmers formed regional control groups, and AI was promoted and used heavily to overcome the spread of disease within the Tasmania herd. Many beef herds were also diagnosed about this time, but the disease was almost eradicated through the vaccination of bulls and culling of empty cows. The cattle reproduction unit was dissolved in the 1980's, but vibriosis had not been systemically eradicated by that time.

Up until the mid to late 1990's, Tasmania had a quarantine policy for introduced cattle, with mature bulls being tested three times for vibrios and trichomonas while in quarantine. Currently there is no routine testing or certification for the introduction of cattle from the mainland for these two diseases, yet diagnostic testing reveals both pathogens to be present on the island.

Over the last 17 years a total of 11 confirmed cases of Campylobacteriosis and 1 confirmed and 1 suspicious case of Trichomoniasis have been documented from Animal Health Laboratory (AHL) in Launceston. In early 2014-2015, 5 herds were diagnosed with Vibriosis throughout Tasmania and Trichomoniasis was suspected as a cause of infertility in a beef herd on Flinders Island. A survey conducted in 2014 by Meat and Livestock Australia included several questions on fertility which identified significant fertility problem throughout Tasmania. A third of producers required extended joining periods and almost 25% of herds reported more than 10% empty cows at pregnancy testing (Sherriff et al. 2014).

Though there are several possible causes of low pregnancy testing rates and abortions, very few producers and veterinarians test for exclusion of vibriosis or trichomoniasis. Thus, the use of preventative programs such as annual vaccination with Vibrio and regular screening of introduced bulls is not frequently performed. Current evidence from confirmed cases submitted in for testing at AHL, suggest that CFV is still present within Tasmanian beef and dairy herds, with some sporadic cases of trichomoniasis as well.

Recommendations

Monitoring and Surveillance

Routine systematic testing of bulls for CFV and *T foetus* can allow for early detection of a disease incursion and is vital for herds in endemic regions (Ondrak 2016). The conduction of sample collection should take place 1-2 after the end of breeding season and prior to the next, this allows for the number of organisms to increase, and improve the likelihood of detecting a positive CFV and/or *T foetus* bull (BonDurant 2005).

Experiments have indicated a significant improvement in detecting both CFV and *T foetus* using PCR directly on preputial washes, compared with that of conventional culture, however conventional PCR techniques for routine diagnostic testing is not ideal. The recent development of real-time PCR for both organisms allows for detection directly from diagnostic specimens and would be most suitable for routine surveillance within Tasmanian natural breeding herds.

For large breeding herds, initial testing of all breeding bulls may prove very costly, for these instances, the pooling of samples may be used without significant reduction in ability to detect positive herds. If positives are identified, the producer may then choose to perform testing on the bulls from the positive pool in order to determine which bulls should be culled (Ondrak 2016).

Conclusion

The bovine venereal diseases, campylobacteriosis and trichomoniasis remain endemic in Northern Australia and are still present in Southern cattle herds, causing mild to severe reproductive losses. To efficiently diagnose, treat and control these diseases, serial cultures, ELISA and/or regular PCR testing of breeding bulls are necessary to identify the responsible pathogens for reproductively poor herds. Currently, culling infected livestock is the most cost-effective method of mitigating prevalence, but regular vaccination in combination with herd management can help to minimise losses from these two organisms and the disease they cause. Prevention and control of these sexually transmitted diseases, through a better understanding of current prevalence rates and regular testing of breeding bulls has the potential to allow for long term increases in the number of cattle produced and more efficient breeding herds, leading to huge economic benefits within the Tasmanian cattle industry.

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