Final report

Innate susceptibility of various cattle breeds to tick fever disease caused by B. bovis and A. marginale

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Abstract

The objective of this study was to compare the relative susceptibilities to tick fever disease of some recently introduced 'exotic' *Bos taurus* breeds of cattle. No information had previously been documented to help assess the risk of tick fever in these breeds. This study is therefore the first detailed assessment reported of the innate resistance of Tuli, Senepol and Wagyu breeds to *B. bovis* and *A. marginale*.

These breeds were shown to be as susceptible to *B. bovis* infection as other more traditional pure *B. taurus* breeds. All the breeds tested were susceptible to virulent *A. marginale* challenge and would be at risk of severe disease if exposed in the field. These results confirm that pure *B. indicus* cattle are relatively resistant to *B. bovis* but susceptible to *A. marginale*. This information can be used by producers who use these breeds or their crosses to better evaluate the risk of tick fever disease and implement appropriate control measures as required.

Executive summary

Background

In Australia, tick fever is a collective name for a disease of cattle caused by any one of three organisms (*Babesia bovis, Anaplasma marginale* and *Babesia bigemina*) carried and transmitted to cattle by the cattle tick. Previous trials have shown *Bos indicus* cattle to be naturally much more resistant to *Babesia bovis*, the most important cause of tick fever in Australia, than British/European *Bos taurus* breeds, which are quite susceptible. *Bos indicus/Bos taurus* crosses have levels of resistance somewhere in between. All breeds are susceptible to anaplasmosis. Since those trials in the mid 1990s, there has been increasing infusion of other, exotic breeds into the northern Australian beef herd. Many northern Australian pastoralists and the larger pastoral companies have also standardised their base breeding herds as composites, some of which contain infusions of these exotic genotypes in combination with *Bos indicus* and British and European *Bos taurus* breeds. However, little information is available on the susceptibility to tick fever of breeds such as Wagyu, Senepol, and Tuli (all regarded as *Bos taurus*).

Objectives

In the trial described here these breeds were compared to a European *Bos taurus* and pure *Bos indicus* breed. In addition the susceptibility of two crossbred (or composite) groups was assessed. Composite A comprised 75% *Bos taurus* genotypes, including 25% Senepol; Composite B contained 50% *Bos taurus* genotypes.

Methodology

Naive cattle representative of these breeds were artificially inoculated with virulent tick fever organisms. Susceptibility to *B. bovis* was assessed first followed by that to *Anaplasma marginale*. Each animal was monitored after inoculation to assess the level of tick fever organisms in the blood, the development of anaemia and, in the case of *B. bovis* infection, also fever.

Results/key findings

The results were consistent with those of the previous trials. Pure *Bos indicus* cattle were quite resistant to *Babesia bovis* infection while pure *Bos taurus* breeds were, as expected, quite susceptible. Interestingly, the Tulis, Senepols and Wagyus were as susceptible to *B. bovis* infection as the European cattle with development of marked anaemia and fever. Similar percentages in each of the breed groups required specific treatment. The composite or crossbred groups had intermediate susceptibility to *B. bovis* infection, based on the criteria of parasite levels and the degrees of anaemia and fever that resulted. All the breeds, even pure *Bos indicus* and crossbred groups, were quite susceptible to infection with *Anaplasma marginale*.

Whilst pure *Bos indicus* breeds are quite resistant to the effects of *B. bovis* infection, this effect declines quite quickly as the *Bos taurus* content increases. Consequently, as the *Bos indicus* content decreases, the tick fever risk will increase. All breeds of cattle, even pure *Bos indicus*, are very susceptible to anaplasmosis. Even exotic and tropically adapted *Bos taurus* breeds such as Tuli and Senepol are susceptible to all forms of tick fever.

Future research and recommendations

This information can be used by producers who use or are considering these breeds to better evaluate their risk of tick fever disease and implement appropriate control measures as required.

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1. Background

Tick fever is the general or collective name given to babesiosis and anaplasmosis, which are economically important tick-borne diseases of cattle. It is caused by any one of three organisms, all of which are spread by the cattle tick, *Rhipicephalus (Boophilus) microplus.* The most important cause of tick fever in Australia is *Babesia bovis,* followed by *Anaplasma marginale. Babesia bigemina,* the third causal organism, is of relatively minor significance. The disease is generally confined to the warmer, humid northern climates of Australia which support the existence of the cattle tick populations.

It has been known for some time that *Bos indicus* cattle are naturally less susceptible to tick fever disease than *Bos taurus* breeds. This knowledge has been used extensively in the cattle tick endemic areas of northern Australia for many years, with the majority of cattle used in these areas containing a high proportion of *B. indicus.* However, research conducted by Bock *et al* [1] in the mid-1990s demonstrated that while the resistance possessed by *B. indicus* cattle (represented by Brahman) to both *Babesia* species is quite strong, they are still quite susceptible to anaplasmosis. They also demonstrated that, as the proportion of *B. indicus* is reduced in crossbred cattle, innate resistance also decreases.

While *B. indicus* breeds seem much better suited to the harsher northern conditions (in terms of resistance to ticks , heat; and the general environment), market forces have led to cross breeding with 'softer' *B. taurus* breeds to improve carcase quality and yield. However, this in turn increased the risk of tick fever.

More recently, exotic breeds containing genetics from African Sanga breeds, such as Tuli (from Zimbabwe) and Senepol (developed in the Caribbean), have been introduced to Australia. These are actually *B. taurus* breeds developed in cattle tick and tick fever endemic regions and are marketed as being more suited to harsher environments than the traditional British or European *B. taurus* breeds.

Many northern Australian pastoralists and the larger pastoral companies have also now standardised their base breeding herds as composites. In some cases, the desirable *B. taurus* production characteristics have been introduced by incorporating these exotic breeds into the composite. These breeds like *B. indicus,* are hardy, adaptable and more suited to the rigors of harsher environments; and thereby allow reduction of the *B indicus* content. These northern composites then might contain infusions of these exotic genotypes in combination with *B. indicus* and British and European *B. taurus* breeds. However, no information is available on the relative susceptibility of these breeds to tick fever.

Also, with the demand for Wagyu cattle increasing, some producers in northern Australia have also recently moved to this breed for selected markets. These are a *B. taurus* breed, developed in Japan from native Asian cattle which were crossed with various European breeds in the late 1800s. Similarly, no information is available regarding their susceptibility to tick fever.

2. Objectives

The objectives of this project were to compare the relative susceptibilities of some of these recently introduced 'exotic' breeds, and two composites used by some of the larger northern Australia pastoral companies, with traditional B. *taurus* and B. *indicus* breeds. This information can then be used by northern beef producers to make informed decisions about tick fever risk management strategies.

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 Experimental animals

At least ten heifers 15-18 months of age of each of Brahman, Charolais, Tuli, Senepol and Wagyu breeds, plus two composite 'breeds' used by large pastoral companies in Northern Australia, were purchased from cattle tick free areas of Australia and transported to the Tick Fever Centre (TFC) in Wacol, southeast Queensland. According to the pastoral companies from where the composite groups were sourced, Composite A contains approximately 75% *B. taurus* genetics (including Senepol as well as British and European breeds), and Composite B contained approximately 50% *B. taurus*, (comprised of the more traditional British and European breeds).

The Charolais group represented the more traditional *B. taurus* breeds and was therefore used as a 'susceptible breed' control group. The Brahman group represented the *B. indicus* animals and was used as a 'resistant breed' control group .The other groups were compared with these two control groups.

Where more than ten individuals from a particular group had been sourced, ten were selected based on body weight and/or phenotypic appearance so as to produce as uniform group as possible. The remaining animals were grouped together to form the uninfected control group against which to compare rectal temperatures for the *B. bovis* challenge trial.

The selected animals were used in two separate trials: firstly, challenge with a virulent isolate of *B. bovis;* and, secondly, challenge with a virulent isolate of *A. marginale.*

All animals were tested for antibody to *B. bovis, B. bigemina* and *Anaplasma spp* using an ELISA to confirm they were naive for tick fever disease prior to commencing the trials. They were maintained in cattle tick free paddocks at TFC for the duration of the project.

Cattle were also assessed for infection with benign *Theileria* species using an ELISA test. As some of the cattle were naive for this blood parasite and it is endemic in paddocks at TFC, the cattle were artificially infected with benign *Theileria* spp, so that concurrent naturally acquired infection with *Theileria* would not confound the results during the challenge trials.

As it turned out, approximately 75% of animals had already been naturally exposed to *Theileria* by the time of inoculation, as determined by ELISA testing of blood samples collected on the day of *Theileria* inoculation. Pack Cell Volume (PCV) monitoring following inoculation showed very little depression, even in the *Theileria*-naïve animals.

Animals were then assigned to groups based on breed/genotype. Selection of individuals for inclusion into the project was performed by the original owners of each group, and whilst probably not truly random, can be considered representative for each genotype.

Animals were assigned a randomly generated number between 1 and 70 which was used to record smear, PCV and rectal temperature results for both trials. The laboratory technicians were thus unaware which animals belonged to which breed group to minimise bias.

Naïve splenectomised *Bos taurus* calves, 3-6 months of age that had been bred in a cattle tick-free area of Queensland and housed in cattle tick free, mesh-floored calf pens at the Tick Fever Centre, were used to prepare the infective blood for each challenge trial.

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3.2 Parasites

The two virulent parasite isolates for the challenge inoculum were derived from blood collected from clinical cases and subsequently inoculated into splenectomised calves, from which blood was collected and cryopreserved in liquid nitrogen for long term storage as a stabilate. The *B. bovis* isolate was obtained in 1988 and designated W isolate [2]. The *A. marginale* parasite was isolated in 1975 in north Queensland and designated Gypsy Plains isolate [3].

To produce infective blood for challenge inoculum in each trial, a splenectomised calf was inoculated with the appropriate stabilate, according to the method of Timms et al [4]. Cattle were challenged by inoculating $1x10^8$ parasites of the appropriate species intravenously for each trial. The *B. bovis* trial (Trial 1) was performed first, followed by the *A. marginale* trial (Trial 2), with a 7 week recovery period between the end of Trial 1 and the start of Trial 2.

3.3 Measurement of response to infection

Criteria to assess reactions after infection included parasitaemia, depression in PCV, the need for specific therapy, and, in the case of *B. bovis,* temperature rise or fever [5].

Packed cell volume (PCV) is the percentage of blood volume that is occupied by red blood cells and is used as an indicator of red blood cell loss or destruction. PCV depression was calculated for each individual as the maximum percentage depression below the mean of preinfection values, which had been established for each animal on five separate occasions prior to infection. The mean of these maximum PCV depressions was calculated for each group. PCVs were measured prior to the start of Trial 2 to confirm that they had returned to within the normal range before this trial commenced.

Temperature rise (fever) in the *B. bovis* trial was expressed as each animal's rectal temperature minus the mean of comparable uninfected cattle (temperature control animals) for that day.

Parasitaemia was determined by examining Giemsa-stained blood films using oil immersion light microscopy under 1000x magnification. However, the parasitaemia was expressed differently for each of the parasites. For *B. bovis,* peripheral (tail tip) blood films were collected for examination and the 'per high power field' parasitaemia recorded each day. These daily parasitaemias were used to calculate a parasitaemia score using the method of Callow and Pepper [5]. For *A. marginale,* blood was collected from the coccygeal (tail) vein/artery to produce the blood smears. Parasitaemia was calculated as the Percentage Parasitised Erythrocytes (PPE), the maximum of which for each animal was used for statistical analysis.

3.4 Treatment of infection

Treatment to control *B. bovis* infection was administered if any one of the following criteria was met:

- PCV less than or equal to 15%
- Cumulative temperature increase above temperature control animals of more than 7.5ºC over 3 consecutive days

- Parasitaemia of more than or equal to six parasites per standard high powered field
- Severe clinical distress

B. bovis infections were treated with Imidocarb (Imidox®, Parnell Laboratories Australia Pty Ltd), at 1.2 mg/kg (1 ml/100 kg) given subcutaneously. All animals were treated with Imidocarb at 3 mg/kg (2.5 ml/100 kg) at the completion of Trial 1 to sterilise the *B. bovis* infection so that all animals were similar with regard to *B. bovis* infection and Imidocarb residues prior to starting Trial 2 [6].

Anaplasma infections were treated with oxytetracycline [6] (Oxytet-200 LA, Ilium Veterinary Products) if any one of the following criteria was met:

- PCV was less than 20% and PPE greater than 5
- PCV was less than or equal to 15%, regardless or PPE
- Severe clinical distress

3.5 Statistical analysis

The significance of differences between groups in their response to infection was determined by analysis of variance (ANOVA) for each variable with error estimated from animal to animal variation. Group means were compared using the protected Least Significant Difference (LSD) procedure at the 5% level [7]. No allowance for treatment was made in analysing data.

4. Results

4.1 Trial 1 - *Babesia bovis*

The results of trial 1 are summarised in table 1.

Table 1: Comparison of the innate immunity of unvaccinated heifers of differing genotype when challenged with a virulent *B. bovis* field isolate (W)

Group	Mean max PCV depression (%)	Total parasitaemia score	Mean max. temperature rise (°C)	Proportion meeting treatment criteria
Charolais	52.7 ± 6.4 [a]	16.2 ± 5.9 [b c]	2.12 ± 0.44 [a]	40%
Tuli	56.7 ± 4.0 [a]	24.8 ± 11.4 [a]	1.95 ± 0.57 [a b]	50%
Senepol	52.1 ± 7.2 [a]	20.8 ± 9.1 [b]	1.39 ± 0.87 [b c]	50%
Wagyu	55.7 ± 5.7 [a]	29.3 ± 12.1 [a]	1.95 ± 0.56 [a b]	60%
Composite A	33.5 ± 7.0 [b]	10.5 ± 5.2 [c]	1.16 ± 0.62 [c]	0%
Composite B	37.3 ± 6.0 [b]	8.9 ± 7.2 [c]	1.15 ± 0.77 [c]	0%
Brahman	20.9 ± 2.6 [c]	1.7 ± 0.9 [d]	0.35 ± 0.34 [d]	0%
LSD's at 5% level	8.3	8.4	0.64	

Notes:

The mean plus or minus the 95% confidence limits are shown for each variable

Within columns, means with a different superscript are significantly different at the 5% level

The Brahman *(Bos indicus)* heifers were significantly more resistant than any other genotype, as evidenced by a lower PCV depression, lower parasitaemia score, lower temperature rise and the fact that none required specific treatment. The Charolais, Tuli, Senepol and Wagyus (all *Bos taurus* breeds) were significantly more susceptible to *B. bovis* than the remaining groups, and 40-60% of the animals in these breeds required treatment. Whilst there was no significant difference between these *B. taurus* breeds in PCV depression, the Tuli and Wagyu animals did have significantly higher parasitaemia scores than the Charolais and Senepols. The only significant difference between these *B. taurus* breeds with respect to fever was that the Charolais group had a statistically higher rectal temperature rise than the Senepols.

The two composite genotypes performed very similarly, with no significant differences between these two groups in any parameter. These composite breeds had an intermediate susceptibility to *B. bovis* and were significantly different to both the *B. taurus* breeds and the Brahman group in PCV depression, parasitaemia (except for the Charolais group) and temperature rise (except for the Senepol group).

4.2 Trial 2 - *Anaplasma marginale*

The results of trial 2 are summarised in table 2.

Table 2: Comparison of the innate immunity of unvaccinated heifers of differing genotype when challenged with a virulent *A. marginale* field isolate (Gypsy Plains)

Group	Mean max PCV depression (%)	Max parasitaemia (PPE)	Proportion meeting treatment criteria
Charolais	39.1 ± 14.8	5.9 ± 6.1	50%
Tuli	48.3 ± 5.6	5.1 ± 2.4	60%
Senepol	35.5 ± 13.3	6.3 ± 5.9	60%
Wagyu	41.1 ± 15.2	7.6 ± 8.6	40%
Composite A	48.6 ± 8.6	5.9 ± 2.6	50%
Composite B	34.7 ± 10.5	3.3 ± 3.6	30%
Brahman	40.1 ± 7.2	3.1 ± 1.3	30%
LSD’s at 5% level	16.3	5.1	

Notes:

The mean plus or minus the 95% confidence limits are shown for each variable

No group differed significantly from any other at the 5% level

There were no statistically significant differences detected between any groups for either variable. Thus, all groups were equally susceptible to *A. marginale.*

4.3 Discussion

The results of this study indicate that breed resistance to *B. bovis* and *A. marginale* varies with the parasite as previously demonstrated [1]. These results are important however as they are the first reports of the relative susceptibility of Tuli and Senepol (two African Sanga-infused breeds bred for hardiness and survival) and Wagyu to *B. bovis* and *A. marginale*. The study therefore provides valuable information for the cattle industry when assessing the use of these breeds in Australia's cattle tick infested areas.

Our results for breed susceptibility to *B. bovis* agree with those obtained by others [1, 8, 9], which showed that *Bos indicus* cattle are significantly more resistant to *B. bovis* than *Bos taurus* cattle. It also confirms that crossbred animals, containing both *B. indicus and B. taurus* genetics, have an intermediate level of resistance. This study extends this work by including the Senepol, Tuli and Wagyu breeds and shows that they are as susceptible as traditional *B. taurus* breeds to *B. bovis* challenge.

It is worth noting that the *B. bovis* challenge in this project appeared to be slightly milder than what is usually seen when working with this particular isolate. This was evidenced by the fact that only 40% of the Charolais group (which was the susceptible control group) met the treatment criteria, whereas it was expected, based on previous experience with susceptible *B. taurus* animals, that 80% or more would have required treatment. The reaction in the splenectomised calf used to produce the infected blood inoculum was also unusual in that the parasitaemia and fever, which are usually features of these infections, declined spontaneously for a few days before increasing again, at which point blood was collected for inoculation of the trial heifers. In hindsight, given the peculiar nature of the progression of this infection, it may have been better to exclude the calf from the trial and inoculate another one in which to produce the inoculum.

Wilson *et al* [10] , Otim *et al* [11] and Bock *et al* [1] reported no statistical difference in susceptibility of *B. taurus* and *B. indicus* cross-bred cattle to *A. marginale*. Our study confirms these reports and indicates that the use of Senepol, Tuli or Wagyu genetics does not change this general breed susceptibility to *Anaplasma spp*. It suggests that *A. marginale* could be a significant cause of disease in all pure and crossbred herds.

The data show that these 'exotic' *B. taurus* breeds are just as susceptible to babesiosis and anaplasmosis as the more traditional *B. taurus* breeds as represented by Charolais in this project. The use of these 'exotic' breeds as pure breeds or as part of a cross or composite breed, therefore, has a similar impact on the risk to tick fever as the infusion of any of the better known taurine breeds. If, for example, Tuli or Senepol are used, because of their tropical adaptability, in composites in lieu of *B. indicus*, the resultant composite may be at greater risk to disease due to tick fever organisms than one which had a greater proportion of *B. indicus.*

5. Conclusion

5.1 Key findings

This project has met its objectives of determining the susceptibility to tick fever of the recently introduced *Bos taurus* breeds, Senepol, Tuli and Wagyu and of commonly used composites comprising 75% and 50% *Bos taurus*. This information will be valuable to producers who are using or are considering introducing these breeds or composites into their operations.

It is evident that tropically adapted *Bos taurus* breeds such as Senepol and Tuli are as susceptible to tick fever as British and European *Bos taurus* breeds. Crossbreeding programs aimed at utilising the hardiness of these breeds in order to reduce the *Bos indicus* content of the desired crossbred or composite may result in animals that are more susceptible to tick fever than crosses with higher proportions of *Bos indicus.*

Likewise, the Wagyu breed is also as susceptible to tick fever as the traditional *Bos taurus* breeds.

5.2 Benefits to industry

Based on the results obtained from this project, producers will be better informed about the risk of tick fever in some of these ‘exotic’ breeds, some of which are promoted as being more suitable to harsher environments. Whilst that may be true in other aspects, it is now known that they are no more resistant to tick fever than other *Bos taurus* breeds.

Producers utilising these breeds, either as purebreds or as part of a cross breeding program, will therefore need to consider other tick fever risk minimisation methods, such as vaccination, if using these breeds. Our results also confirm the susceptibility of all breeds including *Bos indicus* type cattle to anaplasmosis.

The results of this project will be disseminated to northern beef producers and advisors via publication in industry newsletters (for example, Frontier and the Northern Muster) and presentation at the Northern Australian Beef Research Update Conference in 2011. The aim is also to publish the information in a peer reviewed scientific journal.

6. Future research and recommendations

It is therefore recommended that producers take tick fever susceptibility into consideration when introducing these breeds into their beef operation, evaluate the risk of tick fever disease and implement appropriate tick fever control strategies as required.

7. References

1. Bock RE, de Vos AJ, Kingston TG, McLellan DJ. Effect of breed of cattle on innate resistance to infection with *Babesia bovis, B bigemina* and *Anaplasma marginale* [Published erratum appears in *Aust Vet J* 1997;75:449]. *Aust Vet J* 1997;75:337-340.
2. Bock RE, de Vos AJ, Kingston TG, Shiels IA, Dalgliesh RJ. Investigations of breakdowns in protection provided by living *Babesia bovis* vaccine. *Vet Parasitol* 1992;43:45-56.
3. Jorgensen WK, Bock RE, de Vos AJ, Shiels IS. Sheep-adapted *Anaplasma marginale* maintains virulence for cattle. *Aust Vet J* 1993;70:192-193.
4. Timms P, Stewart NP, Dalgliesh RJ. Comparison of tick and blood challenge for assessing immunity to *Babesia bovis*. *Aust Vet J* 1983;60:257-259.
5. Callow LL, Pepper PM. Measurement of and correlations between fever, changes in the packed cell volume and parasitaemia in the evaluation of the susceptibility of cattle to infection with *Babesia argentina*. *Aust Vet J* 1974;50:1-5.
6. Callow LL. Treatment of babesiosis and anaplasmosis in Australia. *the therapeutic jungle*. University of Sydney Post Graduate Committee in Veterinary Science, Sydney, 1978:264-270.
7. Snedecor GW, Cochran WG. *Statistical methods*. 6 edn. Iowa State University Press, Ames, Iowa, 1971.
8. Daly GD, Hall WTK. Note on the susceptibility of British and some zebu-type cattle to tick fever (babesiosis). *Aust Vet J* 1955;31:152.
9. Johnston LAY, Sinclair DF. Differences in response to experimental primary infection with *Babesia bovis* in Hereford, droughtmaster and Brahman cattle. In: Johnston LAY, Cooper MG, editors. *Ticks and Tick borne Diseases Proceedings of a Symposium held at the 56th Annual Conference of the Australian Veterinary Association*. Australian Veterinary Association, Townsville, 1980:18-21.
10. Wilson AJ, Parker R, Trueman KF. Anaplasmosis in *Bos indicus* type cattle. In: Johnston LAY, Cooper MG, editors. *Ticks and Tick borne Diseases Proceedings of a Symposium held at the 56th Annual Conference of the Australian Veterinary Association*. Australian Veterinary Association, Townsville, 1980:26-27.
11. Otim C, Wilson AJ, Campbell RSF. A comparative study of experimental anaplasmosis in *Bos indicus* and *Bos taurus* cattle. *Aust Vet J* 1980;56:262-266.