STUDIES ON THE EPIZOOTIOLOGY OF BOVINE BABESIOSIS IN NORTH EASTERN NEW SOUTH WALES

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Introduction

Babesia argentina and *Babesia bigemina* were probably introduced into northern Australia along with their tick vector *Boophilus microplus* in 1872. They spread from the Northern Territory to Queensland and reached New South Wales in 1916 (Seddon 1952). In New South Wales, babesiosis and *B. microplus* have been confined to the north-eastern corner of that State by rigid control by the Department of Agriculture through the agency of the Board of Tick Control. Whenever infestations of *B. microplus* are found, compulsory dippings are carried out until all cattle on the holding are free of ticks as determined by visual examination. In addition, all cattle in the area controlled by the Board of Tick Control are dipped routinely between October and March each year. Until 1964, whenever babesiosis occurred, a holding was quarantined for three years, but testing by complement fixation (CF) (Mahoney 1962*a*) was commenced in that year, and the quarantine period was then reduced to 10 months. All cattle are now CF tested at the beginning of the quarantine period and again at the end, and dipped fortnightly throughout the period. Animals with subclinical infections are slaughtered.

In the adjoining state of Queensland, ticks are not under intensive control so that cattle are continually infested. Infection of calves with *Babesia* at an early age is considered desirable to ensure immunity in later life and Mahoney (1962*b*) found that 30% of animals in one herd near Brisbane had demonstrable parasitaemias throughout the year. The border between Queensland and New South Wales is in some places on a natural boundary, the Macpherson Range, and has a fenced buffer zone, one chain wide. Thus, there is theroetically no uncontrolled physical contact between the cattle in the two States.

Despite the extreme precautions taken with the control of ticks and tick fever in New South Wales, the vector has not been eradicated and 324 sporadic outbreaks of babesiosis have occurred between 1916 and 1960 with an average of 1.76 deaths per outbreak or 2.5 deaths per 100,000 animals per year (Mackerras *et al* 1961). Whether these sporadic clinical infections are caused by a smouldering enzootic situation or represent new infections introduced from Queensland has not been resolved.

In preliminary studies of this problem the extent of subclinical infection and its relationship to clinical babesiosis were investigated in New South Wales using the thick blood film technique (Mahoney and Saal 1961) and the CF test (Mahoney (1962*a*). This paper presents the results of surveys carried out with these techniques between 1964 and 1970.

Materials and Methods

Tests for Subclinical Infection

Thick blood films — The incidence of demonstrable parasitaemia was determined by the thick blood film technique described by Mahoney and Saal (1961).

The complement fixation test — The method described by Mahoney (1962*a*) for preparing antigens and for conducting the test was used throughout. Two slight modifications were made. Firstly, serums were stored at —20°C without the addition of preservative, and secondly several high and several low titred serums were used to titrate the antigens each day. The method of diluting serums was unchanged. Serums were screened at the 1/5 dilution, and those showing any reaction were retested at high dilutions. A titre of 10 was considered to be positive for *B. argentina* and 5 positive for *B. bigemina*.

Transmission tests — Blood was taken from suspected animals, and injected intravenously into splenectomised calves, 2-12 months of age, at a dose rate of 5 ml of whole blood per kg live weight. Blood films were collected from the splenectomised calves daily for 28 days and were examined for the presence of parasites.

All animals in the field showing a positive CF reaction were subjected to a transmission test. In addition groups of 50 animals negative to the CF test were tested by pooling 20 ml of blood from each, and injecting it into splenectomised calves.

Method of Survey

To control babesiosis in New South Wales, infected herds and neighbouring herds are quarantined. All cattle over one week old in these herds are CF tested soon after the commencement of quarantine. Neighbouring herds are released from quarantine if found to be free of infection. Infected herds are retested at the end of 10 months, and, if further subclinically infected cattle are found, they are slaughtered, and the herd retested every two months until clean. The CF tests carried out soon after the imposition of quarantine restrictions were used in the survey described in this paper.

Thick blood films were collected from selected herds in areas with a high incidence of infected herds and from a herd with a history of a previous infection.

Results

Clinical Babesiosis

Geographical distribution — The boundaries of quarantine areas mentioned in the survey and the location of clinical infections occurring between 1964 and 1970 are shown in Figure 1. The yearly incidence of clinical babesiosis in each area is listed in Table 1.

Only one outbreak resulted in more than one death. This was in the Kyogle area in 1965, and was caused by *B. argentina*. Two herds in the Kyogle area with clinical infections in 1965 had further deaths, one in 1966 and the other herd in 1967. Infections in these two herds were due to *B. argentina*. No herds had clinical cases with both organisms although one herd in the Woodenbong area in 1965 had a clinical case involving a mixed infection.

All clinical infections recorded during the period were in animals over two years of age, and only one animal that developed clinical signs recovered. This animal was infected with *B. bigemina*.

Seasonal distribution — Most clinical infections of *B. argentina* occurred in the summer months. Five were in December, 29 in January, 18 in February and 3 in March. Two *B. bigemina*

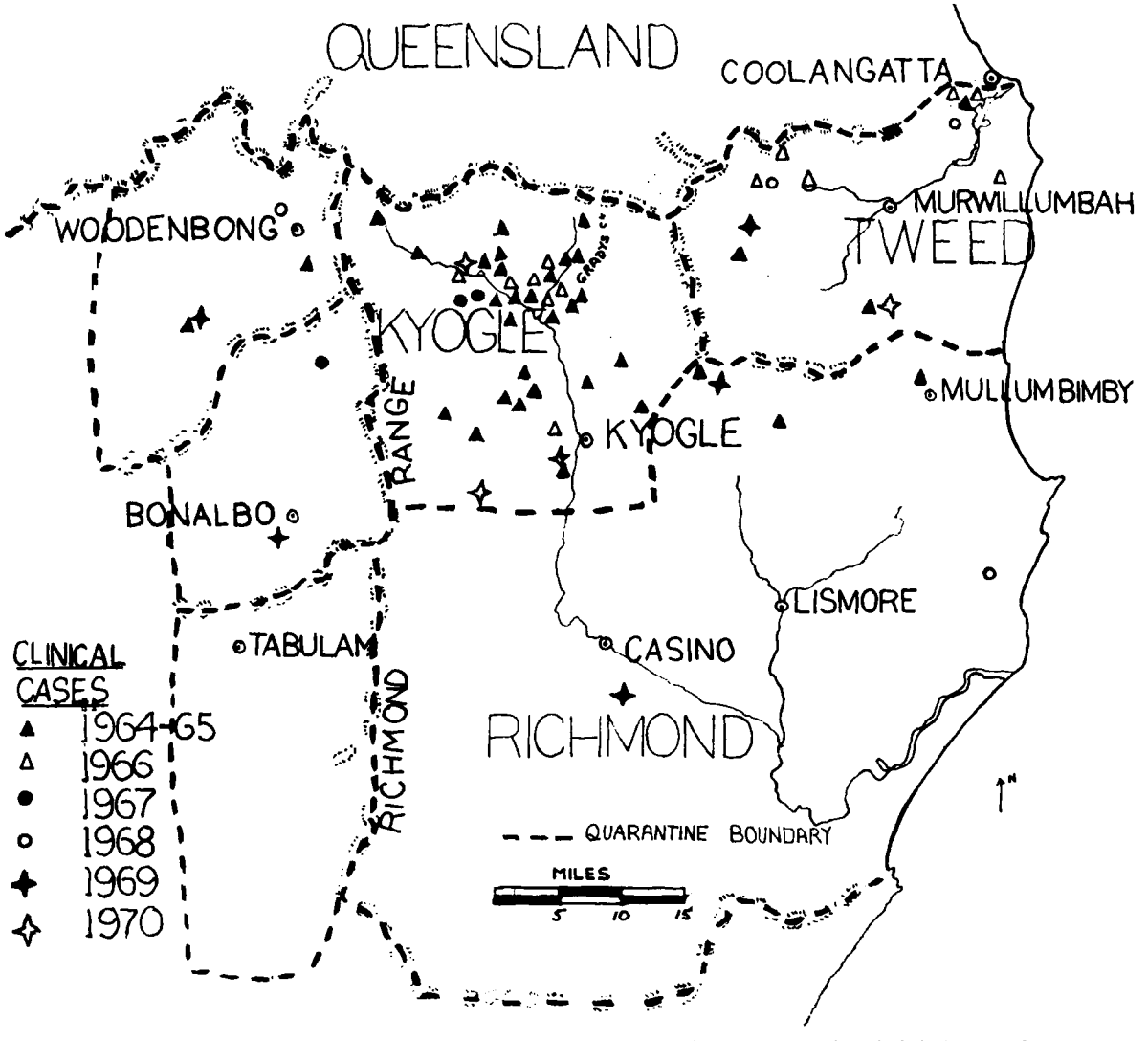

Figure 1. A map showing the tick quarantine areas of New South Wales and the location of clinical cases of babesiosis from 1964 to 1970.

TABLE 1

The Incidence of Clinical Babesiosis in the Tick Quarantine Areas of New South Wales for the Period 1964 to 1970

Tick Quarantine Area	Year	No of Herds with Clinical Babesiosis		
		Causative Organism		
		B. argentina	*B. bigemina*	Mixed Infection
Tweed	1964	—	—	—
	1965	3	—	—
	1966	6	—	—
	1967	—	—	—
	1968	2	—	—
	1969	1	—	—
	1970	1	—	—
	Total	13	—	—
Richmond	1964	—	—	—
	1965	2	1	—
	1966	—	—	—
	1967	—	—	—
	1968	1	—	—
	1969	2	—	—
	1970	—	—	—
	Total	5	1	—
Kyogle	1964	—	1	—
	1965	22	4	—
	1966	7	—	—
	1967	2	—	—
	1968	—	—	—
	1969	—	—	—
	1970	2	1	—
	Total	33	6	—
Woodenbong and Bonalbo (West of the Richmond Range)	1964	—	—	—
	1965	1	—	1
	1966	—	—	—
	1967	—	1	—
	1968	1	—	—
	1969	2	—	—
	1970	—	—	—
	Total	4	1	1
Grand Total		55	8	1

infections occurred in January, two in February and single cases in May, June, July and October. The mixed infection was in February.

Subclinical Babesiosis

The incidence of demonstrable parasitaemias —

1. In July 1964, a group of 80 animals in an infected herd at Cougal (northern end of Grady's Creek in the Kyogle area) were examined for parasitaemias. At the time the blood films were collected, the group was heavily infested with *B. microplus* and one animal had died from a *B. bigemina* infection the week before. Seven were calves under three months of age while the remainder were over three years old. Two of the calves had detectable parasitaemias while the incidence in the adults was 27.4%. Only *B. bigemina* was observed.

2. Three herds at Findon Creek (north-western part of the Kyogle area) were examined in July 1967. The herds appeared to be free of *B. microplus* at the time.

In one herd of 154 animals, all with negative CF reactions and another herd of 151, in which two subclinical infections of *B. argentina* had been detected, no parasitaemias were observed.

In the third herd of 64 animals, one subclinical infection of each organism had been found. A parasitaemia was detected in only the animal infected with *B. bigemina.*

3. In February 1968 a clinical case of *B. argentina* occurred in a herd at Limpinwood in the north-western part of the Tweed area. The herd of 180 head had been infected with the same organism in 1966. On both occasions the herd was free of *B. microplus.* Blood films were collected within a week of the mortality but no parasitaemias were detected.

Complement Fixation Test Survey

The incidence of subclinical infections in New South Wales herds between 1964 and 1970 is shown in Table 2. Only herds experiencing clinical infections and herds adjoining infected herds were tested. Subclinical infections were detected by the CF test and confirmed by transmission tests.

Of 544 holdings tested, 15.8% were found to contain subclinical infections (10.1% of *B. argentina,* 4.6% of *B. bigemina* and 1.1% of both organisms). The highest incidence of herds with subclinical infections was in the Kyogle area (19.1%) and the lowest in the Richmond area (3.8%). The areas west of the Richmond Range (Woodenbong and Bonalbo) in which babesiosis had not been recorded before 1965, had an overall incidence of 9.3%. The incidence in the Tweed area was 14.4%.

Only 73 subclinical *B. argentina* infections were detected in 61 herds, and the greatest number that occurred in a single herd was four. The overall incidence of these infections was approximately one per thousand head.

With *B. bigemina,* 255 subclinical infections were demonstrated in 31 herds. Nine of the herds had a single subclinical infection while five herds in the Kyogle area and two in the Richmond area had more than five. Approximately 3.5 subclinical infections were detected per thousand head tested.

Of 64 herds where clinical infections with either organism occurred, 8 (12.5%) were

TABLE 2

The Incidence of Subclinical Babesiosis in New South Wales Herds, Tested Between 1964 and 1970*

Tick Quarantine Area	Year	No Herds Tested	No Herds with Subclinical Infections			No Animals Tested	No Animals with Subclinical Infections		
			A	B	AB†		A	B	AB‡
Tweed	1965	34	—	3	—	2832	—	3	—
	1966	31	7	1	1	2548	15	2	—
	1967	7	2	—	—	494	2	—	—
	1968	12	—	—	—	987	—	—	—
	1969	3	—	—	—	200	—	—	—
	1970	10	—	—	—	998	—	—	—
	Total	97	9	4	1	8059	17	5	—
Richmond	1965	28	—	1	—	2348	—	8	—
	1968	9	—	—	1	831	1	9	2
	1969	15	—	—	—	979	—	—	—
	1970	1	—	—	—	27	—	—	—
	Total	53	—	1	1	4185	1	17	2
Kyogle	1964	11	3	4	1	3598	5	183	—
	1965	241	28	9	3	33814	30	35	1
	1966	34	7	3	—	3964	7	4	—
	1967	24	3	3	—	2311	5	4	—
	1968	1	—	—	—	88	—	—	—
	1970	29	1	—	—	5279	1	—	—
	Total	340	42	19	4	49054	48	226	1
Woodenbong and Bonalbo (West of the Richmond Range)	1965	22	2	1	—	3811	2	4	—
	1967	5	—	—	—	1118	—	—	—
	1968	12	—	—	—	1530	—	—	—
	1969	15	2	—	—	4040	2	—	—
	Total	54	4	1	—	10499	4	4	—
Overall Total		544	55	25	6	71797	70	252	3

*Herds tested were those with clinical infections and those adjoining infected herds.
†A = of *B. argentina* only B = of *B. bigemina* only AB = of both species (either as mixed infections or separate infections of *B. argentina* and *B. bigemina.*)
‡A = with *B. argentina* only B = with *B. bigemina* only AB = with mixed infections of both species.

found to harbor subclinical infections of *B. argentina* as did 53 (11.5%) of 480 herds adjoining these.

While the results of tests usually indicated that a simple epizootiological situation existed with only one or two subclinical infections per herd, some herds had a fairly high incidence of infection. Three of these had interesting histories and are reported below.

1. The herd at Cougal which contained the group of animals examined for the presence of parasitaemia (see above) was quarantined in 1964 because of a clinical *B. bigemina* infection. Tests carried out within one month of the mortality demonstrated 105 subclinical *B. bigemina* infections in 889 animals (11.8%). Heavy infestations of *B. microplus* (more than 10 engorged ticks per animal) were found on the group examined for parasitaemia and 81% of these were found to be infected with *B. bigemina.* Other groups were lightly infested (less than 5% of animals with *B. microplus*), and the incidence of *B. bigemina* in these was less than 10%. Two subclinical *B. argentina* infections were detected in one of the lightly infested groups.

In two adjoining herds, 49 of 174 (28.3%) and 17 of 303 (5.3%) animals were subclinically infected with *B. bigemina,* but no *B. argentina* infections were demonstrated. Two other adjoining herds of 303 and 154 head each contained two *B. argentina* infections, this time with no evidence of *B. bigemina.*

2. In a herd at Mullumbimby (1965, Richmond area) surrounded by herds negative to the CF test, 8 of 85 animals were found to be subclinically infected with *B. bigemina.* A clinical *B. bigemina* infection occurred in this herd just before it was tested, but no evidence of *B. microplus* could be found on it or neighbouring properties.

3. An outbreak at Knockrow in the Richmond area in 1968 was caused by *B. microplus*

TABLE 3

The Relationship between the Distance from the Queensland Border and the Incidence of Babesia *Infected Herds in the Kyogle Area of New South Wales from 1964 to 1966*

Group of Herds	Distance from Queensland Border	No of Herds Tested	No of Animals Tested	Percentage of Herds Infected*		
				With *B. argentina*	With *B. bigemina*	With Either Organism
A	0-5 miles	48	7348	35.4	18.8	52.1
B	5-10 miles	90	11908	30.0	15.5	38.8
C	10-15 miles	71	11248	19.7	1.4	21.1
D	15-20 miles	56	8131	14.3	—	14.3
E	20-25 miles	21	2741	9.5	—	9.5
Overall Totals and Percentages		286	41376	23.8	8.4	29.7

*A herd was classed as infected if a clinical or subclinical infection was demonstrated in one animal.

larvae carried from Queensland on a seed harvester. Of 18 animals in the paddock in which the harvester was working, three died. Only one of these was examined, and it was shown to have died from a *B. argentina* infection. Nine others were subclinically infected with *B. bigemina,* one with *B. argentina* and two with mixed infections. Although all animals in the paddock were infested with *B. microplus,* three escaped infection.

The Distribution of Infected Herds in the Kyogle Area

More than half the clinical and subclinical infections reported in this paper occurred in the Kyogle area between June 1964 and December 1966. Kyogle herds tested during this period were classified into five groups (A to E) based on a calculation of the shortest distance from each holding to the Queensland border. The percentage of infected herds (either due to a clinical or subclinical infection) in each group is shown in Table 3.

Fifty-two per cent of the herds within five miles of the border (group A) were infected. The incidence declined as the distance from the border increased (groups B to E) so that only 9.5% of herds over 20 miles from the border were infected. While *B. argentina* infections were distributed throughout the Kyogle area, *B. bigemina* was confined to herds within 15 miles of the border.

The Grady's Creek valley was the most heavily infected region with 12 out of 36 herds infected with *B. argentina,* 10 with *B. bigemina* and 4 with both organisms. The valley to the west of this (Findon Creek) was less heavily infected with 3 of 21 herds infected with *B. argentina* and 4 with *B. bigemina.* Both these valleys are within 10 miles of the border.

Transmission Tests from CF Negative Animals

Altogether 1,006 animals, negative to the CF test, were subjected to transmission tests and no infections were demonstrated. The animals were selected from all age groups in 10 herds in the Kyogle and Tweed areas in 1965. Three herds were infected with *B. argentina,* one with *B. bigemina* and one with both species. The other five herds adjoined infected herds but were free of ticks, and there was no history of them having been infected with *Babesia.*

Discussion

The results show that the incidence of subclinical *B. argentina* infections in New South Wales herds associated with outbreaks is low, and, with few exceptions, the same is true of *B. bigemina.* The low incidence of subclinical compared to clinical infections contrasts with the situation in eastern Queensland. The epizootiological situation in the latter area has been well documented (Mahoney 1962*b,* 1969, Johnston 1967).

In the Cougal herd where a high incidence of subclinical infection was found, demonstrable parasitaemias were encountered in 27.4% of the adults. In a herd near Brisbane, Mahoney (1962*b*) found an incidence of parasitaemia of 40% in the calves but of less than 10% in the animals over two to three years of age. He considered that the decline in incidence with increasing age was due to a continued super-infection leading to a build up in immunity to parasitaemia. The high incidence of parasitaemia in the adults in the Cougal herd may have been due to a lack of such immunity. It therefore seemed likely that the infection in this herd was due to a recent introduction of infected ticks rather than to an enzootic state similar to that existing in Queensland.

The origin of the sporadic clinical infections in New South Wales remains obscure. Three possible explanations can be given —

1. Other vectors or hosts act as transmittors or reservoirs of infection.
2. They originate from ticks infected by carriers in New South Wales herds.
3. They are caused by larval ticks carried from Queensland.

The absence of demonstrable tick populations on many herds with clinical or subclinical infections (for example the Mullumbimby herd) suggests the presence of another vector. However, both species may be transmitted by a single tick (Mahoney and Mirre 1971) which may not mature to a point where it is readily visible on the infected animal. In addition, the close association between *B. microplus* and babesiosis in Queensland and the absence of babesiosis in the tick free areas of both States seems to negate the possibility of another vector.

The situation as revealed by the survey was probably caused by the introduction of small numbers of infected larvae onto holdings where infection was either absent or of a low incidence. The introduction of a large number of larvae from Queensland by the seed harvester, for example, resulted in at least one clinical infection, nine subclinical infections of *B. bigemina,* one of *B. argentina* and two of both species. Three of 18 animals were not infected. Small numbers of larvae might cause single infections of either species and an overall scattered distribution of infections as found in the survey.

The origin of the *B. microplus* larvae causing these infections in New South Wales is difficult to determine. The decline in the incidence of infected herds with increasing distance from the Queensland border suggests that some infections might be due to larvae carried from Queensland. The possibility still remains that others are due to larvae infected by animals in New South Wales.

Summary

In New South Wales between 1964 and 1970, 55 herds experienced clinical infections with *Babesia argentina,* 8 herds with *B. bigemina* and one herd a clinical infection involving both species.

During this period surveys of the incidence of subclinical infections were carried out using the complement fixation test, thick blood films and transmission tests. Herds where clinical infections occurred and herds adjoining infected herds were tested soon after the imposition of quarantine restrictions. Of 544 such herds tested, 55 were found to contain subclinical infections with *B. argentina* only and 25 with *B. bigemina* only. Six herds had mixed infections or contained separate infections with *B. argentina* and *B. bigemina.* Of 71,797 animals tested, 70 were found to be subclinically infected with *B. argentina* only, 252 with *B. bigemina* only and 3 with mixed infections of both species. The highest number of subclinical *B. argentina* infections in a herd was four. While most *B. bigemina* herds contained only one or two infections, seven herds did have more than five infections. The highest incidence of subclinically infected herds was in the Kyogle area (19.1%) and the lowest in the Richmond area (3.8%).

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