Effect of breed of cattle on innate resistance to infection with *Anaplasma marginale* transmitted by *Boophilus microplus*

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Objective To assess the innate resistance of and transmission in naive *Bos taurus* cross *Bos indicus* and purebred *Bos indicus* cattle when placed in a paddock with cattle infected with *Anaplasma marginale* and carrying *Boophilus microplus* ticks.

Design A group of 49 purebred *B indicus*, and *48 B indicus* cross *B taurus* (50%, F1 generation) 24-month-old steers were kept in the same paddock with cattle artificially infected with a virulent isolate of *A marginale* and *Boophilus microplus*. The cattle were seronegative for *A marginale* at the start of the trial but had previously been exposed to *Babesia bovis* and *B bigemina*.

Procedure Cattle were inspected twice weekly for 118 days. Whole blood, blood smears and serum samples were collected from the cattle on day 37 after exposure and then at regular intervals to day 83 after exposure to measure packed-cell volumes, parasitaemias and antibody titres to *A marginale*. Any animals that met preset criteria were treated for anaplasmosis. On day 83 all cattle were treated with an acaricide and cattle infected with *A marginale* were removed from the rest of the group.

Results *A marginale* was detected in blood smears from 14 crossbred and 9 *B indicus* steers between days 56 and 72 after exposure. Five and two of the infected crossbred and *B indicus* steers required treatment, respectively. One of the *Bos indicus* cattle died as a result of the *A marginale* infection despite treatment. Antibodies to *A marginale* were detected in the 23 infected cattle. The mean packed-cell volume depression was 40 and 37 % in the affected crossbred and *Bos indicus* groups, respectively. There was no significant difference detected in susceptibility between these two groups.

Conclusions Innate resistance of purebred *B indicus* and crossbred cattle was not significantly different. The results confirm that purebred *B indicus* and crossbred cattle are sufficiently susceptible to warrant the use of vaccination against *Anaplasma* infections.

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Anaplasmosis is a disease of cattle caused by *Anaplasma marginale*. In Australia it is usually transmitted by the vector, *Boophilus microplus*,[1] although mechanical [2] and *in utero* [3] transmission may also be significant. Use of *Bos indicus* cattle as a means of controlling cattle ticks and tick fever has been advocated since 1912,[4] but evidence on the relative susceptibility of *B taurus* and *B indicus* cattle to infection by *A marginale* is contradictory. In Australia, Wilson et al [5] and Otim et al [6] found no breed difference in susceptibility to *A marginale* although Parker et al [7] found *B indicus* cattle to be marginally more resistant to disease than *B taurus*. Others have produced indirect evidence from diagnostic records in Queensland to show that *B indicus* and crossbred cattle were less susceptible to anaplasmosis than *B taurus* cattle.[1, 8]

Because of non-specific immunity in young animals, it is an advantage for cattle to become infected with *A marginale* before 12 months of age.[5] In a serological survey of weaner cattle in 10 shires in northwest Queensland during 1996, Bock et al [8] showed that only 10% had antibody titres for *A marginale*. This was presumed to be due to the combined effects of tick-resistant breeds of cattle and recent droughts, but despite this there are few reports of anaplasmosis from the region.[8] Almost all beef producers in northern Australia now have herds of greater than three-eights *B indicus* infusion.[9, 10]

A live vaccine containing *Anaplasma centrale* has been used in Australia since the introduction of this organism in 1934.[11, 12] However few cattle producers in northern Australia vaccinate their herds.[9, 10]

Bock et al [13] found *B indicus* and crossbred cattle had a significantly lower parasitaemia, but PCV depression was not significantly different from *B taurus* steers after infection with *A marginale*. However, in these trials [13] the innate resistance of some breeds of cattle to *B microplus* and the role this has in the subsequent transmission of tick fever parasites was not considered. The purpose of the present study was to assess the transmission and relative susceptibility to *A marginale* of 24-month-old *B indicus* and crossbred steers following exposure to *A marginale* infected cattle with infestations of *B microplus*. This information is needed to assess the risk of losses due to anaplasmosis following natural transmission and to allow rational decisions on the need for *A centrale* vaccination in *B indicus* and crossbred herds.

Materials and methods

Experimental animals and site

Fifty-one, F1 generation, half *B indicus* (Brahman) cross half *B taurus* (Charolais), and 56 purebred *B indicus* (Brahman) 24-month-old steers from north-western Queensland were purchased from a property that was free of *B microplus*. The crossbred and *B indicus* steers had been used in a similar *Babesia*

TFRC	Tick Fever Research Centre
PCV	Packed-cell volume
CAT	Card agglutination test
DPIQ	Department of Primary Industries Queensland
ELISA	Enzyme-linked immunosorbent assay
BEF	Bovine ephemeral fever
PPE	Percent parasitised erythrocytes
LSD	Least significant difference

spp challenge trial 6 months prior to this study.[14] Between the two studies they were maintained in the same *B microplus* infested 60 ha paddock on a DPIQ research station in south-eastern Queensland. The cattle had no antibodies to *Anaplasma* spp as indicated in a CAT[15] before the trial started. An unvaccinated *B taurus* group was not included because the possible morbidity and mortality represented an unacceptable risk.[13]

Nine 18-month-old *B taurus* (Hereford) steers from the *B microplus* free area of western Queensland were vaccinated with trivalent tick fever vaccine containing *B bovis, B bigemina* and *A centrale* parasites. They were then maintained free of *B microplus* in paddocks at the TFRC for three months before the start of the trial.

Parasites

The *A marginale* isolate was isolated in 1975 in north Queensland in blood collected from a clinical case and was designated Gypsy Plains. At the time of isolation it was inoculated into a splenectomised calf and subsequently stored as a stabilate in liquid nitrogen.[16]

Transmission

The nine vaccinated *B taurus* as well as six *B indicus* steers randomly selected from the trial group were used as carriers of *A marginale*. Each animal was infested with one gram of *B microplus* larval ticks and inoculated with 5 mL of *A marginale* stabilate in groups of three *B taurus* and two *B indicus* on day 0, 2 and 6 of the trial, respectively, before being introduced to the trial paddock. These steers were then grazed with the *B indicus* and crossbred trial steers to facilitate transmission.

Observations

The steers were monitored by twice weekly inspections from horseback for 118 days. They were also mustered 15 times between days 30 and 83 after mixing with the carrier cattle to allow closer examination, sampling and, if necessary, treatment for *Anaplasma*. At each muster, visual estimates of the tick burdens were made. All blood samples were collected from the coccygeal artery or vein using a new 18-gauge needle for each animal. Criteria to assess infection were parasitaemia, depression in PCV and clinical signs. PCV values were determined by microhaematocrit and expressed as a percentage. PCV depression was expressed as the maximum percentage depression below pre-infection values. Parasitaemia was determined by examining thin-blood films stained with Giemsa and expressed as a PPE. Serum antibody to *A marginale* was assessed using the CAT.[15]

Blood for serum, PCV determination and smears was collected from all the trial cattle on days 37, 42, 49, 56, 63, 69, 76 and 83 after exposure. Blood for PCV's and smears was also collected from all animals on days 65 and 72 and from clinical cases only on day 59, 64, 66, 74 and 79.

A marginale infections were controlled with oxytetracycline[17] (Bivatop 200® Boehringer Ingelheim) if one of the following criteria was met: PCV less than 20% and PPE greater than 5, PCV less than or equal to 15% regardless of PPE, severe clinical distress. Infected steers were separated from the remaining cattle and all cattle were treated with moxidectin (Cydectin® pour on, Cyanamid Websters) on day 83. No acaricide treatments were used prior to this. Less intense monitoring was continued for another 35 days at which time all uninfected cattle were vaccinated with *Anaplasma centrale*.

BEF outbreak

Twenty-eight crossbreds and twelve *B indicus* cattle were clinically affected with BEF from days 30 to 42 and treated with the non-steroidal anti-inflammatory drug flunixin meglumine (Finadyne® Novartis Animal Health Australasia). As a result of the outbreak, three crossbreds and one *B indicus* were removed from the trial and euthanased. This reduced the groups to 48 crossbreds and 49 *B indicus*. The BEF outbreak resulted in the trial cattle being yarded on days 30, 31, 35, 39 and 41 for treatments, in addition to the set sampling days.

Analysis

To compare the severity of infection between breeds, the mean PCV decrease and mean maximum PPE were calculated without allowance for treatment of clinical signs, and these data were subjected to one-way analysis of variance. Means were compared using the protected LSD procedure at the 5% level.[18]

Results

A marginale carrier steers

In the unvaccinated *B indicus* carrier steers maximum *A marginale* parasitaemias ranged from 2 to 13 with an average PPE of 5. In the *A centrale* vaccinated *B taurus* carrier steers, four had no detectable parasites, and the range was 0 to 13 with an average PPE of 2.

Measurement of response to infection

A marginale was detected in blood smears from 14 crossbreds and 9 *B indicus* steers between days 56 and 72 after exposure. All 23 cattle had *Anaplasma* antibodies and 5 of the crossbred and 2 of the *B indicus* steers met the preset treatment criteria. One of the *B indicus* steers died from anaplasmosis despite treatment. The results of the *A marginale* infections are summarised in Tables 1, 2 and 3. The mean PCV depression was 40 and 37 % in the affected crossbred and *B indicus* steers, respectively. There was no statistically significant difference detected in susceptibility between the two groups. Light to moderate infestations of *B microplus* were observed in all cattle of both breeds.

Table 1. Effects of A marginale infection transmitted by *B microplus* in *B indicus* and crossbred steers.

Breed	Mean maximum PCV depression (%)	Mean maximum parasitaemia (PPE)	No. treated
B indicus	37	6	2 of 9
Crossbred	40	5	5 of 14
LSD at the 5% level.	14	4	

The F values for both variables were not significant

Table 2. The number of *B indicus* and crossbred steers with detectable parasitaemias and day of first detection after exposure to *A marginale* carrier cattle.

	Number of cattle infected	
Day first detected	Crossbreds n = 48	*B indicus* n = 49
56	8	5
63	5	1
65	1	1
69	0	1
72	0	1
Total infected	14	9

Table 3. Number of *B indicus* and crossbred steers with different degrees of depression in PCV after infection with *A marginale*.

Maximum PCV depression (%)	Number of cattle	
	Crossbreds	*B indicus*
< 20	1	3
21 - 25	1	1
26 - 30	1	0
31 - 35	2	0
36 - 40	2	0
41 - 45	1	2
46 - 50	3	1
> 50	3	2
Total infected	14	9

Discussion

The results of this study show that 29% of the crossbred and 18% of the *B indicus* cattle became infected with *A marginale* under our simulated field conditions. Our results also show that 36% of the crossbred and 22% of *B indicus* infected steers required treatment, but the difference was not significant. This indicates that under similar conditions, *A marginale* could cause significant losses in both crossbred and *B indicus* herds. Our study also showed a marked variation in resistance of individuals within breeds (Table 3) thus confirming the findings of other researchers.[12, 5]

Transmission of *A marginale* by the one-host tick *B microplus* is known to be both stage to stage (transstadial) and within stages (intrastadial) as this species is incapable of transovarial transmission.[19, 20] Mason and Norval [21] demonstrated the interhost transfer of larval and adult male *B microplus* under field conditions and because male *B microplus* survive on cattle for periods exceeding 2 months,[12] they are probably most important in *A marginale* transmission. In our trial, the transmission to purebred *B indicus* and crossbred cattle was essentially the same. *A marginale* parasitaemias in the *A centrale* vaccinated, *B taurus* carriers were lower than those observed in the naive *B indicus* carriers and the *B taurus* cattle tended to segregate from the other two breeds. It is therefore concluded that the six *B indicus* carriers were the main source of infection.

Wagland [22, 23] provided good evidence that *B indicus* cattle previously exposed to *B microplus* acquire high resistance to infestation and about a third as many ticks matured compared to unexposed *B indicus* cattle, but that there is marked individual variation. Johnston [24] also showed that, following initial tick exposure, crossbred cattle are infested with smaller numbers of ticks than *B taurus*. In our trial, all cattle had been exposed to *B microplus* for over 8 months before the trial, but infestation remained clearly visible on both breeds throughout the trial. Because the trial animals were mustered on 15 occasions for sampling and 5 times for other reasons over the trial period of 84 days, opportunities for interhost transfer of *B microplus* would have exceeded those seen under normal field conditions.

Connell [19] showed that the prepatent period averaged 27 and 44 days following intrastadial and transstadial transmission by *B microplus*, respectively. Transmission of Anaplasma can also take place iatrogenically or mechanically by biting insects, but under these circumstances the prepatent period would be expected to be long, because it is dose related. [2] Davis et al [26] put 0.25 mL of *A marginale* infected blood under the lower lid of both eyes in four calves, each of which developed a patent, *A marginale* infection, which peaked in 40, 69, 78 and 144 days. Our trial cattle were sampled from the coccygeal artery or vein on 15 occasions during the trial and mechanical transmission resulting from residual haemorrhage was therefore possible.[25] However, as sampling in the current study did not start till day 37 of the trial and 21 of the 23 cases of anaplasmosis were patent by day 65 (Table 2), we consider it unlikely that iatrogenic transmission was important. There is also little evidence that anaplasmosis is commonly transmitted by biting flies in Australia[12] and we conclude that intrastadial transmission with *B microplus* was the most likely mode of transmission.

DPIQ laboratory records show that of confirmed field outbreaks of tick fever in Queensland, about 11% were due to *A marginale* compared to 82% due to *B bovis*.[8] Because of the relative predominance of *B bovis* and the known resistance of *B indicus* breeds to this parasite,[13] few producers with *B indicus* and crossbred herds in northern Australia vaccinate against tick-borne diseases. By using the results of this study in a Markov chain disease probability model and discounted cashflow analysis,[27] *A centrale* vaccination of *B indicus* and crossbred weaners showed a benefit to cost ratio of 4 to 22 and 7 to 39, respectively in a representative northwest Queensland herd. The range depended on the estimated annual seroprevalence of *A marginale* in yearling cattle in the herd. These results suggest that anaplasmosis may well have a significant effect in these breeds and that preventive vaccination warrants greater consideration than it has received to date.

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BOOK REVIEW

Biological Control of Vertebrate Pests: The History of Myxomatosis, an Experiment in Evolution *by Frank Fenner and Bernardino Fantini, CABI Publishing, UK, 1999, 352 pages, Price: USD110.00, ISBN 0 85199 323 0*

In addition to Pharlap, football and koalas, Australia is famous as the theatre in which was enacted one of the world's most successful biological control attempts against a mammal. I refer, of course, to the rabbit and to the disease myxomatosis which devastated rabbit populations in the early 1950's and continues today as an important aid in rabbit control. 'Myxo' is a household word in Australia today and so too is the name Fenner. Indeed, you cannot think of one without the other. Professor Frank Fenner, in collaboration with Francis Ratcliffe (another Australia icon), wrote his first book on myxomatosis in 1965. It has remained as the standard reference text until this year when Fenner, this time in collaboration with Bernadino Fantini from the University of Geneva, published Biological Control of Vertebrate Pests. Without doubt, this recent book will become the new standard against which all other reviews in this field will be judged.

This updated account is an enormously detailed history of myxomatosis both in Australia and overseas. Here you will also find a scholarly account of the relationship between viruses and their hosts. Fenner and Fantini also give us a fascinating account of a newer biological control agent, Rabbit Haemorrhagic Disease Virus (Calcivirus or RCD in many circles). In three introductory chapters, they give a very useful account of pests in general, of the rabbit in particular, and of various biological control attempts in the past - both the successful and the unsuccessful. The reader is gradually immersed into the main theme so that, by the time he or she gets to the business end of the book, the profound importance of biological control of the rabbit in Australia is made manifest.

In summary, a marvellous book. At a RRP of USD110.00 it may seem expensive but weigh against it lifetimes of experience and toil on the part of people like Fenner and Fantini. Those people who toiled away at the problem, from Guiseppe Sanarelli (who first published on myxomatosis in 1896) through to present-day scientists in Australia, are all in this book which includes a useful 'rogues gallery' of photos with accompanying biographical sketches. Australian veterinarians are well represented. The book is a must for all decent institutional libraries and strongly recommended for all those individuals with an interest in the history of our understanding of diseases.

BL Coman

Dr Coman has a long involvement in vertebrate pest research and is the author of Tooth and Nail, a new book on the history of the rabbit in Australia, which is soon to be reviewed in the Australian Veterinary Journal.