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BABESIA ARGENTINA, PLASMODIUM VIVAX AND  
P. FALCIPARUM: ANTIGENIC CROSS-REACTIONS

Colin G. Ludford, et al

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16. Abstract <p>An indirect fluorescent antibody test was used to analyze the antigenic relationships between <i>Ribesia argentina</i>, a parasite of cattle, and two human malaria parasites, <i>Plasmodium falciparum</i> and <i>Plasmodium vivax</i>. Elevated antibody titers to <i>P. falciparum</i> were found in cattle infected with <i>B. argentina</i>. Some persons infected with <i>P. falciparum</i> or <i>P. vivax</i> were found to produce antibodies to <i>B. argentina</i>. Explanations for the occurrence of these cross reactions are considered.</p>					
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## *Babesia argentina*, *Plasmodium vivax* and *P. falciparum*: Antigenic Cross-Reactions<sup>1</sup>

Colin G. Ludford and W. T. K. Hall

Animal Research Institute, Queensland Department of Primary Industries,  
Queensland, Australia

and

Alexander J. Sulzer<sup>2</sup> and Marianna Wilson

Parasitology Section, Center for Disease Control, Health Services and Mental Health  
Administration, Public Health Service, U. S. Department of Health, Education,  
and Welfare, Atlanta, Georgia 30333

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LUDFORD, C. G., SULZER, A. J., WILSON, M., AND HALL, W. T. K. 1972. *Babesia argentina*, *Plasmodium vivax*, and *Plasmodium falciparum*: Antigenic cross-reactions. *Experimental Parasitology* 32: 317-326. An indirect fluorescent antibody test was used to analyze the antigenic relationships between *Babesia argentina*, a parasite of cattle, and two human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*. Elevated antibody titers to *P. falciparum* were found in cattle infected with *B. argentina*. Some persons infected with *P. falciparum* or *P. vivax* were found to produce antibodies to *B. argentina*. Explanations for the occurrence of these cross reactions are considered.

INDEX DESCRIPTORS: Indirect fluorescent antibody; *Babesia argentina*; *Plasmodium vivax*; *Plasmodium falciparum*; Serology; Human hosts; Cattle; Antigen-antibody reactions; Immunity; Antigens.

The reports of human cases of babesiosis, recently reviewed by Garnham *et al.* (1969), have attracted interest to the *Babesia*, a genus once thought to have a host range restricted to wild and domestic animals. Such infections might be confused with malaria, and correct diagnosis is important for both clinical and epidemiological reasons.

It is of historic interest that Wilson and

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<sup>2</sup>Please address reprint requests to Dr. A. J. Sulzer, Parasitology Section, Center for Disease Control, Atlanta, Ga. 30333.

Chowning (1904) believed that a babesia that they termed *Pyroplasma lecanis* was the causative organism of Rocky Mountain spotted fever. Although this belief was erroneous, their descriptions and illustrations are convincing that they saw babesia in some of their patients, all of whom had been bitten by ticks. This would then be the first record of babesia infecting man.

Four cases of babesiosis in human beings have been reported since 1957. The first three were in splenectomized persons. Skrabalo and Deanovic (1957) reported a case believed to be due to *Babesia bovis*; Scholtens *et al.* (1968) a case in whom an unidentified species of *Babesia* was seen; and Fitzpatrick *et al.* (1968, 1969) a case where the causative organism was *Babesia vivax*.

*gens*. *Babesia microti* was isolated from the fourth case, a nonsplenectomized person, described by Western *et al.* (1970). Serum from the latter case was found by one laboratory to give a positive indirect fluorescent antibody (IFA) reaction with *P. falciparum* antigen.

Babesias differ from plasmodia in vectors, in method of multiplication, and in metabolic activity. In view of these differences, Cox and Milar (1968), seeking an explanation of cross-protection between *Babesia colhouni*, *Plasmodium chabaudi*, and *Plasmodium berghei*, suggested that immune mechanisms were in part nonspecific and independent of the species-specific or genus-specific antigens. However, Cox and Turner (1970) have recently shown by IFA tests that some degree of relationship does occur between parasite antigens of *Plasmodium vivax*, *P. chabaudi*, *P. berghei*, and *B. colhouni* and *B. microti*.

Two of the human cases of babesiosis (Skrabalo and Deanovic 1957; Fitzpatrick *et al.* 1968, 1969) were caused by cattle *Babesia* species. The availability of *B. argentina*, a third cattle parasite, prompted an investigation of the relationship of this *Babesia* and human *Plasmodium*. This paper describes IFA reactions of antisera to *Babesia argentina*, and to the human species *Plasmodium falciparum* and *Plasmodium vivax* with both homologous and heterologous parasite antigens.

#### MATERIALS AND METHODS

##### Antigens

Sindian-adapted strains of *P. falciparum*, *P. vivax* and *P. brasilianum* were used to prepare washed-cell, thick-smear antigens (Sulzer *et al.* 1969).

Blood with 2% of the erythrocytes parasitized by the "C" strain of *B. argentina* was produced in a splenectomized calf in Australia by the technique of Callow and Mellors (1966). Erythrocytes were washed three times in fetal calf serum by centrifug-

ing at 750 g and resuspended in fetal calf serum. Penicillin and streptomycin were added to a final concentration of 100 units/ml to inhibit contamination. The washed cells were sent, cooled on wet ice, by air to the United States. Thick-smear antigens were then prepared as described for the malaria parasites (Sulzer *et al.* 1969).

*Babesia microti*, Gray strain, isolated from a human case of babesiosis by Western *et al.* (1970), can be passaged through monkeys and hamsters. Antigen slides of this strain were prepared from the blood of an infected rhesus monkey by the same technique used for malarial parasites.

##### Antisera

Serum samples collected from 23 nonimmune patients suffering a primary attack of induced *P. falciparum* and *P. vivax* malaria were obtained from Dr. David F. Clyde, Director, Institute of International Medicine, University of Maryland, Baltimore, Md. Preinfection samples were taken from each individual; additional samples were collected approximately every 7 days throughout the course of infection. Samples were tested from three cases of induced *P. falciparum* malaria and 20 cases of induced *P. vivax* malaria. Serum submitted to the Center for Disease Control (CDC) for malaria IFA tests from 29 cases of slide-proven malaria were also examined. Four were from *P. falciparum* cases, one from a *P. ovale* case and 17 from *P. vivax* cases.

Antisera against *B. argentina* were produced in cattle in Australia and sent on ice by air to the United States. On Day 1, 10 Australian Illawarra Shorthorn steers 12-18 months of age were exposed to infection from 2 g, i.e., about 40,000, larval *Boophilus microplus* ticks carrying *B. argentina* strain C. Five blood samples were collected from the jugular veins of each of the 10 cattle: before tick infestation and at Days 13, 26, 35, and 61. Serum samples were stored at -20°C before shipment to the United

TABLE I  
Results of IFA Tests for *Babesia argentina* and *Plasmodium falciparum* Antibodies Using Serum Samples Obtained from Cattle Exposed to Ticks Carrying *B. argentina*

Animal number	Patent parasitemia	Antibody response to <i>B. argentina</i>					Antibody response to <i>P. falciparum</i>				
		Preinfection	Day 13	Day 26	Day 35	Day 61	Preinfection	Day 13	Day 26	Day 35	Day 61
1	Days 13-18			512	512	512	S	S	32	128	32
2	Days 13-17			2048	2048	2048	S	S	128	32	8
3	Days 13-15			512	512	512		S	32	32	8
4	Days 12-15			512	512	128	S		32	128	S
5	Days 13-18			128	128	128	S	32	32	32	32
6	None						32	S	32	32	32
7	Days 12-17			512	512	512	S	32	32	128	32
8	Days 12-16			512	128	128	S	S	32	32	32
9	Days 12-16			512	512	128	S	S	S	128	32
10	none						S		32	32	S

States. Upon receipt they were again frozen until tested.

*Indirect Fluorescent Antibody Tests*

Indirect fluorescent antibody (IFA) tests were performed by the technique described by Wilson *et al.* (1970), using a 4-fold dilution series. All *B. argentina* serum samples from infected cattle (initial serum dilution, 1:8) were tested at the same time against a particular antigen. Serum samples from individual malaria cases were tested with all antigens simultaneously but all cases were not tested on one day because of the large number of specimens.

All slides were randomized, coded, and read on a Leitz Ortholux fluorescence microscope equipped with Schott BG-12 and UG-2 exciter filters and a Leitz K470 secondary filter. Fluorescence of schizonts in *Plasmodium* spp. antigens was sought, as mentioned by Targett *et al.* (1970). *B. argentina* was readily recognized as a bright fluorescent body surrounded by the fluorescent ring of the parasitized erythrocyte, similar to its description by Ludford (1969) after direct fluorescent staining. In the present work, however, using the indirect technique, morphological details were not as apparent as with the direct technique. Inten-

sity of fluorescence was graded from 0 to 4+ as described by Wilson *et al.* (1970).

Preinfection serum samples from the induced cases served as negative controls. As a conjugate control, saline was incubated with antigen slides in place of serum dilutions. Human and bovine sera known to react with *Plasmodium* spp. and *B. argentina* antigens, respectively, were included as positive controls.

RESULTS

*B. argentina* Antisera

As shown in Table I, *B. argentina* was found in blood smears of eight of 10 cattle, 12-18 days after infestation with ticks. Antibodies to *B. argentina* were detected in the sera of these eight cattle on the third bleeding and remained with little alteration in titer until the end of the experiment. The reactions of the sera of the 10 cattle against *P. falciparum* antigen are also shown in Table I. Sera from nine of the 10 cattle reacted at low levels before infestation with ticks carrying *B. argentina*. However, by Days 26 or 35, a 16-fold rise in titer occurred in five cattle; Nos. 1, 2, 4, 7 and 9, their titers falling again by Day 61. A 4-fold rise in titer was found in cattle Nos. 3,

5, 8, and 10; the latter animal, however, did not have antibodies to *B. argentina*. Animal 6 had no antibody against *B. argentina* and did not show a rise in titer to *P. falciparum* antigen.

None of the serum samples from the 10 cattle reacted with *P. vivax* antigen. Representative samples from two cattle (Nos. 4 and 5) reacted with *B. microti* antigen. The preinfection sample from animal 4 was negative at the 1:4 dilutions, but that from animal 5 reacted at 1:4. Samples from both collected on Day 35 reacted at 1:4 and 1:16. Preinfection samples and samples taken on Day 35 from all eight remaining animals were negative at 1:4.

#### *P. falciparum* Antisera (Induced)

Serum samples from one induced *P. falciparum* malaria infection, case B2, gave positive results in the IFA test with *B. argentina* antigen. The results of the IFA tests are shown in Fig. 1. Tests on Days 1, 8, and 15 were negative, but on Day 22, positive reactions were found to all *Plasmodium* antigens and *B. argentina*. The titer to *B. argentina* was 4-fold greater than that against *P. falciparum*. Thereafter, up to Day 85, titers against *B. argentina* were always greater or equal to those against *P. falciparum*. On Days 71 and 85, reactions against the heterologous malarial parasites *P. bras-*

*ilium* and *P. vivax* had noticeably declined, although those against *B. argentina* remained high.

Serum samples from two *P. falciparum* cases, B11 and B20, did not react with *B. argentina*. The serological results from these two cases against the three malaria antigens are given in Table II. As in case B2, the titer against *P. falciparum* rose to 1:256.

#### *P. vivax* Antisera (Induced)

Of 20 induced *P. vivax* infections, serum samples from three cases, Nos. B4, B9, and B12, reacted in the IFA test with *B. argentina* antigen. Results of these tests are shown in Figs. 2, 3, and 4, respectively. In all three cases, antibody to *P. vivax* and to *B. argentina* appeared together and remained throughout the course of infection. In case B4, titers to both *B. argentina* and *P. vivax* reached 1:1024, although on Days 36, 57 and 71, titers to *B. argentina* were higher than those to *P. vivax*. Similarly, in case B12, the maximum titer to both parasites was 1:256, but on Days 23 and 58, the titer to *B. argentina* exceeded that to *P. vivax*. In case B9, the titer to *B. argentina* rose to 1:4096, 16-fold higher than the titer of 1:256 to *P. vivax*. Case B4 showed a residual titer of 1:4 to *P. vivax* after 328 days, but antibody to *B. argentina* was not

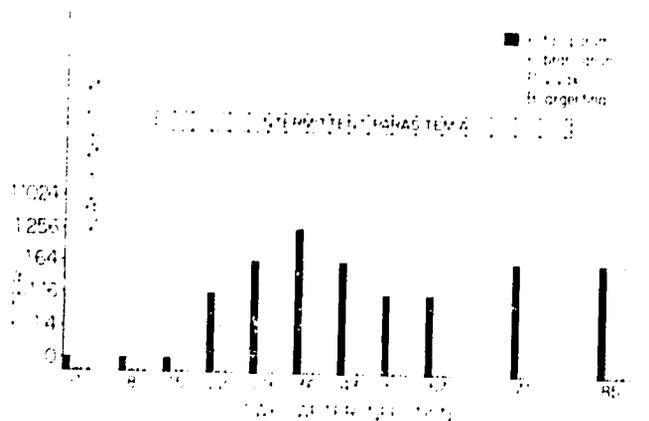


FIG. 1. Case B2 *P. falciparum*, Thailand strain, mosquito induced.

TABLE II  
Maximum IFA Test Titers to Malarial Parasites in Cases of Induced Plasmodium falciparum and P. vivax Malaria with Negative IFA Tests Against Babesia argentina

Case	Method of induction	Species and strain	Number of sera positive to homologous antigen (Titer >16)	Maximum reciprocal titer		
				<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. brasiliense</i>
<i>Falciparum</i>						
B-11	Blood	Thailand	9	256	64	256
B-20	Blood	Thailand	7	256	16	64
<i>Vivax</i>						
B-1	Blood	Chesson	1	64	4096	256
B-3	Blood	Chesson	7	64	1024	256
B-5	Blood	Chesson	7	64	1024	64
B-6	Blood	Chesson	2	64	64	16
B-10	Blood	Merchant	9	256	256	64
B-13	Mosquito	Chesson	6	64	1024	256
B-14	Mosquito	Chesson	1	16	256	256
B-15	Mosquito	Chesson	0	4	16	256
B-16	Mosquito	Chesson	2	4	256	256
B-17	Mosquito	Chesson	1	256	1024	64
B-18	Mosquito	Chesson	3	64	1024	256
B-19	Mosquito	Chesson	3	64	1024	64
B-21	Mosquito	Merchant	5	16	1024	64
B-22	Mosquito	Wenner	8	64	1024	64
B-23	Mosquito	Chesson	1	64	256	256
B-24	Mosquito	Chesson	1	64	1024	256

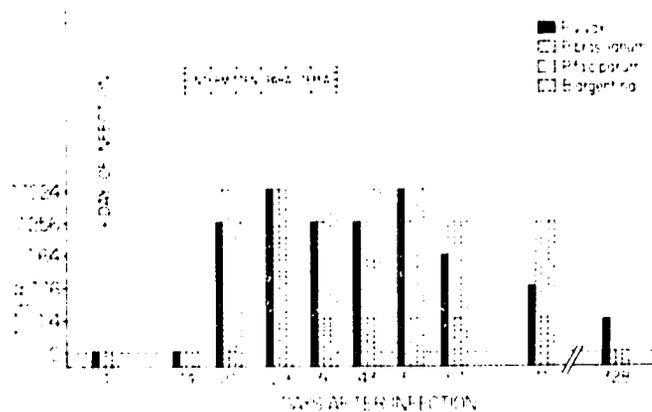


FIG. 2. Case B11 *P. vivax*, mosquito induced.

then detectable. All three cases also had antibody to *P. brasiliense* and *P. falciparum*.

Serum samples from 16 cases of induced *P. vivax* malaria did not react with *B. argentina*. Results of IFA tests with malarial antigens on these are summarized in Table

II. Three strains of *P. vivax* were used for inoculation. In all cases except B15, antibody titers to *P. vivax* were greater than 1:16, and all cases formed antibody to *P. brasiliense* and *P. falciparum*.

Serum samples from one case, B7, gave

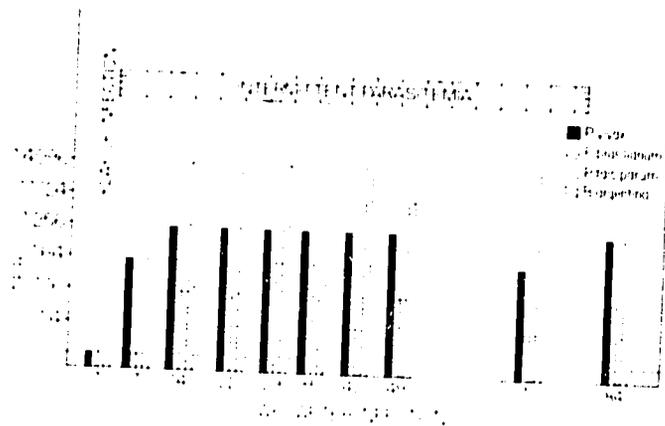


Fig. 3. Case B9 *P. vivax*, Merchant strain, blood induced.

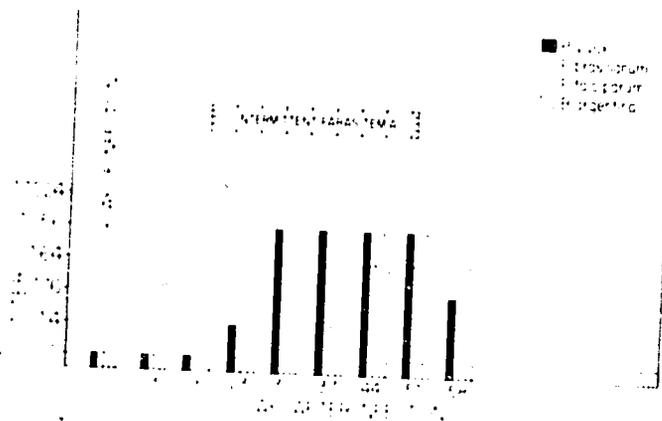


Fig. 4. Case B12 *P. vivax*, Chesson strain, blood induced.

unusual results when tested for antibody against *B. argentina*, although reactions with *P. vivax* were normal. As shown in Table III, there was little or no reaction with *B. argentina* at 1:4 dilution and only partial reactions by some serum samples at 1:16, 1:64, and 1:256 dilutions. The titers to *P. vivax* during infection were 1:256 and 1:1024.

#### Naturally Acquired Malaria

Table IV shows the results of IFA tests for malaria and *B. argentina* antibodies in serum samples from 22 naturally acquired cases of malaria. Sixteen samples had antibody titers of 1:64 or greater to the infecting species of parasite. The six remaining samples with low antibody titers were all

from *P. vivax* infections; two of these showed higher titers to a heterologous parasite, *P. falciparum*. Samples from three *P. vivax* infections reacted with *B. argentina* antigen.

#### DISCUSSION

The results of blood smears and IFA tests showed that eight out of 10 cattle became infected with *B. argentina*. Although preinfection serum samples of most of the cattle reacted in the IFA test with *P. falciparum* antigen at low dilutions, the 16-fold rise in titer in five animals appeared to be a response from infection with *B. argentina*. However, the 4-fold rise in three infected animals is of doubtful significance, as the same rise was found in animal 10 in the

absence of antibody to *B. argentina*. The reactions between sera of two cattle and *B. microti* antigen suggest an antigenic cross reactivity between this parasite and *B. argentina*. Ristic *et al.* (1971) showed by the use of IFA tests that this strain of *B. microti* was antigenically related to *Babesia canis*.

The reactions of human sera with *B. argentina* antigen differed from the reactions of the cattle sera and *P. falciparum* antigen. Serum from only one of seven *P. falciparum* cases reacted with the *Babesia* antigen. Although no reaction was detected between *B. argentina* antiserum and *P. vivax* antigen, serum samples from six of 37 cases of *P. vivax* malaria reacted with *B. argentina* antigen. Malarial antibody patterns of sera positive to *B. argentina* appeared in no way different from those which did not react with *B. argentina*. Although the numbers are small, the incidence of reactors to *B. argentina* appears to be similar in infections from the two malaria species. If results indicate an antigenic cross reaction, it is likely to be between components of the babesia and antibody to a malarial parasite antigen present in both *P. falciparum* and *P. vivax*.

The cross reactivity was best studied in the induced cases, in which antibody responses to *B. argentina* and the malarial parasites were followed throughout the course of infection. Production of antibody to *B. argentina* differed in a number of ways from that of antibody against heterologous malaria parasites. In malaria infections, Gleason *et al.* (1971) showed that 4-fold differences in titer of sera with homologous and heterologous antigens indicated the infecting species in 77% of the sera submitted for routine diagnosis. In the present work, tests with malaria antigens in cases B9 and B12 indicated, by the above criterion, the infecting species on most occasions. The results with sera from case B2 indicated the infecting species on four of eight occasions. However, results obtained with

TABLE III  
Results of IFA Tests on Serum Samples from Case B7, Showing Lack of Reactivity for *Babesia argentina* at Lower Dilutions

Days after infection	Reactions of serum dilutions to <i>B. argentina</i> <sup>a</sup>					Reciprocal Titer to <i>P. vivax</i>
	1:4	1:16	1:64	1:256	1:1024	
-3						1
8						
15	1	2	3	1		1024
22			2	2		1024
29	1	2	2	1		1024
36			2	1		256
43		1	1			256
50			1			256
57		2	1			256
72	1					256
86						256

<sup>a</sup> Reactions graded from - (negative) to 4 (strong positive).

sera from case B4 indicated the correct species in only one of seven samplings. Reactions to *B. argentina* in these four cases followed a different pattern. On all except the final sample from case B4, the serum titer to *B. argentina* equalled or exceeded that to the infecting parasite. The strongest reaction to *B. argentina* did not occur in case B4, where a strong heterologous response against *P. brasilianum* equalled or exceeded that against the homologous species, *P. vivax*. It occurred, however, in case B9, where the antibody pattern against the malaria species correctly identified the infecting species as *P. vivax*. Strong reactions against *B. argentina* were seen with serum samples taken from two of three natural cases of malaria reacting with this parasite, as shown in Table IV.

The irregular occurrence of antibody reacting with *B. argentina* in the malaria antisera was an outstanding feature. Although numbers were small, the frequency of response to *B. argentina* appeared to be about the same, one reactor in seven cases, in both induced and natural malaria. It occurred with both blood-induced and mosquito-in-

TABLE IV  
*Results of Tests for Plasmodia and Babesia argentina Antibody on Serum Samples  
 from 22 Naturally Occurring Cases of Malaria*

Serum number	Infecting species	IFA test results (reciprocal of titer)				
		<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>	<i>P. ovale</i>	<i>B. argentina</i>
1	<i>P. vivax</i>	16	16		NT <sup>a</sup>	
2	<i>P. vivax</i>	64	16	16	NT	
3	<i>P. vivax</i>	256	64	16	NT	
4	<i>P. vivax</i>	256	64		NT	
5	<i>P. vivax</i>	64	64	16	NT	1024
6	<i>P. ovale</i>		64	4	256	
7	<i>P. vivax</i>	64	64	256	NT	
8	<i>P. vivax</i>				NT	
9	<i>P. vivax</i>	4	256		NT	
10	<i>P. vivax</i>	256	64	64	NT	
11	<i>P. falciparum</i>	64	1024	64	NT	
12	<i>P. falciparum</i>	16	1024	64	NT	
13	<i>P. vivax</i>	64	16	64	NT	1024
14	<i>P. vivax</i>	4	4		NT	
15	<i>P. vivax</i>		16	16	NT	
16	<i>P. vivax</i>	16	64	16	NT	
17	<i>P. vivax</i>	256	16	64	NT	
18	<i>P. vivax</i>	1024	64	16	NT	256
19	<i>P. falciparum</i>		64		NT	
20	<i>P. falciparum</i>	64	256	64	NT	
21	<i>P. vivax</i>	64	256		NT	
22	<i>P. vivax</i>	16	16		NT	

<sup>a</sup> Not tested.

duced infections and with more than one strain of *P. vivax*. It differed noticeably from the common response of malaria patients to heterologous plasmodia as well as to the infecting species (Collins *et al.* 1963). The reasons for the irregular occurrence of this reaction are not clear from our work. It is a common immunological phenomenon that not all subjects react alike and that differences may be found in cross reactions of serum samples from different individuals (Landsteiner 1962). When antisera against some antigens are being prepared, many animals may be needed to find individuals producing a good response. Such responses, however, are usually graded, but the reaction between malaria antisera and *B. argentina* antigen was "all or none." The possible exception was in case B7 (Table III) where the reaction resembled a prozone. This

may represent the production of antibody of poor reactivity or avidity to *B. argentina*.

Increased globulin production occurs during malaria infection; not all of this globulin is specifically directed against the parasite (Curtain *et al.* 1964). Kitchen *et al.* (1939) demonstrated positive Wassermann reactions in many malaria patients, and Oliver-Gonzales and Torregrosa (1944) found isoagglutinin titers increased above the normal range in persons who had suffered repeated attacks of malaria. Tick transmission of *B. argentina* to cattle was used in this study rather than blood passage, to prevent the production of isoantibodies that might possibly interfere in IFA tests.

The production of certain antibodies may be related to the presence of Forssman anti-

gen in invading parasites. Cox *et al.* (1966) described the presence of agglutinins for trypsinized erythrocytes in *P. berghei* infections of rats. Kreier (1969) has described a similar agglutinin in *P. gallinaceum* infections of chickens. The reactions described in this paper between *B. argentina* antigen and sera from some malaria cases may be nonspecific or related to the presence of Forssman antigen in these parasites. The occurrence, however, of elevated titers to *P. falciparum* antigen in some cattle infected with *B. argentina* must also be considered as indicating a possible antigenic relationship between the two parasites. The present observations suggest that further investigation is required in this area.

The question of the taxonomy of *Babesia* and their position among the protozoa has been repeatedly examined. In 1964 the Committee on Taxonomy and Taxonomic Problems of the Society of Protozoologists removed the babesia from the sporozoa and established them as class Piroplasma under the superclass Sarcodina (Honigberg *et al.* 1964). At this time the *Babesia* were thought to differ markedly from the plasmodia. Most recently the subject has been reviewed by Levine (1971) who placed the babesia in Class Piroplasma, which, with Class Sporozoa, formed the Subphylum Apicomplexa. Electron microscope studies (Simpson *et al.* 1967, Frerichs 1970) have shown that *Babesia* possess an apical complex resembling that of coccidia, plasmodia, and toxoplasmatids. The occurrence of common or related antigens in *Babesia* and plasmodia is further evidence that these parasites are not as widely separated phylogenetically as was previously believed.

Whether the cross reactivity between *B. argentina* and *P. falciparum* and *P. vivax* is specific or not, it should be kept in mind when interpreting IFA test results from persons thought to be infected with malaria, especially when there has been contact with cattle and ticks.

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