Effect of breed of cattle on transmission rate and innate resistance to infection with *Babesia bovis* and *B bigemina* transmitted by *Boophilus microplus*

RE BOCK, TG KINGSTON and AJ de VOS
Tick Fever Research Centre, 280 Grindle Road, Wacol, Queensland 4076. Bockr@dpi.qld.gov.au

Objective To assess the effect of breed of cattle on the transmission rates of and innate resistance to *Babesia bovis* and *B bigemina* parasites transmitted by *Boophilus microplus* ticks.

Design Groups of 56 purebred *B indicus* and 52 *B indicus* cross *B taurus* (50%, F1 generation) steers were placed in a paddock seeded with and also naturally infested with *B microplus* which were the progeny of females ticks fed on *B taurus* cattle specifically infected with a virulent isolate of *B bovis.* The cattle were placed in the infested paddock 50 days after seeding had started.

Procedure Cattle were inspected from horseback daily for 50 days. Clinically ill cattle were brought to yards and assessed by monitoring fever, depression of packed-cell volume, parasitaemia and severity of clinical signs. Any animals that met preset criteria were treated for babesiosis. Blood samples were collected from all cattle on day 28, 35 and 42 after exposure and antibodies to *Babesia* spp and packed cell volume measured.

Results All steers, except for one crossbred, seroconverted to *B bovis* and *B bigemina* by day 35 and 75% of the crossbred steers showed a maximum depression in packed cell volume of more than 15% due to infection with *Babesia* spp compared with only 36% of the *B indicus* group. Ten of the 52 crossbreds and 1 of the 56 *B indicus* steers showed severe clinical signs. Two of the crossbreds required treatment of which one died 2 weeks after initial treatment.

Conclusions Pure-bred *B indicus* cattle have a high degree of resistance to babesiosis, but crossbred cattle are sufficiently susceptible to warrant the use of preventive measures such as vaccination. Transmission rates of *B bovis* and *B bigemina* to *B indicus* and crossbred cattle previously unexposed to *B microplus* were the same.

Aust Vet J 1999;77:461-464
Key words: Cattle, *Babesia bovis, Babesia bigemina*, breed susceptibility, innate resistance, *Boophilus microplus*.

Tick fever is an endemic disease of cattle in northern Australia caused by the organisms *Babesia bovis, B bigemina* and *Anaplasma marginale* transmitted by the cattle tick *Boophilus microplus*. Use of resistant *Bos indicus* cattle as a means of controlling cattle ticks and tick fever has been advocated since 1912[1] and has been successfully adopted in most parts of northern Australia. Almost all beef producers in far northern Australia have cattle that are more than three-eighths *B indicus*.[2, 3]

Calves from immune dams receive colostral protection to babesiosis. The protection lasts about 2 months and in most cattle this is followed by an age resistance that lasts a further 4 to 7 months.[7] Such calves exposed to babesiosis during the first 6 to 9 months rarely show clinical signs and usually develop a long-lasting immunity. Mahoney et al[5] estimated that if at least 75% of calves in a herd were exposed to babesiosis by 9 months of age a state of endemic stability would prevail and clinical disease would rarely occur. In a serological survey of weaner cattle in northwest Queensland during 1996, Bock et al[4] showed that in 10 shires, *Babesia* transmission rates were much less than those required to achieve endemic stability as defined by Mahoney et al.[5] Similar results from smaller surveys were obtained in this area between 1990 and 1995[4] and during 1997 (our observations). This is presumed to be due to the combined effects of tick-resistant breeds of cattle and recent droughts, but despite the apparent endemic instability in the region, there has been a marked reduction in the number of tick fever outbreaks identified by submissions to DPIQ diagnostic laboratories.[4]

Vaccines containing attenuated strains of *B bovis* and *B bigemina* as well as *Anaplasma centrale* have been available in Australia since 1964,[6,7] but few cattle producers in northern Australia vaccinate their herds.[2,3] Bartholomew and Callow[8] conducted a cost-benefit study of the development and introduction of a vaccine against *B bovis* infection and found a high return. However, their study was based on data obtained almost exclusively from *B taurus* cattle breeds known to be highly susceptible. An economic analysis of the consequences of tick fever in *B indicus* and crossbred cattle in northern Australia has not been attempted to date because quantitative measurement of the effect of disease under conditions of extensive management has been difficult.

Bock et al[9] showed that *B indicus* cattle overcame an inoculation of *B bovis* much more readily than crossbred cattle. In the same study[9] both *B indicus* and crossbreds easily overcame a mild infection of *B bigemina*. However, 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.[9] As part of a continuing study to assess the risk of tick fever outbreaks in northern Australia, we undertook to assess the transmission rates and relative susceptibility to tick fever parasites in 15 to 18-month-old *B indicus* and *B indicus* cross *B taurus* cattle (crossbreds) from this region. The purpose of the present study was to assess the response of naive cattle in a paddock containing large numbers of *B microplus* larvae infected with *B bovis*. This information is needed to assess the

TFRC	Tick Fever Research Centre
PCV	Packed-cell volume
CAT	Card agglutination test
DPIQ	Department of Primary Industries Queensland
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
LSD	Least significant difference

likely risk of losses due to babesiosis and to allow rational decisions on the need for vaccination in extensive crossbred and *B indicus* herds. Because the susceptibility of *B taurus* cattle is well known they were not included in this comparison.

Materials and methods

Experimental animals

Fifty-two half *B indicus* cross half *B taurus* (F1 generation Brahman cross Charolais), 56 *B indicus* 15 to 18 month-old steers from north-western Queensland and nine 18-month-old *B taurus* (Hereford) steers from western Queensland were purchased from properties that were free of *B microplus*. The cattle were assembled and maintained free of ticks in paddocks at the TFRC and examined with an ELISA[10,11] and CAT[12] and found to have no antibodies to *B bovis* and *B bigemina* or to *A marginale*, respectively. The nine *B taurus* steers were vaccinated with trivalent tick fever vaccine containing *B bovis*, *B bigemina* and *A centrale* parasites. Approximately 3 months after vaccination the *B taurus* steers were used to infect ticks with a virulent *B bovis* isolate to seed the trial paddock. Vaccinated steers were used due to welfare concerns because of the known virulence of the *B bovis* isolate. Our previous observations showed that heterologous infections produced sufficient parasitaemias in vaccinated steers to infect ticks feeding on them.

Trial paddock

The trial paddock was naturally infested with *B microplus* and was located at the DPIQ Mutdapilly research facility in south-east Queensland. Previous outbreaks of tick fever in cattle grazing this paddock indicated that ticks already in the paddock could be carrying both *B bovis* and *B bigemina* parasites (our observation).

Parasites

The *B bovis* isolate, designated W, had been obtained in 1988 in blood collected from a clinical case and stored as stabilate in liquid nitrogen.[13] It is well defined and highly virulent.[13,14] To produce *B bovis* infected ticks, the nine vaccinated *B taurus* steers were infested with 1 g of *B bovis*-free larval ticks in groups of three at 70, 64 and 36 days before the start of the trial, respectively. Ten days later these steers were inoculated with 5 mL of thawed stabilate of *B bovis* isolate W so that peak *B bovis* parasitaemias would occur in the steers within 24 to 48 h of the female ticks undergoing their final engorgement. This was calculated to result in a patent parasitaemia of *B bovis* in ticks at detachment and therefore ensure transovarial transfer to the next generation of larval ticks.[15] The *B taurus* steers were allowed to graze the paddock to seed infected female ticks at approximate intervals of 50, 40 and 16 days before the trial steers were to be introduced.

Measurement of response to infection

Cattle were inspected daily from horseback, as well as during weekly musters for the duration of the trial (50 days). Animals noticed to be depressed, standing away from the mob, not eating or reluctant to move were brought to a crush and assessed by measurements of body temperature, PCV, parasitaemia and severity of clinical signs. Parasitaemias were determined by examining peripheral blood films stained with Giemsa and were graded according to the method of Callow and Pepper.[16] Animals were inspected for ticks at each weekly muster and visual estimates were made of the tick burdens.

Cattle of each breed were allocated to one of five categories depending on the maximum percent PCV depression, calculated using pre-trial and minimum PCVs in response to *Babesia* spp infection. The categories used were: unaffected, mild, moderate and severe, and cattle were treated when maximum percent PCV depression was less than or equal to 15%, 16 to 25%, 26 to 35%, 36 to 45% or greater than 45%, respectively.

Treatment of *Babesia* infection was administered if any of the following criteria were met: actual PCV less than or equal to 15%, parasitaemia equal to or more than 2%, and severe clinical distress. The treatment was Imidocarb (Imizol® Coopers Animal Health, Division of Schering-Plough).[17]

Serum samples were collected from all cattle on days 28, 35 and 42 to check for antibodies to *B bovis* and *B bigemina* in an ELISA[10,11] and *A marginale* in a CAT.[12] PCVs were determined at the same time. Ten mL of blood was collected in EDTA vacutainers from 11 clinically ill cattle on day 22 and DNA extracted to allow typing of *B bovis* parasites using PCR to amplify variable lengths of repeat regions in the Bv80 and BvVA1 genes.[18,19]

Analysis

To compare the severity of infection between breeds, the mean maximum percent PCV depression for the two breeds was calculated without allowance for treatment, and these data were subjected to one-way analysis of variance. Means were compared using the protected LSD procedure at the 1% level of significance.[20]

Results

Serology

Serological analysis showed that 73 and 95% of the *B indicus* and 73 and 87% of the crossbreds had seroconverted to *B bovis* and *B bigemina,* respectively by day 28 after exposure to infected ticks. By day 35, all had seroconverted to both parasites except for one crossbred steer that had seroconverted to *B bovis* by day 42. All the cattle remained seronegative for *Anaplasma* for the duration of the study.

Measurement of response to infection

The *Babesia* infections resulted in 75% of the crossbred and 36% of the *B indicus* steers showing a maximum depression in PCV of more than 15%. Ten of the 52 crossbred steers and 1 of the 56 *B indicus* steers showed severe clinical signs and two of the crossbred steers required treatment (Table 1). The mean maximum percent PCV depression was significantly higher in the crossbred steers than the *B indicus* steers when tested in an analysis of variance. One of the treated crossbred steers failed to recover and died 2 weeks after initial treatment. Smears were only taken from clinical cases, but microscopy showed mixed infections in a number of animals and this was confirmed by seroconversion to both parasites. Counts of ticks were not made, but burdens were assessed as high on all groups and all animals were treated with Moxidectin (Cydectin® Pour-On, Cyanamid Websters Pty Ltd, Australia) 33 days after the trial started. There was no apparent difference in the number of ticks carried by *B indicus* and crossbred steers.

Parasite typing

B bovis DNA was extracted from the blood of 11 crossbred steers and one *B indicus* steer on day 22 after tick exposure and tested by PCR assays. PCR product sizes observed for the isolates from these clinical cases all matched the profile for *B*

Table 1. Number of purebred *B indicus* and crossbred (50% *B indicus*) steers that showed different degrees of maximum percent PCV depression after infection with *Babesia spp* transmitted by *B microplus*.

Categories	Maximum percent PCV depression	Number of animals in each category	
		Crossbreds n = 52	B indicus n = 56
Unaffected	≤ 15	13	36
Mild	16-20	7	11
	21-25	9	4
Moderate	26-30	6	3
	31-35	7	1
Severe	36-40	5	0
	41-45	3	1
Treated	46-50	2	0
Mean percent PCV depression		25	14
LSD at 1 percent level		5	

bovis isolate W, the field isolate used to infect ticks before the study. No evidence of other field isolates or Dixie *B bovis* vaccine strain were detected.

Discussion

The results confirm that infections of *Babesia* can have a significant effect on crossbred steers with a 50% *B indicus* infusion whereas purebred *B indicus* steers are relatively resistant. The *B bovis* isolate used to infect the ticks was the same as that used by Bock et al[9] and has consistently induced pathogenic infections in inoculated *B taurus* cattle.[13,14] However, the number of crossbred steers requiring treatment (4%) in the current trial was much smaller than the 20 to 30% reported by Bock et al.[9] The reason for the difference is unclear but there were a number of differences in the trial designs. Bock et al[9] used 10^8 *Babesia* parasites in splenectomised calf blood instead of a tick challenge procedure. However, Timms et al[21] reported that *B bovis* infection transmitted by ticks or by inoculation were of similar pathogenicity. Furthermore, the crossbred steers used in the current trial contained Charolais as the *Bos taurus* component, not Angus that was used by Bock et al[9] and originated from a different region. In a more recent trial (unpublished), a group of crossbred steers with the same genetic composition, age and origin to the current trial group was challenged with *B bovis* isolate W. They showed a susceptibility range after inoculation with *B bovis* that was intermediate between that observed in the current trial and that reported by Bock et al.[9] One of seven steers required treatment and the mean PCV decrease for the group was 42.7%.

DPIQ laboratory records indicate that of confirmed tick fever outbreaks in Queensland, approximately 82, 11 and 7% are due to *B bovis*, *A marginale* and *B bigemina*, respectively.[4] Further analysis of these data for outbreaks, in which a breed type is known, shows that 68, 25 and 7% of *B bovis* and 75, 19 and 6% of *B bigemina* outbreaks occur in *B taurus*, crossbred and *B indicus* cattle, respectively. This indicates that by far the majority of confirmed outbreaks of babesiosis occur in *B taurus* cattle and is caused by *B bovis*.

We were unable to accurately determine which *Babesia* parasite had the greatest effect in this particular trial because mixed infections predominated in serological tests and smear examinations. *B bigemina* has been found to be usually poorly pathogenic in Australia even when fully susceptible cattle are introduced to an endemic area.[22,23] Also, *B indicus* cattle almost invariably experience mild primary reactions with both species of *Babesia*.[1,7,24-26] In cattle, with the same origin and age as those in the current study, Bock et al[27] showed that, when inoculated with *B bigemina*, unvaccinated *B indicus* and crossbred cattle were significantly more resistant than *B taurus* cattle with mean maximum PCV deceases of 22.4, 31.4 and 50.9%, respectively. This and field evidence in Queensland[4] suggest that *B bovis* would have been the principal pathogen in the current study, although *B bigemina* as shown by Bock et al[27] and even blood loss from tick infestation would have contributed to the clinical signs. Because of the known virulence of the *B bovis* in *B taurus* cattle[9,13,28] and our reliance in this trial on daily visual observations only, use of a control group of naive *B taurus* cattle was precluded on animal welfare grounds.

Three months before inoculation with isolate W the *B taurus* steers used to infest the paddock with ticks had been vaccinated with trivalent tick fever vaccine which includes the Dixie *B bovis* strain. Vaccination was done to protect the cattle and we know from previous studies that isolate W will produce detectable parasitaemias suitable for infection of cattle ticks in vaccinated cattle.[13,14] The Dixie *B bovis* strain has been shown to be transmissible by ticks under laboratory conditions and to increase in virulence following tick transmission (our observations). Despite this, the PCR assays of 11 isolates from clinical cases detected only isolate W. This trial therefore offers circumstantial evidence that the presence of Dixie *B bovis* strain in vaccinated cattle is unlikely to alter the dynamics of transmission of parasites under field conditions or constitute a significant risk to naive cattle grazing with vaccinated cattle once vaccine induced parasitaemias have fallen to undetectable amounts.

The 'G' strain of *B bigemina* used in the trivalent vaccine[29] is poorly if at all transmissible by ticks.[30,31] Also cattle infected with *B bigemina* remain infective for ticks for only 4 to 7 weeks.[32,33] Because the cattle used to 'seed' the paddocks were vaccinated 3 months before infection with *B microplus*, the origin of the *B bigemina* is presumed to be from 'wild' ticks that were in the paddock before the start of the trial. Because *B bovis* infects less ticks and is transmitted less readily than *B bigemina*,[34] this is assumed to be the reason why 'wild' *B bovis* parasites were not also detected.

None of the cattle had previously been exposed to tick infestations so that a 'worst case scenario' for *Babesia* spp infection could be assessed. Wagland[35,36] provided good evidence that *B indicus* cattle, not previously exposed to *B microplus*, are as susceptible to ticks as are *B taurus* cattle. This was reflected by the heavy tick burdens acquired by both breeds and the very rapid transmission of *Babesia* spp. Johnston,[37] however, showed that, following initial tick exposure, crossbred cattle are infested with smaller numbers of ticks and have a lower incidence of *B bovis* parasitaemia than *B taurus* cattle.

Mahoney et al[38] compared transmission of *B bovis* in *B taurus* and in three-eighths to half *B indicus* crossbred cattle in south-east Queensland and concluded that, in an environment unfavourable for tick survival, stocking with crossbred cattle will over several seasons almost lead to the disappearance of the ticks. However, our results show that if such cattle are moved to a paddock with a high *B microplus* infestation, *Babesia* transmission rates can be very high.

By using the results of this study in a disease prediction-vacci-

nation model with disease state probabilities in a Markov chain linked to an 8 year discounted cashflow analysis,[39] *B bovis* vaccination of *B indicus* and crossbred weaners showed a benefit to cost ratio of 0.2 to 1.2 and 0.9 to 4.8, respectively in a representative northwest Queensland herd. The range depended on the estimated annual seroprevalence to *B bovis* in yearling cattle in the herd. So, while vaccination of crossbred cattle is probably beneficial, vaccination for babesiosis alone may not be economical in the majority of purebred *B indicus* cattle. Because the vast majority of properties in far northern Australia have crossbred cattle, with *B indicus* infusions ranging from three-eights to five-eights, and few herds are endemically stable for tick fever, most are potentially at risk for outbreaks of babesiosis and could benefit from vaccination. The situation with *A marginale* is currently under investigation.

Acknowledgments

This work was supported by the Meat and Livestock Australia (formerly Meat Research Corporation) and Queensland Department of Primary Industries. We thank Denise Stevenson and Neil Goetsch for their technical assistance, Dr Wayne Jorgensen for supplying *B microplus* larvae, Dr Ala Lew for the *B bovis* PCR assays and Warren Lehmann and the Australian Agricultural Company for their assistance in obtaining trial cattle.

References

1. Francis J. Resistance of zebu and other cattle to tick infestation and babesiosis with special reference to Australia: an historical review. *Br Vet J* 1966;122:301-307.
2. O'Rourke PK, Winks L, Kelly AM. *North Australian beef producer survey, 1990.* Queensland Department Primary Industries and Meat Research Council, Brisbane, 1992.
3. Bock RE, Blight GW, Kingston TG, de Vos AJ. A survey of cattle producers in the *Boophilus microplus* endemic area of Queensland to determine attitudes to the control of and vaccination against tick fever. *Aust Vet J* 1995;72:88-92.
4. Bock RE, de Vos AJ, Rayner AC et al. Assessment of the risk of tick fever mortalities in north-western Queensland beef industry. In: *Challenging the boundaries*, Proceedings of section of Annual Conference, Australian Association of Cattle Veterinarians, Brisbane, 1997:175-182.
5. Mahoney DF. The epidemiology of babesiasis. In: Johnston LAY, Cooper MG, editors. *Ticks and tickborne diseases.* Proceedings of a symposium held at the 56th Annual Conference of the Australian Veterinary Association, Townsville, 1980:9-11.
6. Callow LL, Dalgliesh RJ. The development of effective, safe vaccination against babesiosis and anaplasmosis in Australia. In: Johnston LAY, Cooper MG, editors. *Ticks and tickborne diseases.* Proceedings of a Symposium held at the 56th Annual Conference of the Australian Veterinary Association, Townsville, 1980:4-8.
7. Callow LL. Piroplasms. In: *Protozoal and rickettsial diseases*. Animal Health in Australia. Australian Bureau of Animal Health, AGPS, Canberra, 1984;5:121-160.
8. Bartholomew RB, Callow LL. A benefit-cost study (ex-post) of the development and introduction of a new vaccine against bovine babesiosis. In: *Proceedings of the 2nd International Symposium of Veterinary Epidemiology and Economics*, Canberra, 1979:448-457.
9. Bock RE, de Vos AJ, Kingston TG, McLellan DJ. Effect of breed of cattle on innate resistance to infection with *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale. Aust Vet J* 1997;75:337-340.
10. Molloy JB, Bowles PM, Bock RE et al. Evaluation of an ELISA for detection of antibodies to *Babesia bovis* in cattle in Australia and Zimbabwe. *Prev Vet Med* 1998;33:59-67.
11. Molloy JB, Bowles PM, Jeston PJ et al. Development of an ELISA for detection of antibodies to *Babesia bigemina* in cattle in Australia. *Parasitol Res* 1998;84:651-656.
12. Amerault TE, Roby TO. A rapid card agglutination test for bovine anaplasmosis. *J Amer Vet Med Assoc* 1968;153:1828-1834.
13. 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.
14. Bock RE, de Vos AJ, Kingston TG, Lew AE, Fraser IR. Studies on failure of T strain live *Babesia bovis* vaccine. *Aust Vet J* 1995;72:296-300.
15. Dalgliesh RJ, Stewart NP. Some effects of time, temperature and feeding on infection rates with *Babesia bovis* and *Babesia bigemina* in *Boophilus microplus* larvae. *Int J Parasitol* 1982;12:323-326.
16. 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.
17. Callow LL. Treatment of babesiosis and anaplasmosis in Australia. In: *The therapeutic jungle.* University of Sydney Post Graduate Committee in Veterinary Science, Proceedings 1978;39:264-270.
18. Lew AE, Bock RE, Croft JM et al. Genetic diversity in field isolates of *Babesia bovis* from cattle with babesiosis after vaccination. *Aust Vet J* 1997;75:575-578.
19. Lew AE, Dalrymple BP, Jeston PJ, Bock RE. PCR methods for the discrimination of *Babesia bovis* isolates. *Vet Parasitol* 1997;71:223-237.
20. Snedecor GW Cochran WG. *Statistical methods*. 6th edn. Iowa State University Press, Ames, 1971:272.
21. Timms P, Stewart NP, Dalgliesh RJ. Comparison of tick and blood infection for assessing immunity to *Babesia bovis. Aust Vet J* 1983;60:257-258.
22. Callow LL. Some aspects of the epidemiology and control of bovine babesiosis in Australia. *J S Afr Vet Assoc* 1979;50:353-356.
23. Meehan JF. Field proof for the tick fever vaccine. *Qld Agric J* 1969;95:768-771.
24. 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.
25. Lohr KF. Susceptibility of non-splenectomised and splenectomised Sahiwal cattle to experimental *Babesia bigemina* infection. *Z Veterinarmed* 1973;20:52-56.
26. Queensland Department of Primary Industries. Studies on cattle tick, tick fever and tick control. *Australian Meat Research Committee Project DAQ12, Final Report*, Brisbane, 1981.
27. Bock RE, Kingston TG, Standfast NF, de Vos AJ. Effect of cattle breed on innate resistance to *Babesia bigemina* infection. *Aust Vet J* 1999;77. In press.
28. de Vos AJ, Jorgensen WK. Protection of cattle against babesiosis in tropical and subtropical countries with a live, frozen vaccine. In: Fivaz BH, Petney TN, Horak IG,editors. *Tick vector biology, medical and veterinary aspects.* Springer Verlag, London, 1992:159-174.
29. Dalgliesh RJ, Callow LL, Mellors LT, McGregor W. Development of a highly infective *B bigemina* vaccine of reduced virulence. *Aust Vet J* 1981;57:8-11.
30. Dalgliesh RJ, Stewart NP, Rodwell BJ. Increased numbers of strahlenkörper in *Boophilus microplus* ticks ingesting a blood-passaged strain of *Babesia bigemina*. *Res Vet Sci* 1981;31:350-352.
31. Mason TE, Potgieter FT, Van Rensberg L. The ability of a South African *Babesia bovis* vaccine strain to infect *Boophilus microplus*. *Onderstepoort J Vet Res* 1986;53:143-145.
32. Mahoney DF. Bovine babesiosis: A study of factors concerned in transmission. *Ann Trop Med Parasitol* 1969; 63:1-14.
33. Johnston LAY, Leatch G, Jones PN. The duration of latent infection and functional immunity of Droughtmaster and Hereford cattle following natural infection with *B argentina* and *B bigemina*. *Aust Vet J* 1978;54:14-18
34. Mahoney DF, Mirre GB. Bovine babesiosis: estimation of infection rates in the tick vector *Boophilus microplus* (Canestrini). *Ann Trop Med Parasitol* 1971;65:309-317.
35. Wagland BM. Host resistance to cattle tick (*Boophilus microplus*) in Brahman (*B indicus*) cattle I. Responses of previously unexposed cattle to four infestations with 20 000 larvae. *Aust J Ag Res* 1975;26:1073-1080.
36. Wagland BM. Host resistance to cattle tick (*Boophilus microplus*) in Brahman (*B indicus*) cattle II. The dynamics of resistance in previously unexposed and exposed cattle. *Aust J Ag Res* 1978;29:395-400.
37. Johnston LAY. Epidemiology of bovine babesiosis in northern Queensland. *Aust Vet J* 1967;43:427-431.
38. Mahoney DF, Wright IG, Goodger G et al. The transmission of *Babesia bovis* in herds of European and zebu x European cattle infested with the tick *Boophilus microplus*. *Aust Vet J* 1981;57:461-473.
39. Ramsay GC. Setting animal health priorities: *A veterinary and economic analysis with special reference to the control of* Babesia bovis *in central Queensland* [PhD thesis]. The University of Queensland, 1997.

(Accepted for publication 19 April 1999)