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Investigation of balanitis in beef herds in southern Australia

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Executive summary

Veterinarians and beef producers in north-east Victoria and southern New South Wales have observed lesions on the penis ('balanitis') of beef breed bulls early in the mating period. This often leads to a reluctance of bulls to mate with cows ('bull breakdown'), hence poor conception rates. The penile lesions had an inconsistent appearance not always typical of known viral causes of balanitis in cattle in Australia, namely Bovine Herpersvirus-1 and -5 (BHV-1 and -5). Additionally, veterinarians from different regions described the lesions and the effect on bulls differently.

There are two types of BHV that can cause venereal diseases in cattle, BHV-1 and -5. Clinical signs include reddening of the preputial or vaginal mucosae, pain associated with pustules or ulcerative lesions and abortion. In bulls, the pain caused by the lesions can lead to reduced serving capacity, hence reduced conception rates in the cow herd.

The initial studies identified that BHV-1 was associated with balanitis in some bulls, however not all the bulls with clinical signs were positive for BHV-1. Therefore, the primary aim of this project was to identify the cause of mid-season bull breakdowns caused by this syndrome, and establish if cows play any role in the transmission.

There were two parts to this project. Firstly, a producer survey to determine which farms may or may not have bulls affected with balanitis, followed by a serological survey to determine the prevalence of BHV in Victoria and southern New South Wales. Secondly, we attempted to identify farms which had a history of problems with bull breakdowns due to the syndrome described by veterinarians, serial sample a mob of cattle, bulls and cows and describe the progression of the syndrome, and risk factors associated with it, on these farms.

Samples were collected from four farms, two were located near Coolac in southern New South Wales, and the other two located at Ournie, just across the Murray River from Walwa in north east Victoria. Five visits occurred; before joining, at the start of joining, mid-joining, the end of joining and at pregnancy testing although, not all these visits occurred on all farms. All cattle had swabs taken of either their penile or vaginal mucosae, were visually assessed for lesion scores on this mucosa, had blood taken from their tail vein and a small number of bulls had biopsies taken of the lesions on their penile mucosae.

Swabs were used for a PCR that identified the presence of BHV-1 and -5 and a universal PCR was used to identify the presence of BHV. Samples which were negative for BHV-1 or -5, but positive on the universal PCR were assumed to be BHV-6 after sequencing of a proportion revealed this. The bloods were sent to IDEXX laboratories for immunoserological testing using an ELISA which detected glycoprotein-B-specific antibodies to Bovine Herpesvirus 1.

Results from the serological survey showed a wide range of sero-prevalence within herds tested, from 5.8 to 97.8%. There was a significant difference in the sero-prevalence of BHV in affected/suspected herds and non-affected herds, the overall average was 51.8% (range 5.8 to 97.8%) and 35.0% (range 5.8 to 84.0%), respectively ($P<0.0001$).

The second part of the project identified the presence of BHV-6 in penile and vaginal swabs. The significance of this finding is yet to be determined. Bovine Herpesvirus-1 and -5 were not identified in any of the samples collected, even though on two of the farms, the producers commented that the lesions present were typical of the syndrome.

Bull breakdowns can be devastating for beef enterprises, because no calf equates to no income. This project highlights the need for producers to get bulls examined by veterinarians, especially if there is no obvious reason for their breakdown. The financial benefit to producers is avoiding a disaster, either through poor conception rates or the need for an extended mating period due to a change of bulls halfway through the joining period. An extended mating period also creates problems with late-born steers not reaching turn off weight, and late-born heifers failing to reach mating weight.

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1 Background

Veterinarians and beef producers in north-east Victoria and southern New South Wales have observed lesions on the penis ('balanitis') of beef breed bulls early in the mating period. This often leads to a reluctance of bulls to mate with cows ('bull breakdown'), hence poor conception rates.

Investigations conducted from 2005 to 2011 by general practitioners and veterinarians employed by NSW Livestock Health and Pest Authorities found a variable association between the presence of Bovine Herpesvirus (BHV) and poor bull performance on 14 farms. The penile lesions had an inconsistent appearance not always typically of known viral causes of balanitis in cattle in Australia, namely Bovine Herpersvirus-1 and -5 (BHV-1 and -5)..

Bovine Herpesvirus can cause several diseases, including Infectious Bovine Rhinotracheitis (IBR), encephalitis in calves, Infectious Pustular Vulvovaginitis (IPV) of cows and infectious pustular balanoposthitis in bulls (Engels and Ackermann 1996, Vogel, Flores et al. 2004). Historically all these diseases were thought to follow infection with BHV-1, but recent studies have implicated another strain of herpesvirus, BHV-5, in venereal diseases (Kirkland, Poynting et al. 2009). Venereal diseases of BHV are typically transmitted by direct contact, with the virus replicating in epithelial cells of the prepuce or vagina, producing both sub-clinical (inapparent) or clinical disease. Typical clinical signs include reddening of the preputial or vaginal mucosae, pain associated with pustules or ulcerative lesions and abortion. In bulls, the pain caused by the lesions can lead to reduced serving capacity, hence reduced conception rates in the cow herd.

The initial infection is thought to be short-lived, although infected animals can develop a latent infection whereby the virus remains inactive within neural cells and can become re-activated during periods of stress. This enables continual circulation of the disease within a herd through the infection of naïve animals. There are no recent studies which have documented the sero-prevalence of BHV-1 in beef herds in southern Victoria. A serological study from 1967 indicated that 21% of all animals tested in southern Australia (2866 serum samples) had antibodies to IBR virus (BHV), and 57% of 185 herds tested had animals with antibodies to IBR (St George, Snowdon et al. 1967), and it is considered to be a widespread and common infection.

The initial studies identified that BHV-1 was associated with balanitis in some bulls, however not all the bulls with clinical signs were positive for BHV-1. Consequently, additional research was needed to better define the syndrome described by veterinarians, investigate potential risk factors and explore the association between BHV-1 or -5 and the inconsistent presentation of balanitis in affected herds.

2 Project objectives

Primary aims of the research included;

2.1 Module 1

a. Identification of a sample of herds, with a survey, that have or have no cases of balanitis, and
b. Determination of the sero-prevalence of Bovine Herpesviruses (BHV-1 and -5), within herds that have or have no cases of balanitis in three regions of Victoria and southern NSW.

2.2 Module 2

a. Describe the progression of clinical signs in bulls affected with balanitis in south-eastern Australia, and its effects on serving capacity.
b. Investigate risk factors that may be associated with the syndrome.

Describing the progression and presentation of this syndrome would improve the reproductive performance and profitability of affected beef herds across southern Australia. For example, early recognition of the syndrome would allow replacement bulls to be deployed sooner, whilst an improved understanding of risk factors and how the syndrome circulates through affected herds would enable early and more effective treatment and control options to be developed. Outcomes from this research would benefit beef producers in the affected regions, but the information could also be disseminated nationally through established beef networks, such as Better Beef groups.

Concern from beef producers in the affected areas, and more broadly through the Cattle Council of Australia (CCA), was the primary driver behind this project application. For example, many bull buyers had expressed concerns about the potential for inadvertent spread of this syndrome from bull breeding operations. The syndrome was poorly understood, and so these risks were not easily measured. The outcomes from this project should facilitate a better understanding of these risks, inform beef producers about the syndrome and identify what may be appropriate precautions to take. If bull producers were identified as a potential source of the syndrome, this study should help prevent further spread within the beef industry. As part of the extension process, an advisory group including beef cattle producers, DEDJTR (DPI Vic) beef officers and private practitioners would be kept informed of the findings to facilitate early intervention and development of preventative strategies.

In the short term, this project aimed to identify the cause of mid-season bull breakdowns caused by balanitis and establish if cows play in role in the transmission of this disease. This information could then be used to make producers aware of the disease and its potential impacts on the reproductive performance and profitability of their enterprise.

In the longer term, improved understanding of the epidemiology and risk factors of this disease would allow producers to make informed management decisions, although further studies would be needed to assess proposed control and prevention methods.

3 Methodology

MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research needs to be done under the auspices of a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained.

3.1 Module 1 (June 2013–March 2014)

3.1.1 Producer survey

3.1.1.1 Recruitment of contacts for producer survey

Contacts details were sourced from the Meat & Livestock Australia (MLA) members' database. Meat & Livestock Australia provided The Mackinnon Project with a total of 13,968 contacts. Of these, 1321 contacts were sent surveys in the post and 2367 were sent a link to an online survey, conducted via Survey Monkey. The paper survey is provided in Appendix 1.

Two groups were determined: contacts with email addresses, 7373 and contacts without, 6596. Surveys were posted to 20% of the contacts without email addresses and 32% of contacts were sent an email with the link to the survey. Contacts for the survey were randomly chosen by grouping into postal codes and then selecting every 3rd or 5th contact with that postcode for email or postal delivery, respectively.

3.1.1.2 Response rate

There was a response rate of about 5%, equating to 181 returned surveys. However, there were a number of surveys returned to sender due to wrong address or person (58) and a large number of email addresses were incorrect/not used.

3.1.2 Sero-prevalence survey

Producers were only included in the serological testing if they provided their contact details and agreed to further involvement. The immediate application of the producer survey was to facilitate selection of herds suspected to be affected by balanitis and herds that were not affected. Herds were selected randomly, stratified across three regions of south-eastern Australia (north-east Victoria/ south-eastern NSW, Gippsland and Central Victoria). Only those producers who maintained a breeding herd of ≥ 100 females were included. A list of eligible herds for each of the three regions was constructed and these herds were visited during Jan-Mar 2014 for the collection of blood samples. Blood was collected from approximately 50 females and a selection of up to 10 available bulls on all properties. If a producer did not want their herd tested, the next eligible herd on the list was chosen. Sera were sent to IDEXX laboratories for immunoserological testing using an ELISA which detected glycoprotein-B-specific monoclonal antibodies to BHV-1.

3.2 Module 2 (May- October 2015)

The aim of module 2 was to identify if BVH-1 and -5 were the cause of, or associated with, reproductive failure in affected beef herds in southern Australia, using a longitudinal serial sampling protocol. Herds with or without cases of balanitis were identified with bulls and cows from each herd selected for monitoring. The aim was to use single-sired mobs, although on one farm (farm B) two bulls were used in the mob of cows being tested.

Five visits occurred; before joining, at the start of joining, mid-joining, the end of joining and at pregnancy testing. Due to difficulties encountered mustering cattle for sampling during the winter, not every visit occurred on each farm and, on Farm C, the same animals were not consecutively sampled at each visit due to the large number of animals in the mob.

A pre-joining sample was collected on farms A, C and D, but this sample was not collected on Farm B. A second sample was collected at joining in the monitor herds, which commenced in mid-May on farms B-D and later, early to mid-June, on Farm A. On this farm, joining dates for the two monitor mobs were two weeks apart. The third sample collection on all farms occurred mid-joining, approximately 3 to 4 weeks after the bull was put in with the cows. A fourth sample was collected at the end of joining. The length of joining ranged from six to 15 weeks. A sample was also collected at pregnancy testing. This sample was only collected on farms B and D.

The dates that the visits to each farm were conducted are summarised in Table 7 in Appendix 2.

3.2.1 Number of monitor herds and mobs

Ten monitor mobs from four farms (farms A to D) were included in the study, with two mobs on farms A and C, one mob on Farm B mob and five mobs on Farm D.

Farms A and B were located near Coolac in southern New South Wales, and Farms C and D were located at Ournie in southern NSW, across the Murray River from Walwa in north east Victoria.

3.2.2 Number of animals

The number of bulls and cows sampled on each farm is summarised in Table 1

Table 1: The number of bulls and cows in the monitor herds on each farm.

	Bulls	Females
Farm A	4	20
Farm B	4	10
Farm C	3	35
Farm D	5	25

Only cows that had already had one calf were sampled on Farm A, but heifers were included on farms B and C, and a mix of heifers and cows was sampled on Farm D. The bulls were of mixed ages on farms A and D, whereas 2 to 3 year old virgin bulls were used on farms B and C.

All mobs in the study were single-sire mated with the exception of a mob on Farm B that had two bulls running with ten heifers.

On Farm B, the original bulls were replaced with fresh bulls after visit 3, which corresponded with mid-joining (four weeks after the start of joining). These bulls were replaced due to their inability or unwillingness to serve due to lesions on their penis and injuries on their prepuce. Similarly, on Farm C one bull was replaced at visit 3 due to characteristic balanitis lesions. An 'outbreak' of balanitis was recorded in a separate mob on Farm C, and additional samples were collected from three bulls and eight cows at this time.

3.2.3 Measurements

At each of the visits, blood samples were collected into plain tubes from the tail vein and swabs were taken from the penile or vaginal mucosae. The blood samples were centrifuged to separate the sera, which were sent to IDEXX laboratories for immunoserological testing using an ELISA which detected glycoprotein-B-specific monoclonal antibodies to Bovine Herpesvirus 1. The swabs were placed in universal transport medium to inhibit the growth of contaminants, before being placed in the -80°C freezer until further testing of these samples.

Additionally, at each visit a scoring system was used to grade the severity of lesions present on the penile and vaginal mucosae. This was on a scale of 0 to 6, with 0 being no lesions and 6 the most severe lesions (shown in Table 2). Photos of each lesion score on the penile mucosa are shown in Appendix 3, and the observations at each visit for each farm are summarised in Table 3.

Table 2: The scoring system for the lesion scores to describe balanitis or vulvovaginitis

Score	Description
0	No trace of lesions
1	Trace of lesions (a few red lesions)
2	Mild, mostly penis, especially the ventral aspect
3	Inflamed penis ± the prepuce
4	Both the penis and the prepuce are severely inflamed
5	Coalescing lesions on the penis and prepuce
6	Secondary infections

Table 3: A summary of data collected at each visit and on which farm these occurred (a tick designates all 4 farms)

	Lesion score	IBR ELISA	BHV 1	BHV 5	BHV 6
Pre-joining	A	A,C,D	A	A	A
Start of joining	✓	✓	✓	✓	A,B,D
Mid-joining	✓	✓	✓	✓	✓
End of joining	✓	A,B,C	A,B	A,B	A,B
Pregnancy testing	B,D	B,D	B	B	B

4 Results

4.1 Module 1

4.1.1 Producer survey

The results for the producer survey are summarised in Appendix 4.

4.1.2 Sero-prevalence survey

A total of 1221 animals in 24 herds were tested with an ELISA, with a wide range of sero-prevalence found within these herds, from 5.8 to 97.8% (Table 4). There was a significant difference in the sero-prevalence within herds determined to be affected or suspected to be affected with atypical balanitis and herds not-affected, with the overall average being 51.8% (range 5.8-97.8%) for affected herds and 35.0% (range 5.8-84.0%) for non-affected herds ($P<0.0001$). In general there was a higher sero-prevalence detected in bulls and adult cattle compared to heifers, although one herd with a very high overall sero-prevalence (97.8%) also had a high prevalence in the heifer mob (99.9%).

Table 4: Sero-prevalence for Bovine Herpesvirus by region in affected/suspect and non-affected herds

Region	Affected/suspect herds		Non affected herds	
	Total tested	Total (%) positive	Total tested	Total (%) positive
1	223	116 (52.0)	210	65 (31.0)
2	206	109 (52.9)	221	47 (21.3)
3	204	103 (50.5)	157	94 (59.9)
Total	633	328 (51.8)	588	206 (35.0)

4.2 Module 2

4.2.1 Summary of samples collected

A total of 339 samples were collected. ELISA was performed on 295 of these samples, a PCR test for BHV-1 and -5 was conducted on 201, a universal BHV PCR was conducted on 108, and there were 288 records of lesion scores. Histology was conducted on biopsies

collected from six bulls with lesion scores, with two of these samples further analysed by electron microscopy.

4.2.2 Serology using an IBR ELISA

All the blood samples collected were submitted for analysis with an ELISA. On three of the four farms, farms A, B and D, BHV was present in both the bulls and cows tested, although the ELISA used does not differentiate between BHV-1 and -5. Surprisingly, antibodies to BHV were not present in either the bulls or cows tested on Farm C, even though an outbreak of the balanitis syndrome occurred during the study. This farm has a long history of the atypical balanitis syndrome, and was one of the farms from which clinical material was originally studied more than ten years ago.

At the end of joining, 90% of the cows and all the bulls on Farm A were serologically positive to BHV, 88% of cows and all bulls were sero-positive on Farm B, and 24% of cows and all bulls were sero-positive on Farm D. Subsequently, at the end of the study 86%, 80% and 36% of bulls and cows were sero-positive for BHV, and 40%, 78% and 3% of animals had sero-converted during the study, on Farms A, B and D, respectively.

4.2.3 Histology and electron microscopy

Biopsies were taken from six bulls, four from Farm C and two from Farm D. These biopsies were submitted for histological examination, which indicated a herpesviral infection in all six biopsies (the histopathology reports from Farm D are attached as an example in Appendix 5). However, whereas inclusion bodies are a classic sign of herpesviral infections, no inclusion bodies were seen. Two of these samples were sent for examination by electron microscope (EM), with the hope that any herpes or other viral agents would be seen, but none were detected.

4.2.4 Lesion scores

The lesion scores were recorded at each visit for both the bulls and cows. The number of animals observed at each visit, and the number with a lesion score of 1 or more, are shown in Table 5

The proportion of animals with lesion scores on farms A and B was higher than on farms C and D. The proportion of animals with a lesion score greater than 0 ranged from 50 to 80%, 70 to 100%, 5 to 35% and 0 to 31% on farms A, B, C and D, respectively. On Farm B, both bulls at visit 3 had lesions typical of the syndrome and were replaced. One

On Farm A, lesion scores ranged from 0 to 2 at the pre-joining and joining visits in the cows, and 0 to 1 in the bulls. At the end of joining, one of the mobs on this farm had more cows with lesions, including some with lesion scores of 3.

On Farm B, the lesion scores also increased in severity in the cows from the start of joining until the end, with more cows showing scores 2 and 3 by the end of joining.

On farms C and D, the highest lesion score observed in a small number of cows in the monitored mobs was 1. On Farm C, one bull had a lesion score of 2 at the mid-joining visit, and so this bull was treated with oxytetracycline and replaced with another bull. This was a normal management procedure on this farm, where any bulls with lesions at a routine mid-joining examination were treated with oxytetracycline and replaced by an unused bull.

Table 5: The number of animals examined and the number with a lesion on each farm at each visit

		Visit number				
		1	**2**	**3**	**4**	**5**
Farm A	Number of animals	4	20	20	11	-
	Number with a lesion score	**2**	**16**	**16**	**9**	**-**
Farm B	Number of animals	-	12	10	10	8
	Number with a lesion score	**-**	**9**	**7**	**10**	**8**
Farm C	Number of animals	-	19	35	19	-
	Number with a lesion score	**-**	**1**	**12**	**3**	**-**
Farm D	Number of animals	-	29	30	30	30
	Number with a lesion score	**-**	**9**	**4**	**6**	**0**

The proportion of animals in each lesion score category for farms A and B is shown in Table 6. There was no obvious change in the pattern of lesion scores on these farms throughout the study, although many more animals had lesions, and had a lesion score > 1, compared to farms C and D.

Table 6: The proportion of animals in each lesion score category at each visit on farms A and B

	Visit number	Lesion score						
		0	**1**	**2**	**3**	**4**	**5**	**6**
Farm A	**1**	50%	50%	0%	0%	0%	0%	0%
	2	20%	40%	40%	0%	0%	0%	0%
	3	20%	40%	40%	0%	0%	0%	0%
	4	17%	42%	17%	25%	0%	0%	0%
	5	-	-	-	-	-	-	-
Farm B	**1**	-	-	-	-	-	-	-
	2	42%	33%	25%	16%	0%	0%	0%
	3	30%	40%	30%	0%	0%	0%	0%
	4	0%	100%	0%	0%	0%	0%	0%
	5	-	-	-	-	-	-	-

4.2.5 PCR

All of the samples taken on farms A and B and 43% of those from farms C and D were tested for BHV-1 and -5. All of the samples tested were negative for both of these.

In addition to the PCR used to test for BHV-1 and -5, a universal PCR for herpesvirus was used on all the samples from farms A and B, and a proportion of those from farms C and D (36 and 32, respectively). There were 108 animals tested in total, and 44 were positive to the universal PCR. Some of the positive samples were sequenced to determine which herpesvirus was present. BHV-6, or Bovine Lymphotropic Herpesvirus (BLHV), was identified in 26 of 33 (79%) samples sequenced. This is a gamma-herpesvirus, unlike BHV-1 and -5 which are alpha-herpesviruses.

Figure 1 and Figure 2 show the results for the universal PCR for Herpesvirus, ELISA and lesion score at each visit on farms A and B for both the bulls and cows. On Farm A, sero-conversion occurred in 35% of animals between the start of joining and mid-joining (visits 2 and 3), however this did not relate to a change in lesion score, and there was only a small increase in the number of animals that were actively shedding BHV.

On Farm B, there appeared to be an increase in the number of animals that seroconverted, had a lesion score and were actively shedding BHV between mid-joining and the end of joining (visits 3 and 4). On this farm at visit 3, both bulls were observed to have clinical signs described by the veterinarian taking the samples as typical for the syndrome and was therefore replaced with previously unused bulls. However, only 12 animals were tested on this farm, and so the significance of this observation is difficult to interpret.

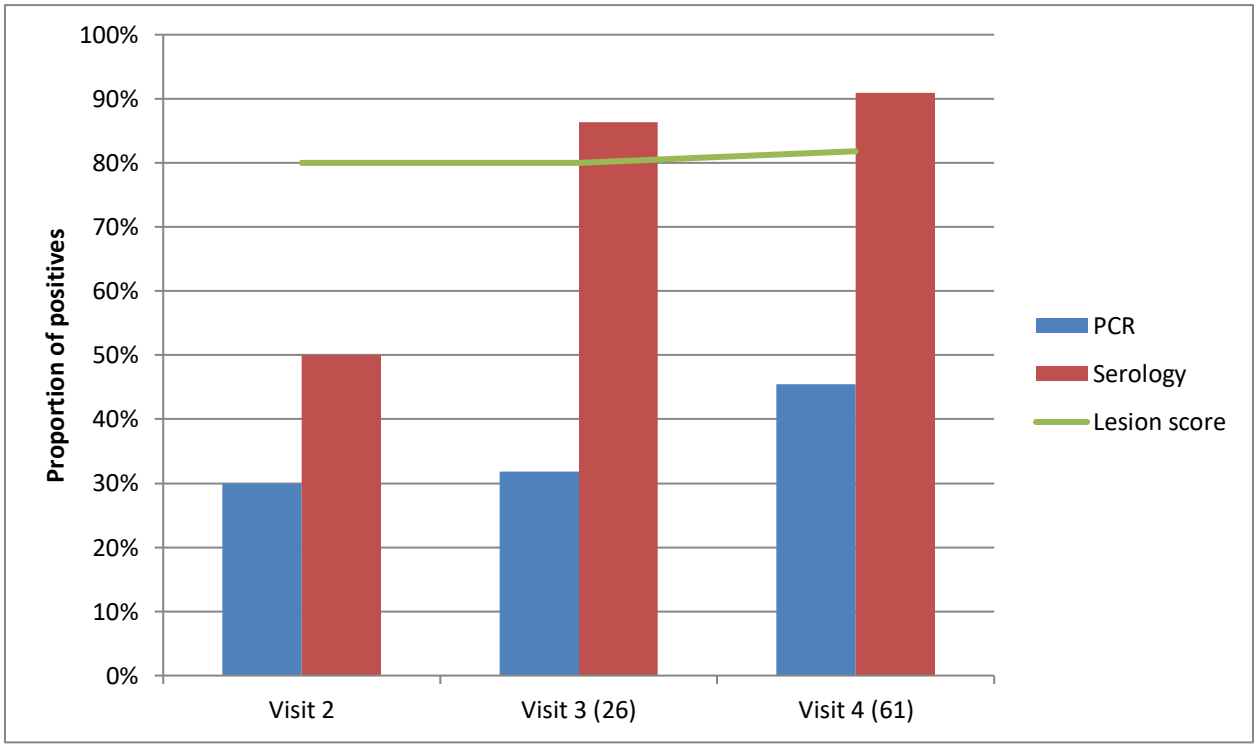

Figure 1: The proportion of positive test results for Bovine Herpesvirus on the universal PCR for Herpesvirus, serology and lesion score at each visit on Farm A (The number of days since last visit are in brackets)

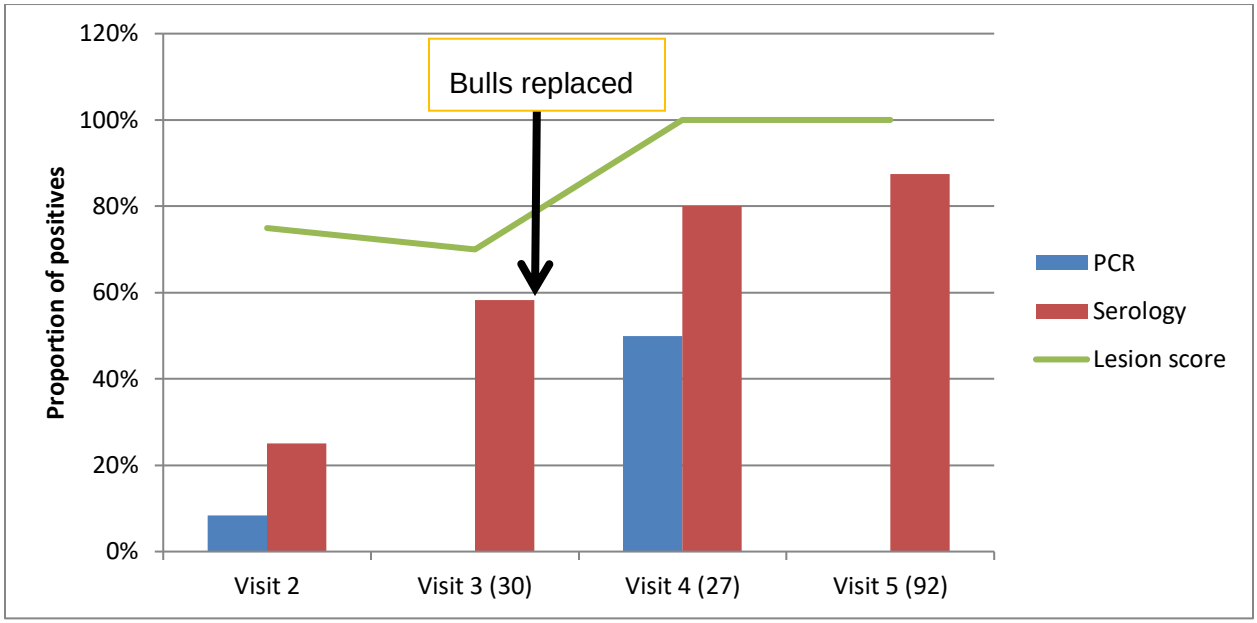

Figure 2: The proportion of positive test results for Bovine Herpesvirus on the universal PCR for Herpesvirus, serology and lesion score at each visit on Farm B (The number of days since last visit are in brackets)

5 Discussion

Veterinarians in southern New South Wales and northeast Victoria are describing a syndrome that is causing 'breakdown' of bulls during the joining period. The description of the syndrome is inconsistent between the regions but both descriptions include balanitis, vulvovaginitis, an unwillingness to mate and reduced conception rates.

In the bulls, the penile lesions have an inconsistent appearance not always typically of known viral causes of balanitis in cattle in Australia, namely Bovine Herpersvirus-1 and -5 (BHV-1 and -5). In southern New South Wales, the reproductive performance of bulls with mild lesions is more severely affected. In comparison, in northeast Victoria bulls are severely affected by balanitis before it is observed that they are unwilling to join.

Some initial studies identified that Bovine Herpesvirus 1 (BHV-1) was associated with balanitis in some bulls, however not all the bulls with clinical signs were positive for BHV-1. More recent studies have implicated another strain of Bovine Herpesvirus, BHV-5, in venereal diseases in Australia (Kirkland, Poynting et al. 2009). Consequently, this study was set up to explore the relationship between Bovine Herpesvirus-1 and -5 and balanitis in more detail.

5.1 Module 1

Module 1 set out to identify evidence of exposure to Bovine Herpesvirus in Victoria. No recent studies had been conducted in this area, with the last investigation completed in 1967 by St. George *et al* (1967). In the current study, evidence of exposure was identified using an ELISA test, which detected glycoprotein-B-specific antibodies to Bovine Herpesvirus 1 (also known as Infectious Bovine Rhinotracheitis (IBR)). However, this ELISA may not be

specific for BHV-1, with Wellenberg et al. (2001) noting that calves experimentally infected with BHV-5 did cross-react to this test. It was unknown whether BHV-6 also cross reacts with the ELISA, however after personal communication with the virologist at The Melbourne University, it is unlikely that there is cross-reaction with BHV-6 based on work they do with equine herpesviruses. There are assays available which can detect the presence of BHV-6 in blood, a quantitative PCR (qPCR) and a nested PCR, with the qPCR being more sensitive (Kubiś, Materniak et al. 2013).

In the study by St. George *et al*, a serum neutralisation test was used to determine the distribution and prevalence of Infectious Bovine Rhinotracheitis (IBR) throughout Australia. They found that the sero-prevalence of IBR in Victoria was 24% of 1382 animals tested, with 38% of 78 herds tested having evidence of past exposure to IBR. However, because the neutralising antibody is not detectable in all cattle after a variable time, these estimates are a minimum. The results from the current study demonstrated that the prevalence of BHV was much higher, with all herds tested having evidence of exposure to BHV. Over half the herds tested had a sero-prevalence of over 50%, confirming that widespread exposure to this potential pathogen is occurring. The overall sero-prevalence was 75% of 1,221 animals tested in southern NSW and Victoria.

A wide variation was seen between mobs, sexes and age groups within herds in all regions. These are likely to be due to differences in management between the herds, and between mobs and age groups within a herd, that will influence the level and frequency of exposure to BHV.

The project objectives for module 1 included the use of a survey to identify a sample of herds that have or have no cases of balanitis, and determination of the sero-prevalence of bovine herpesviruses (BHV-1 and -5) within herds that have or have no cases of balanitis in three regions of Victoria and southern NSW.

The survey was successful in identifying farms which were suspected or affected with balanitis and those which were non-affected. These herds were then sampled to determine the sero-prevalence of BHV in Victoria and southern NSW.

5.2 Module 2

Preliminary investigations into the mid-season breakdown of bulls, conducted by veterinarians in the upper Murray and central NSW areas between 2005 and 2011, indicated that the syndrome they were describing was perhaps not typical of Infectious Pustular Balanoposthitis (IPB) of bulls caused by BHV-1 or -5. The penile lesions had an inconsistent appearance not always typically of known viral causes of balanitis in cattle in Australia, namely Bovine Herpersvirus-1 and -5 (BHV-1 and -5).

Module 2 set out to identify the cause of mid-season breakdown of bulls due to balanitis, and the role that cows may have in the transmission of the syndrome. However, unfortunately this part of the study did not identify any clear aetiology or risk factors for the mid-season breakdown in bulls due to balanitis.

The results from farms A and B will be primarily used in this discussion because balanitis and vulvovaginitis, typical of what was described in past investigations, was identified in a number of bulls and female cattle on these farms.

A universal PCR for herpesvirus was initially used to identify if this virus was present in the penile and vaginal swabs. A third of the initial samples tested were positive, indicating the

presence of a herpesvirus. However, the universal PCR does not identify which herpesviruses are present, and so a PCR specific for BHV-1 and -5 was then used. Interestingly, all the samples which were positive on the universal PCR were negative on the PCR for BHV-1 and -5. The samples to the universal PCR were sequenced, demonstrating the presence of BHV-6 (in 26 of 33 tested). Following this, all the samples on farms A and B were tested using both the universal PCR and the PCR for BHV-1 and -5. If positive for universal PCR, but negative for BHV-1 and -5, they were assumed to be positive for BHV-6. At the start of joining, mid-joining and the end of joining there were 20, 20 and 48% of samples which tested positive to the universal PCR, with all samples returning a negative result for BHV-1 and -5.

There are only a limited number of articles published about BHV-6, which is also referred to as Bovine Lymphotropic Herpesvirus (BLHV). This virus was first identified in the United States of America, with data collected between 1985 and 1997 showing it to be ubiquitous, with up to 91% of cattle positive in Colorado, New York and New Jersey. Subsequently, it has been detected in peripheral blood mononuclear cells from 52-87% and 30% of healthy adult cattle and calves, respectively (Rovnak, Quackenbush et al. 1998, Collins, Bruns et al. 2000, Kubiś, Materniak et al. 2013). The relationship between BLHV and other diseases in cattle, particularly lymphoproliferative disease, is still unclear. It has since been found in cattle in the United Kingdom, Canada and Belgium (Cobb, Banks et al. 2006, Gagnon, Allam et al. 2010, Garigliany, Bayrou et al. 2013). It has also been suggested that BLHV is associated with non-responsive metritis of dairy cattle in the United Kingdom and New Zealand (Banks, Ibata et al. 2007, de Boer, Zheng et al. 2014).

Over 80 herpesviruses have been characterised, and found in insects, reptiles and amphibia, and nearly all bird and mammalian species. Herpesvirus is fragile and doesn't survive well outside of the body, with transmission through close contact. Herpesviruses are classified under the family *Herpesviridae*, with the classification of three subfamilies, *Alphaherpevirinea, Betaherpesvirinae and Gammaherpesvirinae.* BHV-1 and -5 are classified under the subfamily *Alphaherpesviridae* and BHV-6 under *Gammaherpesviridae* (Fenner, Bachmann et al. 2014).

The pathogenesis of BHV-1 and -5 probably follow a similar pattern (Fenner, Bachmann et al. 2014). At the site of infection the virus replicates in epithelial cells, causing disease at this site. However, in some cases it can cause a viraemia and spread to other organs of the body. Generally the incubation period for BHV is two to four days, with the infection lasting from five to ten days in uncomplicated disease. After infection, the virus replicates in the mucous membranes and travels to the ganglia were it becomes latent. Stress, such as mating, can induce reactivation of the virus which can result in shedding of the virus. Sero-conversion occurs within seven to 14 days after infection, with the immune-response presumed to persist life-long, meaning that it can be detected long after the initial infection. Shedding of the virus can be detected five to 14 days after infection or reactivation (Beer 2016). Information on BHV-6 is very limited. Other gammaherpesviruses have a narrow host range with a slow replication cycle. Viruses in this family are specific for either B or T lymphocytes transforming them into tumours. The latent phase is in lymphoid tissue rather than neural tissue like alphaherpesviruses (Fenner, Bachmann et al. 2014).

Due to the time between visits during the joining period (from 26 to 61 days), and the limited number of animals included in this study, it is difficult to draw any strong conclusions about the potential association of BHV-6 with signs of balanitis or vulvovaginitis. Ideally, as was

described in the project protocol, sampling should have occurred 7 to 14 days after the bull was put in with the female cattle. At this time active shedding of the virus would be expected, either from stress, and hence reactivation of disease, or new infections due to transmission from bulls to cows or vice versa. However, sampling at this short interval did not occur due to difficulty with re-mustering and disruption it would have caused to these and other mobs on the farms at this time.

On Farm B, 78% of the animals tested seroconverted throughout the course of the study. The number of animals that had active lesions and were shedding BHV-6 also increased, particularly between visits 3 and 4 (increased from 70 to 100% for lesion score and 0 to 50% for shedding of BHV-6), which corresponded with a change of the bulls following the breakdown of the bulls used at the start of joining. Additionally, only 70% of the heifers pregnancy tested were in calf. The veterinarian who collected these samples reported that this was a classic example of the syndrome he had observed. However, due to the small number of animals tested on this farm, it is difficult to make any strong conclusions about these observations.

From the current study, it is difficult to determine the significance of the BHV-6 infections, and whether or not it is associated with the atypical balanitis reported on farms in southern New South Wales and the upper Murray region of north-east Victoria. However, it is clear from this study that BHV-1 and -5 are not causing the mid-season breakdown of bulls due to this syndrome on these farms.

A recommendation made in a report from Dr. Colin Palmer, an associate professor of Theriogenology (animal reproduction) at Western College of Veterinary Medicine was to check bulls daily during the first three to four weeks after the bulls go out (Palmer 2011). The first month is critical because it is expected that the bull gets 70 to 75% of the cows pregnant in their first cycle. It is during this time bulls are most likely to breakdown, which was observed in our project on two of the four farms. Producers should observe if bulls are injured, settling well with the cows and mounting and serving cows successfully. This is particularly important for single-sire mated mobs. Immediate action needs to be taken to replace bulls which are failing to work and investigate the cause with a thorough clinical examination of the bull.

Throughout the study, more female cattle had lesion scores on farms A and B, compared to female cattle on farms C and D. Keeping this in mind, it was the bulls on farms B and C which were replaced due to lesions on the penis indicative of the syndrome. Due to the inconsistent nature of finding lesions on female cattle and the breakdown of bulls, assessing females for lesions may not be an early indicator of a breakdown in bulls. However, results from this study can't rule in or out females as a mode of transmission and spread of the syndrome.

The project objectives of module 2 were to describe the progression of clinical signs in bulls affected with balanitis in south-eastern Australia, and its effects on serving capacity, and investigate risk factors that may be associated with the syndrome.

There were only two out of 14 bulls which showed signs of balanitis and were replaced soon after the producer identified it. There were insufficient data on lesion score from these two bulls to describe the clinical progression of the syndrome. It was only on Farm B that the syndrome had an effect on serving capacity with only a 70% conception rate at pregnancy testing. This project did not identify any risk factor due to the small number of bulls affected.

6 Conclusions/recommendations

Bovine Herpesvirus-1 and -5 were not found to be associated with mid-season breakdown of bulls on farms in southern New South Wales and the upper Murray region of north-east Victoria. However, an additional Herpesvirus was identified, BHV-6, but the significance of this virus was unable to be determined within the scope and activity of this project.

Additional investigations are needed to fully understand the cause of mid-season breakdown of bulls and the reproductive failure associated with these breakdowns. This syndrome continues to be a major concern for producers in southern New South Wales and north-east Victoria, and possibly other regions of Australia. Some producers are becoming more concerned about purchasing bulls from bull breeding operations for the fear of these bulls breaking down or (re)introducing the syndrome.

This project has raised many questions around bull management and the cause of balanitis in beef herds. Further research and development is needed to answer these questions, with some areas of particular interest outlined below:

1. Rather than concentrating solely on the syndrome, investigate reproductive failure of bulls in beef herds across south-east Australia more broadly. This would include:
    a. A ‘convenience survey’ conducted through Better Beef Networks and key Veterinarians and producers, in the beef industry, including large scale bull breeders, on the causes of bull breakdown due to problems with the penis, and how producers manage these bulls during the mating period. For example, what proportion of producers get bulls examined by a Veterinarian? Also, investigate when large producers cull surplus bulls or bulls which have broken down during the joining period. .
    b. An abattoir surveillance study, if feasible. This would include a quick survey of abattoirs and large scale bull breeders about the patterns of bull kills, and visits to selected abattoirs to observe and take samples from bulls.
        i. Information from this survey would be used to determine if balanitis is more wide spread then north-east Victoria/southwest NSW.
        ii. The samples collected could include the penis and sacral nerves and lymph nodes (potential reservoirs of infectious agents including bovine herpes viruses), depending on logistics.
            - These samples would be used to further work up the cause of the syndrome, particularly the potential involvement of BHV-6 and other bacterial agents, both through the use of a PCR on swabs from the penis but also a PCR of blood to determine the presence of BHV-6 in lymphocytes
2. Examination of bulls if an outbreak occurs in any herds, but particularly in those ‘sentinel’ herds with a known history of this syndrome:
    a. Physical examination of the bull and specific examination of the penis and prepuce
    b. Collection of bloods for both ELISA and PCR to identify the presence of BHV-6 in lymphoblastoid cells
    c. Collection of penile swabs for PCR to identify the presence of viruses or bacteria
    d. Collection of biopsies of lesions from bulls with clinical signs for additional histological and electron microscopy studies

3. As a follow on from the additional research and development suggested above, the development of agreed ‘best practice’ protocols for the management of bulls before and during mating in beef herds would be a useful resource for the industry.

Practical application of knowledge gained from this project is that the involvement of BHV-1 and -5, which were originally proposed as potential causal agents of this syndrome, was ruled out (at least in the material collected from the 4 suspect herds studied). However, the role of BHV-6 needs to be more fully defined by additional research and clinical investigations as outlined above.

Some simple technology transfer activities that the red meat industry could use to get full value from the current project’s findings would be to:

1. Initiate a program to educate producers about how to best identify bulls which are failing to work early in the mating period, which is the highest risk period for bull breakdowns. This is often not done, but could be achieved by routinely undertaking more intensive surveillance of bulls in the first two to three weeks of joining. The (likely) increased detection of compromised bulls during these examinations may then lead producers to investigate the cause using a competent veterinarian if it is not immediately obvious.
    a. A summary of the checklist might include:
        i. Is the bull obviously injured, e.g.. does the bull appear lame?
        ii. Is the bull mixing with the female cattle?
        iii. Do you observe the bull mounting and successfully mating?
        iv. Do you observe cows which are on heat but the bull is not present?
2. An education program could include:
    a. Technical notes and/or information on websites
    b. Presentations via seminars and/ or webinars
3. Present the results and conclusions from the current project to cattle veterinarians at the cattle stream of the AVA annual conference.
4. Develop a course for veterinarians on the safe and effective examination of bulls. This was a limitation encountered in examining bulls in some herds during this project. For example, a full reproductive examination (penis, prepuce, testes and internal glands) and the safe collection of samples is made much easier and safer by competent handling of bulls in crushes, including effective roping techniques.

7 Key messages

From the results of this project it is highly recommended that beef producers should monitor mobs more closely during mating. If they suspect a problem with bulls failing to work, they should immediately respond by removing bulls and having the problem investigated by a competent veterinarian skilled in beef reproduction. This is particularly important in mobs of cows that are single-sire mated.

In addition, whilst not a direct finding from this project, a pre-joining examination of bulls is an initial and critically important step in addressing sub-optimal reproductive performance in a beef herd. This should include an overall examination (health and locomotion, mating/joining competence), plus a physical examination of the penis and testicles, to determine if the bulls are fit for service.

Bull breakdowns can be devastating for beef enterprises, because no calf equates to no income. This project highlights the need for producers to get bulls examined by veterinarians, especially if there is no obvious reason for their breakdown. The financial benefit to producers is avoiding a disaster, either through poor conception rates or the need for an extended mating period due to a change of bulls halfway through the joining period. An extended mating period also creates problems with late-born steers not reaching turn off weight, and late-born heifers failing to reach mating weight.

8 Acknowledgements

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10 Appendix

10.1 Appendix 1: Producer survey

1. Property details

Property location				
Phone number (optional, for contact if further information is required)				
Total property area (Hectares or Acres)		**Hectares**		**Acres**
Total grazing area (Hectares or Acres)		**Hectares**		**Acres**

2. Enterprise details

Please indicate with the number 1, which description best suits your cattle enterprise. If your enterprise comprises of more than one system please indicate the importance using numbers, 1 being your main income source, 2 being your secondary income source, etc.

Stud breeder	**Breeding**	**Breeding and growing**	**Breeding and finishing**	**Specialised growing and backgrounding**	**Specialised finishing on pasture**	**Specialised finishing in feedlots**
	Turning off vealers/ Weaners (<1 yo)	Turning off store yearlings/feeders steers (>1 yo)	Turning off finished steers/bullocks for slaughter	Purchase weaners/yearlings to grow out for specialised finishers	Finish store cattle for domestic or export markets	Feedlots which finish cattle on high-energy diets for domestic or export (JapOx) markets

Other (please describe and indicate what income source it is; i.e. main, secondary, etc)

3. Number of animals

Please indicate the number of animals you TYPICALLY have in each livestock class on the 30th of June

Livestock class	**Number of animals**		**Livestock class**	**Number of animals**
1 yo bulls			**2012-born steers**	
2 yo bulls			**2012-born heifers**	
3 yo bulls			**2013 born calves**	
4+ yo bulls			**Ewes**	
1st calvers (heifers)			**Rams**	
2nd and subsequent calvers			**Other livestock class (please specify _____)**	

If you DO NOT mate cattle on your farm, then the remainder of the survey is not relevant. Thank you for taking the time to fill out the survey. Your contribution is greatly appreciated. Please return the survey in the reply paid envelope supplied. We welcome any comments. A space is provided on page 6.

If you DO mate cattle on your farm, please continue with the survey.

4. Management details

TYPICAL mating start date (dd/mm/yyyy)?	TYPICAL length of mating period (days)?	TYPICAL number of females mated per bull?

Do you have some/all of your females artificially inseminated (AI)?	YES/ NO (circle the applicable answer)
- If YES, what age group(s) of females do you TYPICALLY get AI (i.e. heifers, 2-3 yo, all age groups, etc)?	
How many cycles of AI are performed?	
Do you use "mop-up" bulls to mate those which do not get pregnant from the AI?	YES/ NO (circle the applicable answer)

5. Bull information

Do you breed your own bulls?		YES/ NO (circle the applicable answer)		
Do you lease/share replacement bulls?		YES/ NO (circle the applicable answer)		
Do you purchase replacement bulls?		YES/ NO (circle the applicable answer)		
- If YES, when was the last time you bought a bull? (If you only lease/share bulls, go to section 6. Cow information)				
How many bulls do you TYPICALLY purchase each year?				
Do you TYPICALLY purchase bulls from the same breeder each year?				
Are all your bulls run together when they are not needed for mating?		YES/ NO (circle the applicable answer)		
- If NO, please indicate with a tick how you TYPICALLY manage bull groups? (Please tick one box only)	**Bulls in the same age groups**	**For convenience**	**Based on paddock size**	**Other (specify_____)**
At what age do you TYPICALLY stop using your bulls (years)?				

6. Cow information

Is your herd self-replacing (i.e. do you retain heifer calves for breeding)?	YES/ NO (circle the applicable answer)			
- If YES, how many heifer calves do you TYICALLY retained as replacements each year?				
Do you purchase replacement females?	YES/ NO (circle the applicable answer)			
- If YES, please indicate with a tick what age group(s) of females you TYPICALLY purchase? (Please tick one box only)	**1-2 yo heifers**	**2-3 yo cows**	**4-5+ yo cows**	**Mixed age**
Do you purchase pregnancy tested in calf (PTIC) replacement females?	YES/ NO (circle the applicable answer)			
At what age do you TYPICALLY join your heifers (months)?				
Please indicate with a tick what age group of bulls you would	**2 yo**	**3-4 yo**	**4-5+ yo**	**Mixed age**

TYPICALLY use to mate the heifer portion? (Please tick one box only)				
At what age do you TYPICALLY cull your females (years)?				

7. Reproduction data

Do you routinely have your females pregnancy tested?	YES/ NO (circle the applicable answer)			
- If YES, indicate what age group(s) are TYPICALLY pregnancy tested (i.e. heifers, 2-3 yo, all age groups, etc)				
Please indicate with a tick at what stage during pregnancy are they TYPICALLY pregnancy tested? (Please tick one box only)	**6 weeks**	**2-3 months**	**4-5 months**	**6+ months**
Please indicate with a tick what your TYPICAL calving percentage is? (Please tick one box only)	**<70%**	**70-80%**	**80-90%**	**90-100%**
If you have had years with low calving percentages, have you had problems with any of the following?	**Under weight heifers/cows at mating**	**Bull break down**	**Abortion**	**Other (Specify)**
How many females were mated in 2011 and were due to calve late in 2011 or in 2012?				
How many females were pregnancy tested in calf (PTIC) after mating in 2011?				
Please indicate with a tick what your calving percentage was in late 2011 or 2012?	**<70%**	**70-80%**	**80-90%**	**90-100%**

For each mob managed during the most recent mating period please indicate (for more mobs, go to the page 5):

	Mob 1	**Mob 2**	**Mob 3**	**Mob 4**	**Mob 5**	**Mob 6**
Mating start date?						
Length of mating period (days)?						
How many cows in this mob?						
Age of the cows (years)?						
How many bulls used in this mob?						
Age of the bull(s) used (years)?						
Bull(s) bred on farm? (Yes/No/Some – circle the appropriate answer)	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some
- If NO or SOME, bull(s) purchased (P) or leased/ shared (L/S)?						
- If purchased, indicated YEAR						
If you had any problems which affected the reproductive performance of your bull(s) during mating?(Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
What was the problem? (Lameness, broken penis, failure to work, specify other)						

Continue questions for:	Mob 1	Mob 2	Mob 3	Mob 4	Mob 5	Mob 6
How many days after mating started did you notice problems?						
If the bull(s) was removed during mating? (Yes/No/Some)	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some
If the bull(s) was replaced with another? (Yes/No/Some)	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some
Age of bull(s) removed?						
If you had the bull(s) examined by a vet? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
- If YES, did the vet give a diagnosis? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
- If YES, what was the diagnosis?						
As a result of this mating period, please indicate for each mob (if known):						
How many females were PTIC?						
How many calves were born?						
How many calves were marked?						
If you had any abortions? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
If you lost any calves due to difficult calvings? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No

8. Reproduction data from previous years (Specify for each year if you had problems in more than one year)

In the 5 years prior to your most recent mating, have you had any problems affecting reproductive performance of your bulls?	YES/ NO (circle the applicable answer)		
- If YES, indicate what year(s) you had problems	Year:	Year:	Year:
For each year indicate (if more than one year):			
How many days after mating started did you notice problems?			
Indicate what the problem was? (Lameness, broken penis, failure to work, specify other)			
Did you have the bull(s) examined by a vet?	YES/ NO	YES/ NO	YES/ NO
- If YES, did the vet give a diagnosis?	YES/ NO	YES/ NO	YES/ NO
- If YES, what was the diagnosis?			
Was this bull replaced by another?	YES/ NO	YES/ NO	YES/ NO
As a result of this mating period indicate (for each year if more than one):			
How many females were PTIC?			
How many calves were born?			
How many calves were marked?			
If you had any abortions?	YES/ NO	YES/ NO	YES/ NO

Thank you for taking the time to fill out the survey. Your contribution is greatly appreciated. Please return the survey in the reply paid envelope supplied. We welcome any comments. A space is provided on page 6.

For each mob managed during the most recent mating period please indicate (only relevant if more than 6 mobs):

	Mob 7	Mob 8	Mob 9	Mob 10	Mob 11	Mob 12
Mating start date?						
Length of mating period (days)?						
How many cows in this mob?						
Age of the cows (years)?						
How many bulls used in this mob?						
Age of the bull(s) used (years)?						
Bull(s) bred on farm? (Yes/No/Some – circle the appropriate answer)	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some
- If NO or SOME, bull(s) purchased (P) or leased/ shared (L/S)?						
- If purchased, indicated YEAR						
If you had any problems which affected the reproductive performance of your bull(s) during mating?(Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
What was the problem? (Lameness, broken penis, failure to work, specify other)						
How many days after mating started did you notice problems?						
If the bull(s) was removed during mating? (Yes/No/Some)	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some
If the bull(s) was replaced with another? (Yes/No/Some)	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some	Yes/No/ Some
Age of bull(s) removed?						
If you had the bull(s) examined by a vet? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
- If YES, did the vet give a diagnosis? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
- If YES, what was the diagnosis?						
<u>As a result of this mating period, please indicate for each mob (if known)</u>:						
How many females were PTIC?						
How many calves were born?						
How many calves were marked?						
If you had any abortions? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No
If you lost any calves due to difficult calvings? (Yes/No)	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No

If you would like to make a comment, please do in the space provided below

10.2 Appendix 2: The date and outline of each visit on each farm

Table 7: The date of each visit and an outline of the activities at each visit on each farm (in brackets are the number of animals tested at each visit)

	Farm A.	Farm B.	Farm C.	Farm D.	Activities-BULLS	Activities-COWS
1. Pre-join	19-May-15 (4)		19-April-15 (20)	01-May-15 (30)	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the penis and prepuce placed in virus transport media (VTM) for virology; examination and photograph of the penis and prepuce	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the vulva and placed in virus transport media (VTM) for virology; examination of the vulva
2. Bulls in	Mob 1 2-Jun-15 Mob 2 18-Jun-15 (20)	19-May-15 (12)	21-May-15 (22)	18-May-15 (29)	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the penis and prepuce placed in virus transport media (VTM) for virology; examination and photograph of the penis and prepuce	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the vulva and placed in virus transport media (VTM) for virology; examination of the vulva
3. Mid-join	Mob 1 and 2 14-Jul-15 (22)	18-Jun-15 (12)	5-Jun-15 (29)	4-Jun-15 (30)	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the penis and prepuce placed in virus transport media (VTM) for virology; examination and photograph of the penis and prepuce	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the vulva and placed in virus transport media (VTM) for virology; examination of the vulva
4. End of joining	Mob 1 14-Sep-15 (11)	15-Jul-15 (10)	01-Jul-15 (19)	30-Jun-15 (30)	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the penis and prepuce placed in virus transport media (VTM) for virology; examination of the penis and prepuce	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the vulva and placed in virus transport media (VTM) for virology; examination and photograph of the vulva
5. Pregnancy testing		29-Oct-15 (8)		01-Sep-15 (30)	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the penis and prepuce placed in virus transport media (VTM) for virology; examination of the penis and prepuce	Collection of blood for serology (Bovine Herpesvirus ELISA); Swabs taken of the vulva and placed in virus transport media (VTM) for virology; examination of the vulva

10.3 Appendix 3: Lesion score system

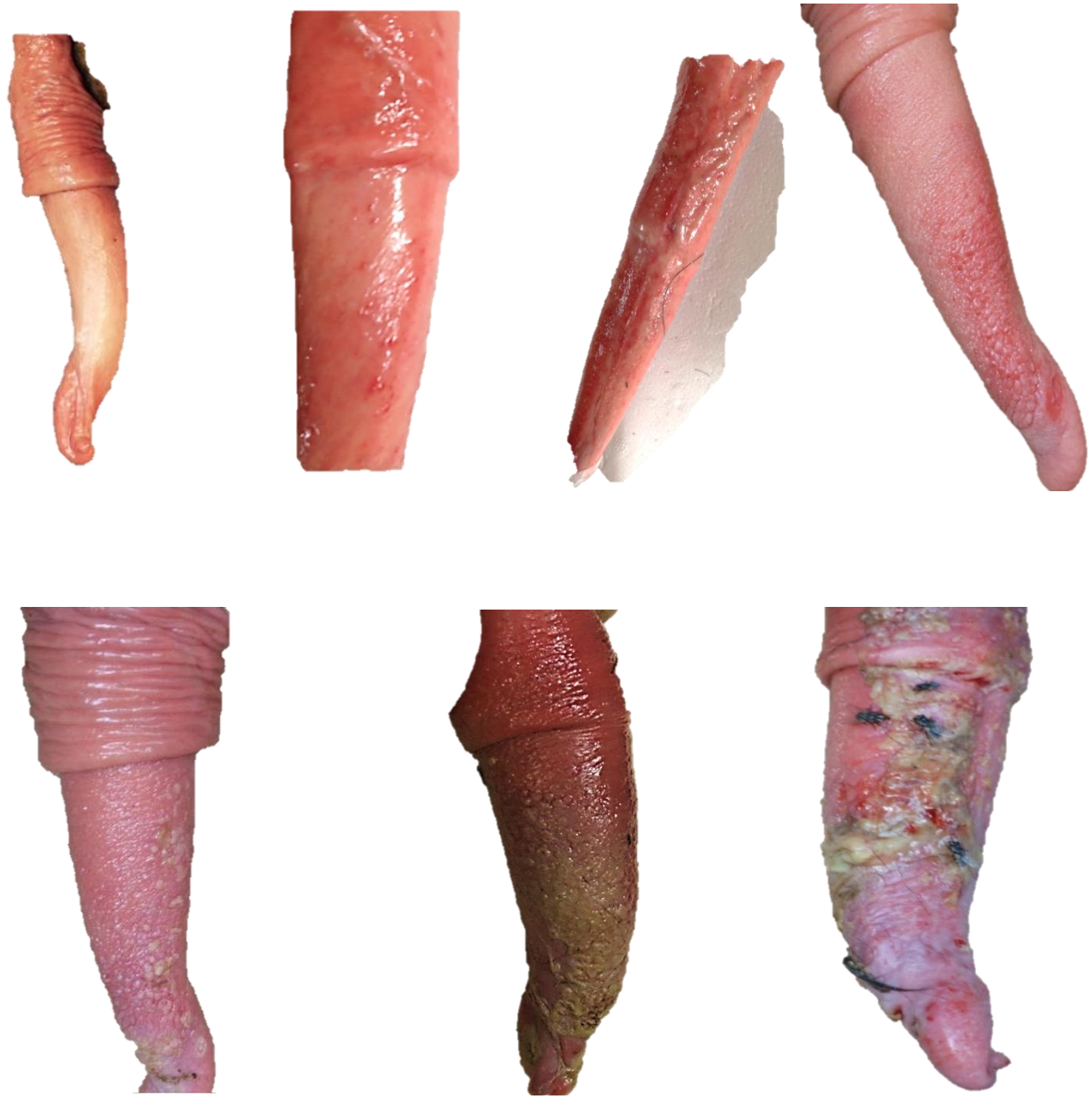

Figure 3: Balanitis lesion scoring system (from left to right lesion score 0 to 6)

10.4 Appendix 4: Survey responses

Below is a summary of the responses from the producer survey. One hundred and eighty one surveys were returned, however only 161 of these were useable due to insufficient data or that breeding cattle was not part of the enterprise. Also, some of these could not be used in every analysis due to missing responses.

10.4.1 Farm characteristics

10.4.1.1 Number of farms per state

Enterprises in higher rainfall areas of south eastern Australia were targeted for the survey. A majority of the producer's farms were located in Victoria, followed by New South Wales, Tasmania, South Australia and a small number in southern Queensland. Figure 4 summarises the location of the farms of producers surveyed.

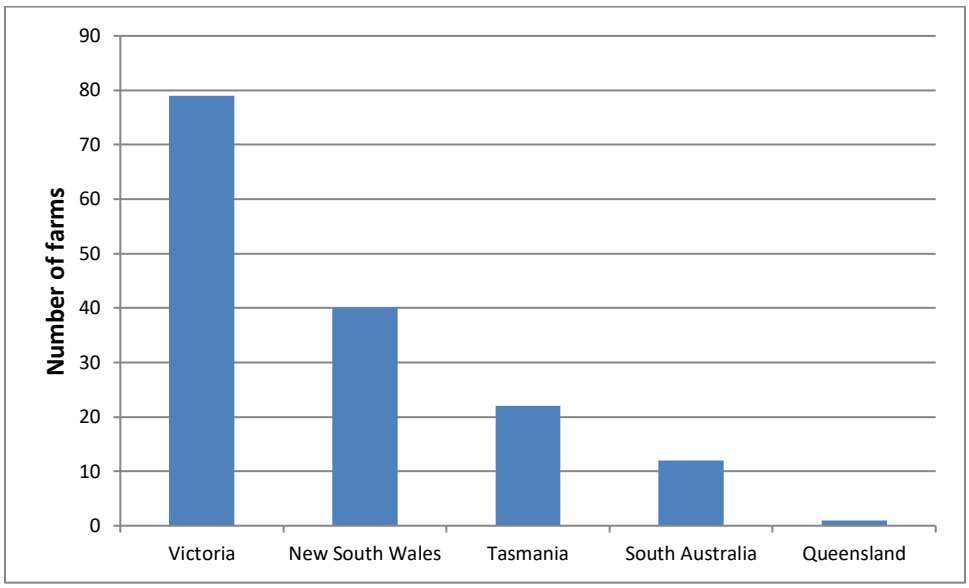

Figure 4: Number of farms surveyed per state

10.4.1.2 Enterprise types and income source

There were eight options for enterprise type and each producers was asked which enterprise contributed as their main, secondary or tertiary income source. These enterprise types are indicated inTable 8: Enterprise type and their description

Table 8: Enterprise type and their description

Enterprise	Description
Stud breeder	Breeds and sells breeding stock
Breeding	Turns off vealers/weaners; less than one year old
Breeding and growing	Turns off store yearlings/feeder steers; older than one year old
Breeding and finishing	Turns off finished steers/bullocks for slaughter
Specialised growing and backgrounding	Purchases weaners/yearlings to grow out for specialised finishers
Specialised finishing on pastures	Finishes store cattle for domestic or export markets
Specialised finishing in feedlots	Finishes cattle on high-energy diets for domestic or export (JapOX markets)
Other	Please specify type of enterprise. Eg. sheep meat/wool, dairy, cropping, off farm income

There were 159 producers who bred cattle as part of their enterprise, 110 indicated that breeding was their main income source and 49 indicated their main income came from a different source. Alternative sources of main income came from sheepmeat or wool (17), while 32 either did not indicate what their main income source was or indicated one of the following as their main income source:

- Dairying,
- Cropping,
- Contracting, or
- Other – off farm income.

Figure 5 shows the numbers of producers indicating which enterprises are their main, secondary or tertiary income sources. Fifty six producers indicated breeding and selling vealers/weaners was their main income source, followed by breeding and growing, breeding and finishing, stud breeding and specialized finishing on pasture, with 33, 18, 9 and 5 producers, respectively. Ten producers indicated more than one enterprise as their main income source.

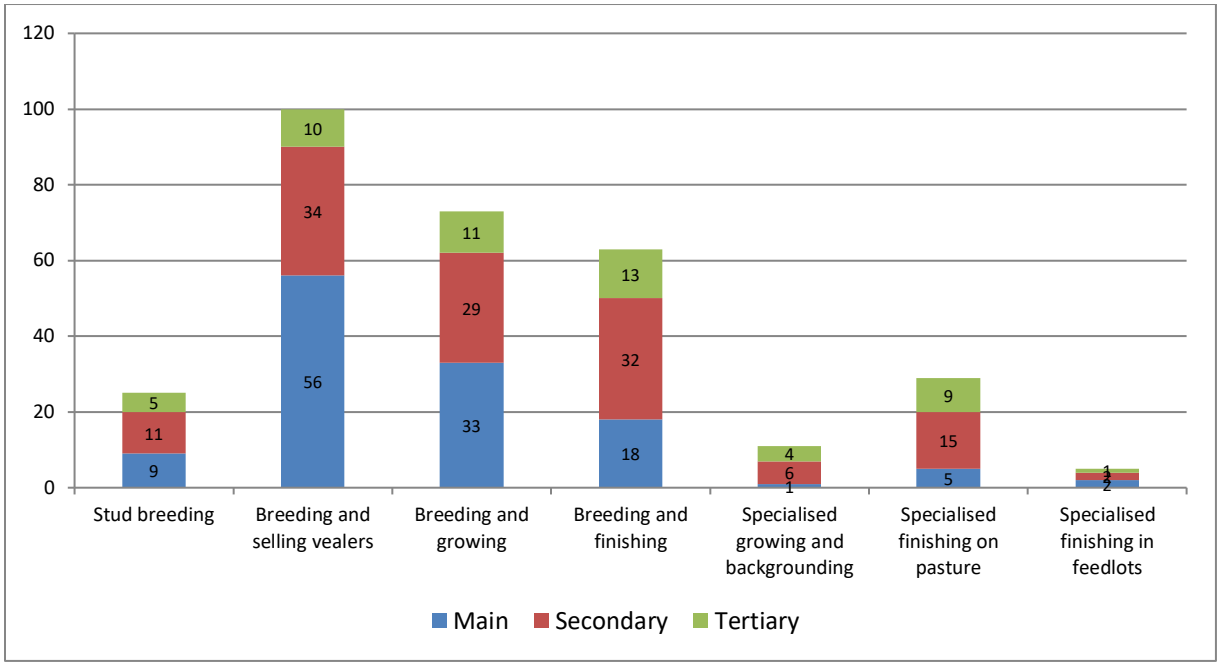

Figure 5: The enterprise producers have indicated as their main, secondary or tertiary income source

10.4.1.3 Farm size

The total grazing area on the farm managed by the producers ranged from 11 to 32000 hectares. Figure 6 shows the number of farms in each property size category. Two thirds of these farms had a total grazing area less than 500 hectares, with a third of farms having between 101 to 500 hectares of total grazing area, and a third having less than 100 hectares.

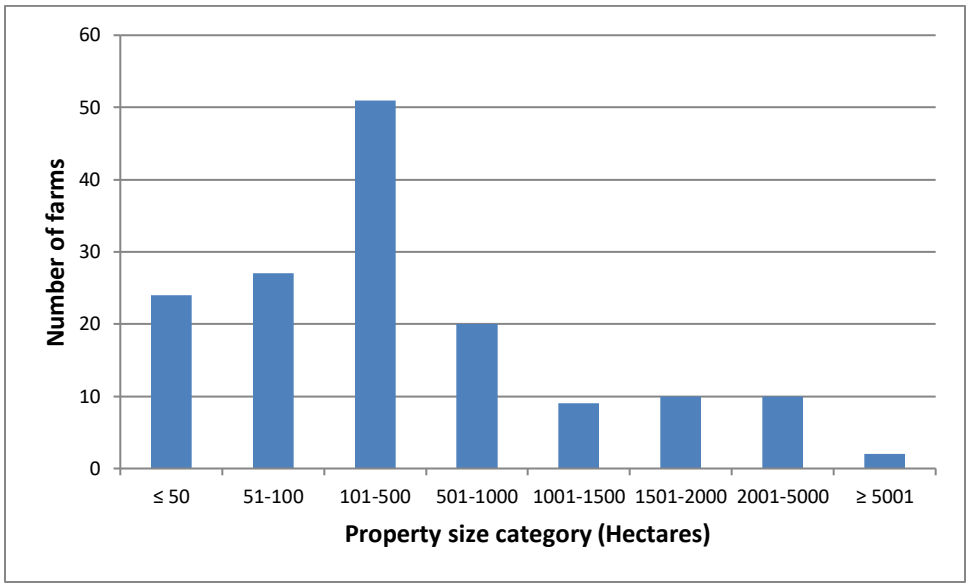

Figure 6: Number of farms in each property size category

10.4.2 Cattle management

10.4.2.1 Reproduction details

One hundred and twenty-six producers ran self-replacing herds, with 25 producers buying some or all of their replacement females.

10.4.2.1.1 Calving start date

The month calving started was taken from the 15th of the allotted month to the 14th of the following month. Figure 7 shows two calving periods, January/February and July/August, with 90 out of 147 producers indicating their cows start calving at one of these times of the year. Fifty producers indicated their cows start calving in late summer (January/February) and 40 in late winter/early spring (July/August). There was no option in the survey to capture those producers who might split join their herd.

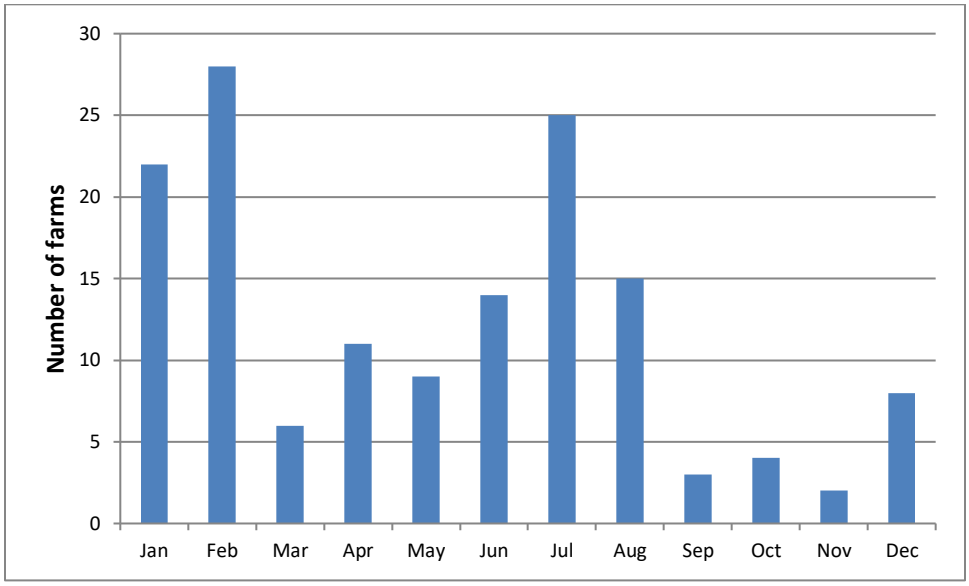

Figure 7: Month of calving (from the 15th of the month to the 14th of the following month)

10.4.2.1.2 Length of joining

At least half of the respondents indicated that they left bulls in with the cows in the range of six to nine weeks as shown in Figure 8. In general, the remaining half joined for either 12 or 13 weeks. Only six producers indicated they joined for longer than five months, with three of these joining all year round.

There was no option in the survey to indicate if there was a different length of joining for second and subsequent calvers compared to heifers, or first time calvers.

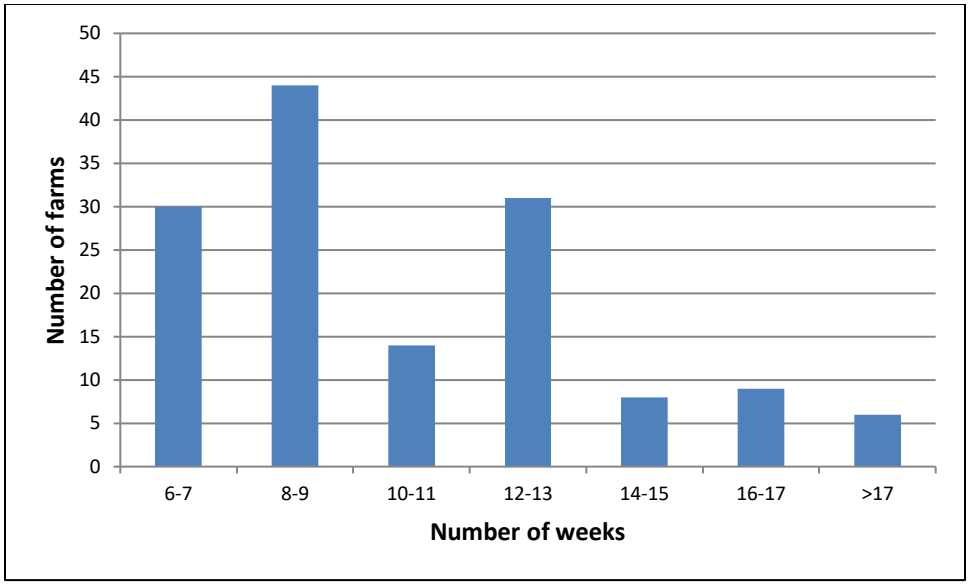

Figure 8: The number of weeks bulls are left in with the cows (joining period)

10.4.2.1.3 Number of cows mated per bull

There were 44 producers (31%) who indicated they run between 31 and 40 cows with one bull at mating time, with 33 producers running between 21 and 30 cows per bull, 25 running between both 11 and 20 cows per bull and 41 and 50 cow per bull (Figure 9). Some producers (6) run more than 50 cows per bull.

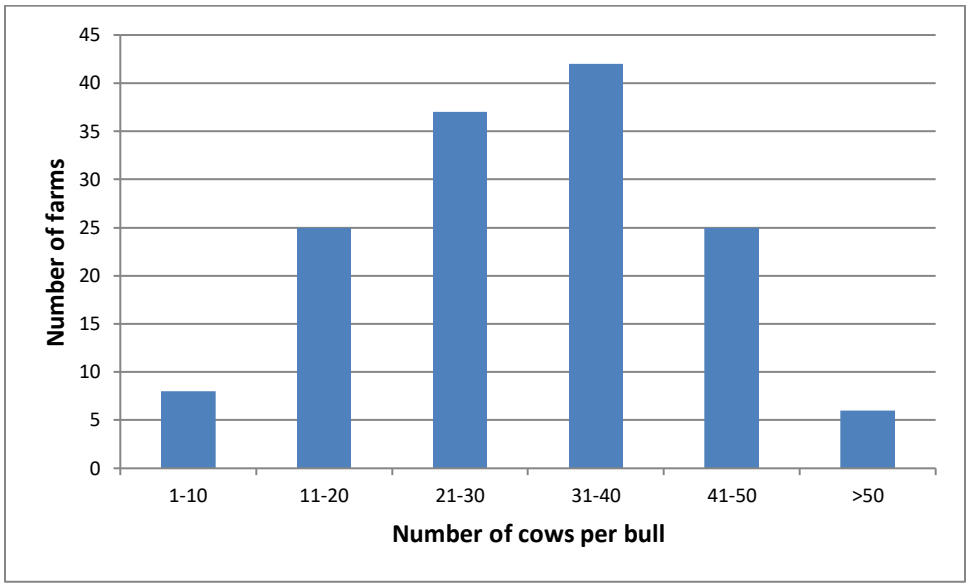

Figure 9: The number of cows mated per bull

10.4.2.1.4 Artificial insemination

Artificial insemination (AI) was performed by 32 of 154 producers. Of these, seven indicated they used AI on 100% of their cows, 11 indicated between 1 and 25% of their cows and five

indicated between 26 and 50%. In general, most producers only used AI for one cycle, with some performing AI for two or three cycles. Most producers, 27, used bulls as 'mop ups' after they had performed AI.

10.4.3 Bull details

10.4.3.1 Number of bulls

The number of bulls run on each farm ranged from 0 to 300, with the median being four bulls. Figure 10 shows the number of farms in each bull number category, with 84 farms running between 1 and 5 bulls. Thirteen producers indicated they didn't have any bulls on their farm, but they leased or shared bulls.

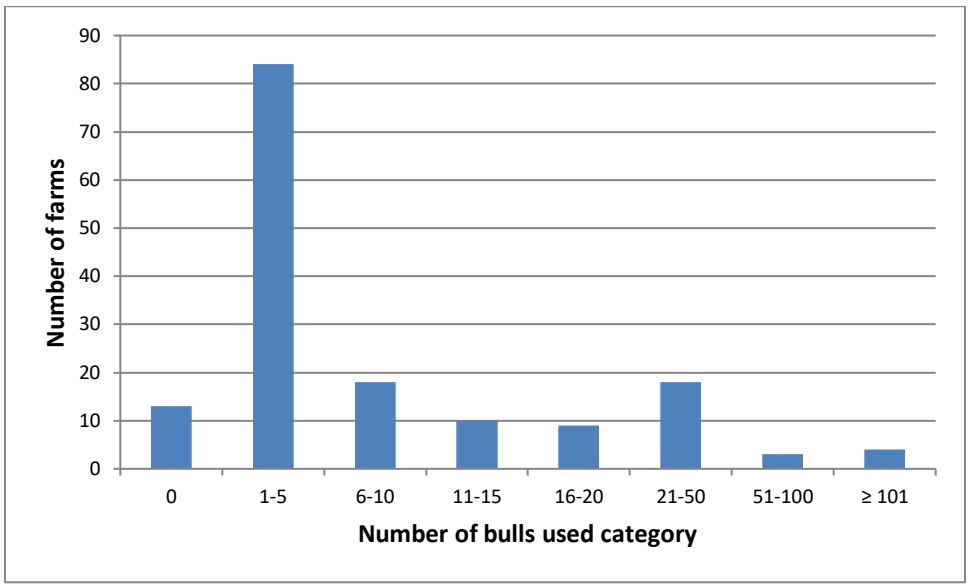

Figure 10: The number of farms in each number of bulls used category

10.4.3.2 Origin of bulls

Of 154 producers surveyed, 48 breed their own bulls. The average number of male calves these respondents kept was 3%, with a range of 1 to 29%. Eight of these producers used only their own bulls, the remaining producers who bred their own bulls, also purchased bulls.

Over half, 55%, of the producers indicated that they only purchased their bulls, 11% both purchased and leased/shared bulls and 5% only leased/shared bulls, as shown in Figure 11.

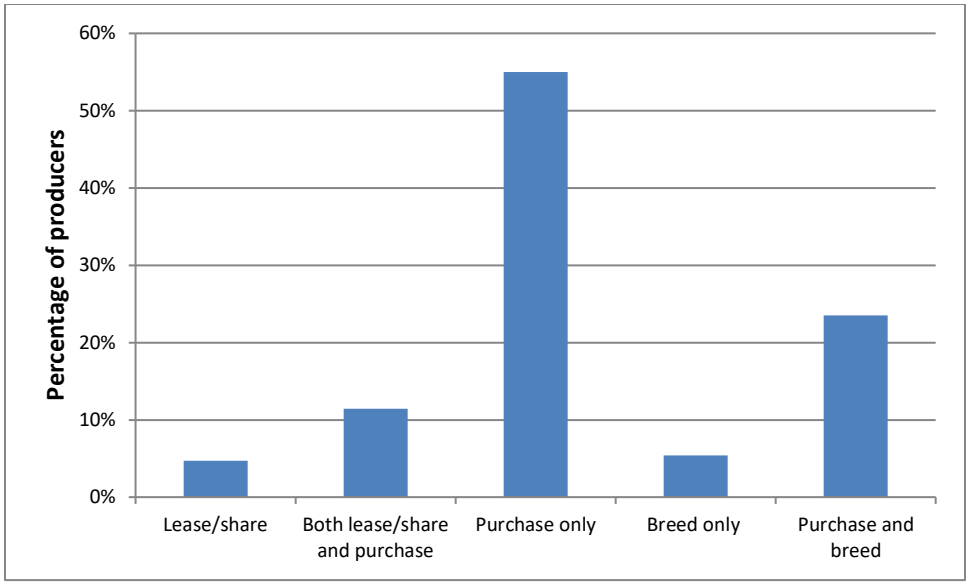

Figure 11: The percentage of producers who lease/share, both lease/share and purchase, purchase only, breed only or purchase and breed bulls

10.4.3.3 Management

When bulls were not used for mating, 84 producers managed all their bulls together in one mob and 49 producers managed bulls in separate mobs. The producers who managed bulls separately typically managed bulls separately for convenience or based on age of the bull.

The average age that producers typically culled their bulls was seven years old with the most being culled between five and eight years. Figure 12 shows the distribution of ages which bulls were culled. Some producers typically culled their bulls as young as three to four years of age. One of these producers had the largest herd and bred their own bulls.

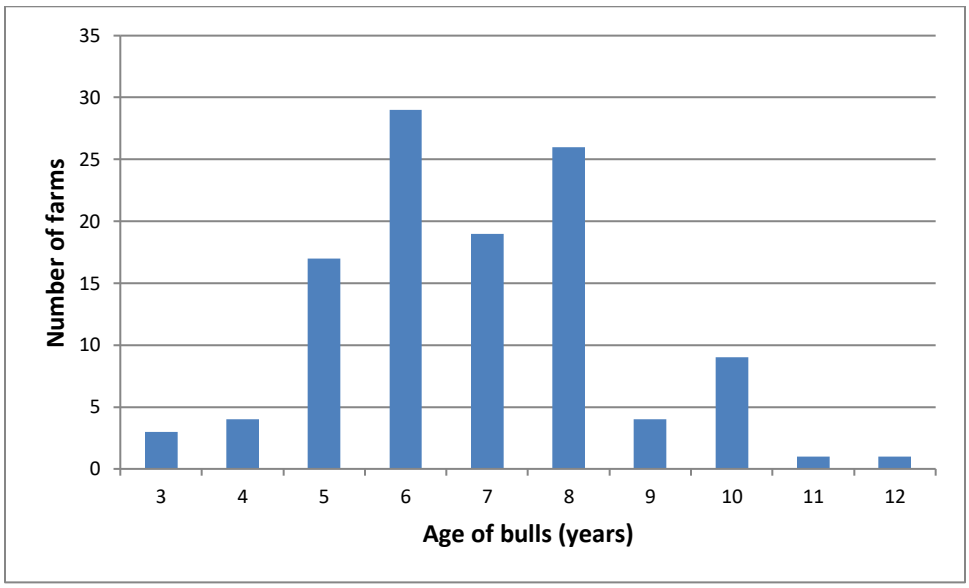

Figure 12: The age bulls are typically culled

10.4.4 Cow details

10.4.4.1 Number of cows

The total number of farms in each breeding cow category is indicated in Figure 13. The number of cows in each enterprise ranged from 2 to 3200 with a median of 79 breeding cows. A large majority of the producers (100) had less than 150 breeding cows in their enterprise. There were 52 enterprises that had more than 150 breeding cows with 10 of these having more than 1000 breeding cows.

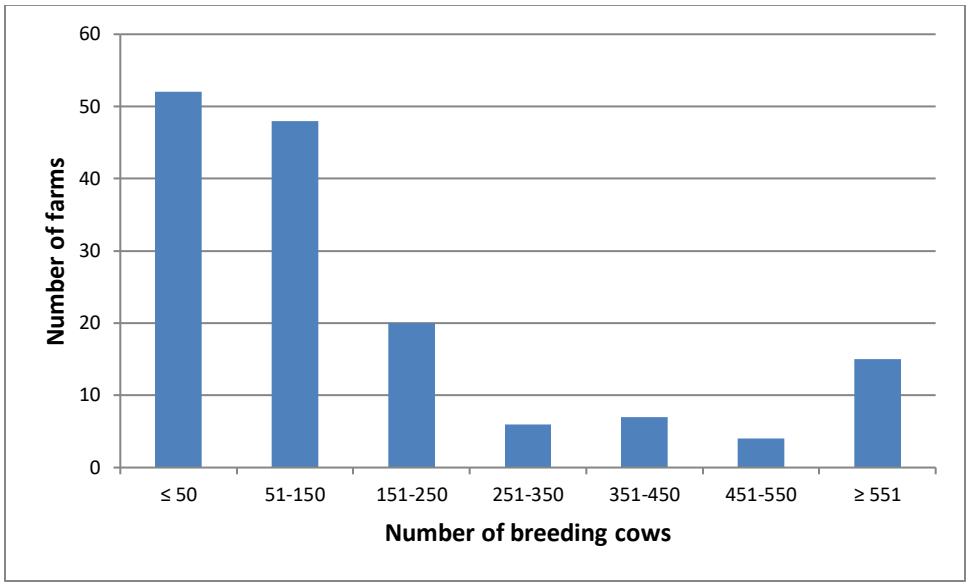

Figure 13: Number of farms in each category of breeding numbers

10.4.4.2 Origin of breeding females

One hundred and five producers did not purchase replacement females, with 47 indicating they did. Of these producers, 23 indicated they purchased 1 to 2 year old heifers, 14 indicated they purchased 2 to 3 year old cows and four indicated they purchased cows four years or older. Six producers purchased replacement females from more than one age group. More than a third (29) of the producers who purchased replacement females, bought females which were already in-calf.

There was no defined pattern to the proportion of heifers typically kept for breeding each year (Figure 14). More than half of the producers (68%) kept 50% or less of the total number of heifers, with 18% of producers keeping between 41 to 50% of heifers.

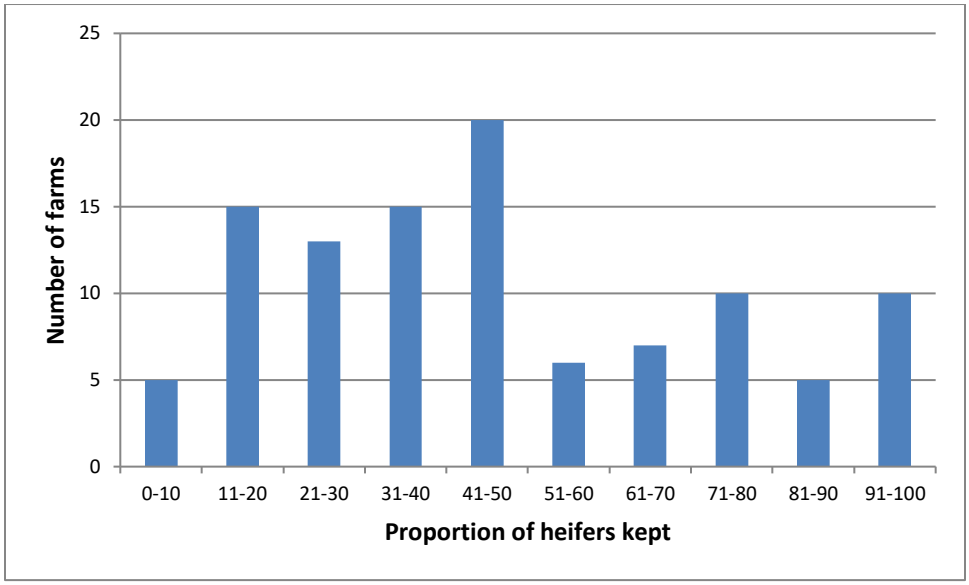

Figure 14: The proportion of heifers, out the total number of heifers, kept for breeding each year

10.4.4.3 Management

The proportion of first calvers compared to second and subsequent calvers on each farm is shown in Figure 15. Forty percent of the producers indicated that between 21 and 30% of the breeding herd was made up of first time calvers, with 90% of producers indicating that 40% or less of the breeding herd was made up of first time calvers.

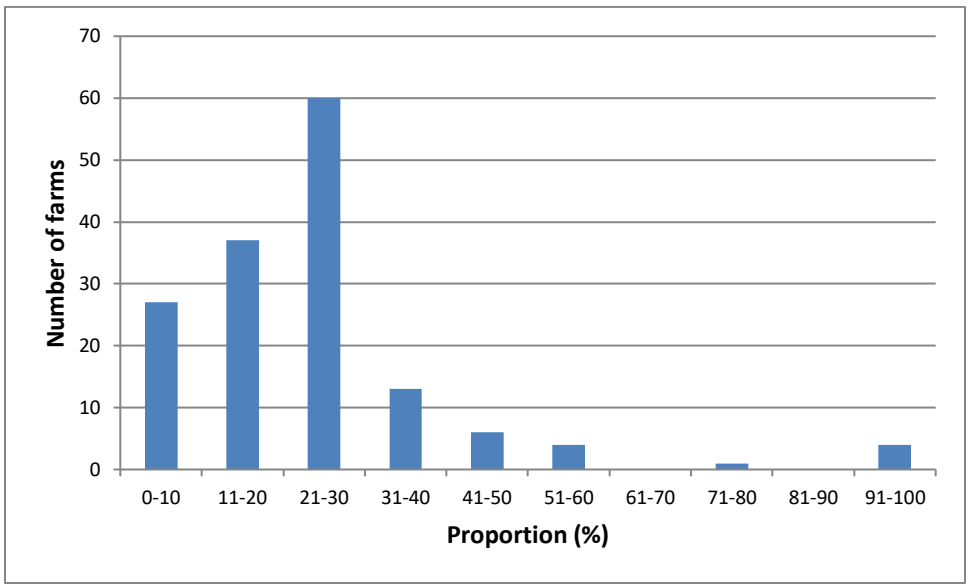

Figure 15: The proportion of first calvers to second and subsequent calvers

Heifers were typically joined at 15 months to calve at 24 months of age, as shown in Figure 16. The age which heifers were joined ranged from 12 months through to 48 months. The age group of bulls used to join to heifers was generally the 1 to 2 year olds (63% of respondents), with 14%, 2% and 21% using 3 to 4, 4 to 5 year olds and mixed aged bulls, respectively.

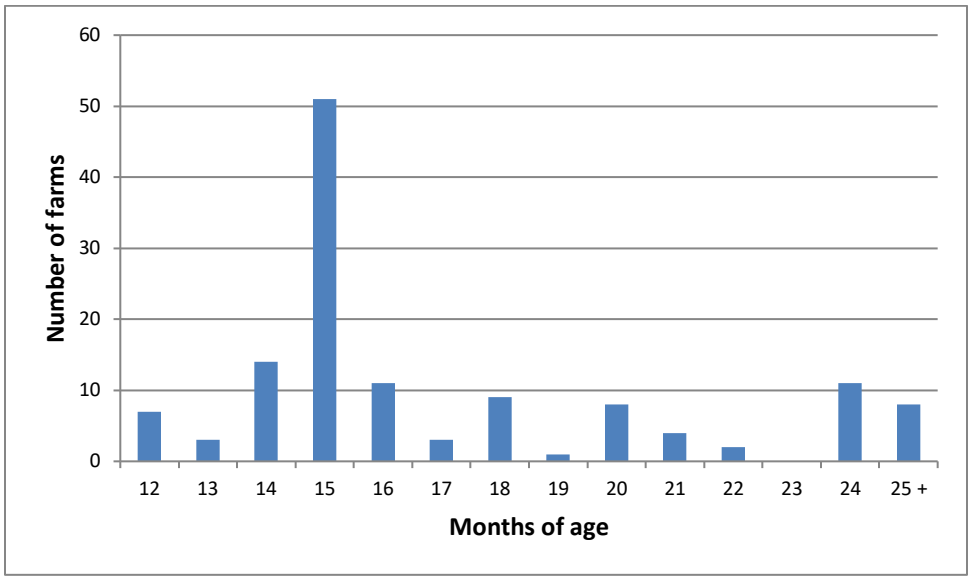

Figure 16: The age heifers are typically joined

The most common age to cull cows was between 8 and 10 years, with almost 60% of producers indicating they cull their cows in this age bracket (Figure 17).

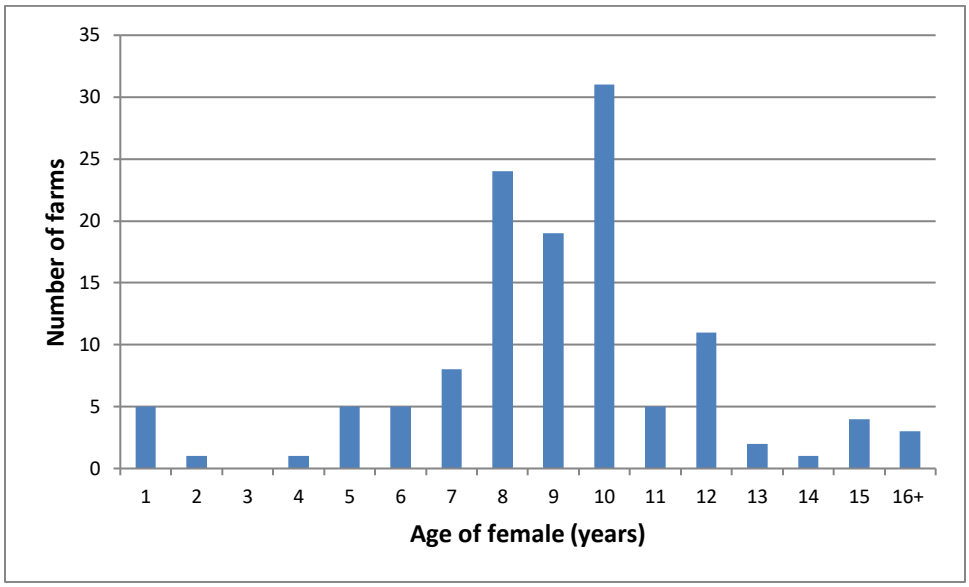

Figure 17: The age females are typically culled

10.4.5 Reproduction details

10.4.5.1 Pregnancy testing

Almost half of the producers (70) had joined females, pregnancy tested, with 50 of these producers indicating all age groups were tested. Some of the other producers indicated they only tested; heifers, mature cows, those up for sale and late joined females.

Typically, females were pregnancy tested between 2 and 5 months after the bull was removed, with 24 producers indicating they had their females tested 2 to 3 months after and 26 indicating 4 to 5 months after. Some producers had their females pregnancy tested 6 plus months after (18) and a smaller number indicated they had their females tested 6 weeks after the bull was removed (8). Some producers had different times for pregnancy testing different age groups, with heifers often tested 6 weeks after the bull was removed and mature cows tested within 2 to 5 months after.

10.4.5.2 Calving percentage

The majority of the producers indicated that the typical calving percentage in their mobs was between 90 and 100% (77%). Twenty percent indicated a calving percentage between 80 and 90% and 3% indicated that between 70 and 80% was typical.

The most common cause of reproductive failure was bull break down, followed by underweight females at joining, abortion and other reasons, 67, 40, 17 and 8%, respectively. Some of the other reasons indicated where Pestivirus, the cows were too old at joining and wet weather at joining.

10.4.6 Bull breakdown

Some producers indicated their bulls had some problem affecting their reproductive performance during mating (15), with 6 producers reporting they had bulls break down in more than one mob. Lameness was the most common cause, followed by problems with the penis and over worked bulls, 10, 7 and 2 producers, respectively.

Of the bulls that had problems with their penis, four had a broken penis and two had a cork screw penis. Unfortunately, only one producer indicated they had the problem investigated by a vet, which was diagnosed as balanitis.

10.5 Appendix 3: Pathology reports

VETERINARY ANATOMIC PATHOLOGY

Faculty of Veterinary Science
University of Melbourne
250 Princes Highway
Werribee Vic 3030
Telephone: (03) 9731 2274
Fax: (03) 9731 2366

Submission Date: 09/06/15
Pathology Number: 506-15
Submitter: L.Tyrell, J. Larsen

Client Details:	
Farm D	Telephone:
Ournie, NSW	
	Fax:
	E-mail:

Patient Details:	Patient Number:	
	Species: Bovine	
Breed: Angus ID #53	Age:	Sex: Male

CLINICAL HISTORY:

Suspect balanitis due to herpesvirus. Samples for histo and EM.

GROSS PATHOLOGY:

Multiple tissue fragments

HISTOPATHOLOGY:

In all the sections examined the epithelium is focally thickened forming elongated rete ridges and there is a mild to moderate hyperkeratosis. The superficial dermis is infiltrated by a moderate amount of inflammatory cells, composed of neutrophils and lesser numbers of lymphocytes, plasma cells and macrophages. The neutrophils also cross the epithelium and form little nests within the epithelium. Focally the thickened epidermis is also eroded. Beneath the eroded area keratinocytes display a moderate ballooning degeneration.

DIAGNOSIS:

Focal, subacute to chronic neutrophilic and erosive epidermitis.

COMMENTS:

The presence of hyperkeratosis and erosions in the epidermis is suggestive of herpesviral infection, although inclusion bodies could not be visualised. Ballooning degeneration, usually suggestive of viral cytopathic effect, was present but possibly related to the ulcerated/eroded area nearby.

Pathologist: Barbara Bacci

Date: 11/6/2015

FINAL REPORT

VETERINARY ANATOMIC PATHOLOGY

THE UNIVERSITY OF MELBOURNE

Faculty of Veterinary Science
University of Melbourne
250 Princes Highway
Werribee Vic 3030
Telephone: (03) 9731 2274
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Submission Date: 09/06/15
Pathology Number: 506-15
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Client Details:	
Farm D	Telephone:
Ournie, NSW	
	Fax:
	E-mail:

Patient Details:	Patient Number:	
	Species: Bovine	
Breed: Angus ID #56, #58	Age:	Sex: Male

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In all the sections examined the epithelium is focally thickened forming elongated rete ridges and there is a mild to moderate hyperkeratosis. The superficial dermis is infiltrated by a large amount of inflammatory cells, composed of neutrophils and lesser numbers of lymphocytes, plasma cells and macrophages. The neutrophils also cross the epithelium and form little nests within the epithelium. Focally the thickened epidermis is also eroded. Beneath the eroded area keratinocytes display a moderate ballooning degeneration.

DIAGNOSIS:

Focal, subacute to chronic neutrophilic and erosive epidermitis.

COMMENTS:

The presence of hyperkeratosis and erosions in the epidermis is suggestive of herpesviral infection, although inclusion bodies could not be visualised. Ballooning degeneration, usually suggestive of viral cytopathic effect, was present but possibly related to the ulcerated/eroded area nearby.