CHARACTERISTICS OF THE PIROPLASMS BABESIA ARGENTINA AND B. BIGEMINA IN THE UNITED STATES[1]

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PURPOSE OF INVESTIGATION

The purpose of the present investigation was to determine (1) what species of *Babesia* occur in this country, (2) what their morphological and physiological characters are, and (3) whether there is any variation in their reaction to trypan blue, which thus far has been the most promising means of treating clinical cases of babesiasis. In order to ascertain whether *B. argentina* is a valid species, the morphological characters of piroplasms occuring in Europe, Africa, and South America were also studied.

REVIEW OF LITERATURE

VALIDITY OF PROPOSED GENERA AND SPECIES

In 1893 Smith and Kilborne (*13*)[2] stated that the shape of piroplasms resembles that of apple seeds. Subsequent investigators have shown that the piroplasm nucleus is composed of chromatin particles occurring in more than one body; in other words, the chromatin material is scattered. Up to the present time nothing else of morphological importance has been added. Therefore, there appear to be no valid criteria for a number of genera that have been proposed in the family Babesiidae Poche, 1913, and the writer concurs in the views of Wenyon (*15*) and Reichenow (*10*) that only one generic name, *Babesia* Starcovici, 1893, is valid, and that the following names are synonyms: *Piroplasma* Patton, 1895; *Nicollia* Nuttall, 1908; *Nuttallia* França, 1909; *Smithia* França, 1909; *Rossiella* Nuttall, 1912; *Microbabesia* Sohns, 1918; *Babesiella* Mesnil, 1919; and *Françaiella* Yakimoff, 1926. Under these names are reported parasites of bovines, ovines, equines, canines, and rodents.

The occurrence in Argentina of two species of *Babesia, B. bigemina* and *B. argentina,* was first reported by Lignières (*5*). In material collected from cases of piroplasmosis in Louisiana, the writer (*8*) noted that the piroplasms were scanty in the peripheral blood; that they were abundant post mortem in the organs, minute in size when compared with other piroplasms known to be *B. bigemina,* and that quadruple infections of erythrocytes were common. Dennis,[3] of the University of California, noted a resemblance to the genus *Nuttallia* and regarded the species as different from *B. bigemina.* Becker,[3] of the Iowa State College of Agriculture, noted that the angle between the longitudinal axes of members of the intraglobular couple was usually about 180°, whereas in *B. bigemina* which he obtained from Texas it was seldom greater than 60°. Therefore, Becker and Rees

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[2] Reference is made by number (italic) to Literature Cited, p. 437.

[3] Personal communication.

(*2*) reported the occurrence in Louisiana of a species of *Babesia* (syn., *Piroplasma* Patton, 1895) in addition to the well known *B. bigemina*. This other species, reported by Rees (*8*) as *B. bigemina*, has been determined as *B. argentina* Lignières, 1903.

It appears advisable to recognize *Babesia bigemina* and *B. argentina* as valid species, although Wenyon (*15*), Schilling (*11*), and Reichenow (*10*) do not concur in this view. *B. bovis* Starcovici, 1893, is commonly recognized as a valid species; it is a very small piroplasm like *B. argentina*, and resembles the latter also in that the angle between longitudinal axes of the couple of intraglobular parasites is usually about 180°, but it differs from *B. argentina* in that it is located in the margin of the infected erythrocyte, whereas *B. argentina* is located in the center. As is shown by the data of the present paper, *B. berbera* Sergent and collaborators, 1924, (*12*) resembles *B. argentina*. Yakimoff and collaborators (*16, 17, 18, 19*) have proposed several new species for forms occurring in northern Europe and in Asia that are practically indistinguishable from *B. argentina*.

PIROPLASMS STUDIED BY SMITH AND KILBORNE

The report of Smith and Kilborne (*13*) carries adequate evidence that they were dealing with both *Babesia bigemina* and *B. argentina*. They show in plate 7, figure 2, drawn from a capillary in the kidney, what is clearly a pure infection with *B. argentina;* the parasites are uniform in size, being not more than 2½μ long, which is much too small for *B. bigemina*. In plate 4, figure 4, and plate 6, figures 1 and 2, they show the commonly occurring quadruple infection of erythrocytes which characterizes an infection with *B. argentina*. They report very severe infections and state (*13, p. 61*) that "a long search is necessary before one (parasite) is brought into view", and they say further in the same paragraph: "When present in considerable numbers in the blood the infected corpuscles usually appear in groups in the field of the miscroscope as is shown in the figure referred to (pl. 5, fig. 2), and not uniformly distributed." In further explanation of the figure they state: "The appearance of the infected corpuscles in groups * * * was especially marked in this animal." Lignières (*5*) pointed out that the latter phenomenon is a characteristic of infections produced by *B. argentina* but not of infections produced by *B. bigemina*. Although it has been copied in text books as a figure of *B. bigemina*, this illustration of Smith and Kilborne (pl. 5, fig. 2) is clearly a figure of *B. argentina*. The following statement occurs in their paper (*13, p. 61*):

> With only 1 or 2 per cent, or even 10 per cent, of infected corpuscles in the circulating fluid, it would be difficult to account for the enormous daily losses of blood corpuscles in the acute fever. The difficulty is cleared up by sacrificing an animal in the earlier days of the fever and examining the internal organs for infected corpuscles. Large numbers of parasites are found within corpuscles in the capillary blood of congested areas, such as those of the heart muscle and of the omentum.

The foregoing citations are good descriptions of a typical infection with *Babesia argentina*, but not of one with *B. bigemina*.

Intraglobular forms of the parasite are illustrated in Smith and Kilborne's paper (*13*) in text figure 3, which shows that the angles between the longitudinal axes of the couples are about 150°, 160°, and 180°, respectively; the organisms figured are *Babesia argentina*.

On the other hand, in plate 5, figure 3, a smear of peripheral blood, the organisms are *B. bigemina;* a solitary spindle-shaped form is about 7μ long, which is longer than the diameter of the normal bovine corpuscle, whereas the other parasites in grouped couples are 4μ long. The unstained parasites figured in plate 8, figures 1–5, are *B. bigemina.* Though other pertinent citations might be furnished, the data presented by Smith and Kilborne show adequately that two species of *Babesia, B. bigemina* and *B. argentina,* were seen and described by them under the name *Pyrosoma bigeminum.*

EFFECT OF TRYPAN BLUE ON PIROPLASMS

A number of prominent veterinarians who have large cattle practices in Louisiana have told the writer they they do not use trypan blue in cases of bovine piroplasmosis. These practitioners stated that whereas in certain outbreaks clinical cases were cured by intravenous injections of the drug, in other outbreaks it had no apparent effect.

Trypan blue was found by Nuttall and Hadwen (*7*) and by Theiler (*14*) to be a specific in infections with *Babesia canis* (Piana and Galli-Valerio, 1895) and *B. bigemina,* but was found by Brumpt (*3*) to have no marked therapeutic action in infections with *B. argentina.*

MATERIAL

The material used in the present investigation was obtained from the following sources: (1) Smears from the heart of a cow that was in a herd of purebred Holstein-Friesians, at Lafayette, La., in which 12 out of about 13 cases of infection with *Babesia argentina* proved fatal; (2) a strain of this species of *Babesia* obtained by infecting another cow at the Jeanerette station with the progeny of a tick, *Boophilus annulatus* (Say, 1821), Curtice, 1891, taken from one of these cows; (3) 35 other cases of infection with *B. argentina,* all occurring in Louisiana; (4) 8 cases of infection with a strain of *B. bigemina* shipped to Jeanerette, La., in blood from Texas; (5) 2 naturally occurring Louisiana cases involving *B. bigemina;* and (6) smears of the following piroplasms which were sent to the writer through the courtesy of the investigators named: *Babesia bovis* from Dr. R. Wetzel of the Tierärztliche Hochschule, Hannover, Germany; *Babesiella berbera* from Dr. Sergent, of the Institut Pasteur d'Algérie; *Babesiella minor* Quevedo, 1918, and *Babesia bigemina* from Dr. F. Rosenbusch, of Buenos Aires, Argentina. The writer believes that all these forms belong in the genus *Babesia* and considers *Babesiella minor* a synonym of *Babesia argentina.*

MORPHOLOGICAL CHARACTERS OF THE PIROPLASMS

BIOMETRY

Table 1 shows that *Babesia bigemina* is longer than *B. argentina,* the latter being about the same length as *B. berbera,* and, furthermore, that *B. bovis* is the smallest of the four species. The various forms of *Babesia* occurring in the peripheral blood have mean lengths that vary from 2.26 $\pm$ 0.04μ (smear of *B. bigemina* from Argentina) to 5.00 $\pm$ 0.05μ for *B. bovis.* There were three length groups, about 4, 3, and 2.25μ, respectively, with *B. bigemina* in the first group, *B. argentina* and *B. berbera* in the second, and *B. bovis* in the third. The ranges in length (table 1) were from 2.5 to 5.5μ for *B. bigemina,* 2 to 4.5μ for *B. argentina,* 2 to 5μ for *B. berbera,* and 2 to 3μ for *B. bovis,* so that,

except for *B. bovis*, there was almost complete overlapping. In smears of *B. bigemina* from Argentina the parasites were longer than in smears of the same species from Texas, the means being $5.00 \pm 0.05\mu$ and $4.02 \pm 0.04\mu$. This fact supports the statements which are found generally in the literature that discontinuous variations occur between different strains in the same species.

Table 1 shows that *B. bigemina* occurring in smears made from the heart is smaller than in those made from the peripheral blood, the mean lengths being $4.02 \pm 0.04\mu$ and $3.70 \pm 0.04\mu$; this applies also to *B. argentina*, with corresponding lengths of $3.14 \pm 0.04\mu$ and $2.75 \pm 0.03\mu$. However, in each species these differences of less than 1μ were not so great as those shown in the drawings by Smith and Kilborne (*13*) all of which were supposed to be of *B. bigemina*.

TABLE 1.—*Length of specimens of Babesia and magnitude of angle of the intra-globular couple*

Kind of smear and source	Length		Magnitude of angle	
	Microns	Frequency	Degrees	Frequency
BABESIA BIGEMINA				
Peripheral blood (Texas)	2.5	8	20	40
	3.0	18	30	34
	3.5	33	45	13
	4.0	34	60	12
	4.5	17	90	37
	5.0	38	120	4
	5.5	2	150	6
			180	4
Mean or total	4.02±0.04	150	57.0	150
Heart blood (Texas)	2.5	12	20	12
	3.0	11	30	35
	3.5	21	45	13
	4.0	41	60	8
	4.5	10	90	26
	5.0	5	150	3
			180	3
Mean or total	3.70±0.04	100	56.8	100
Peripheral blood (Argentina)	4.5	6	20	14
	5.0	13	30	5
	5.5	6	45	2
			60	2
			90	2
Mean or total	5.00±0.05	25	32.8	25
BABESIA ARGENTINA				
Peripheral blood (Louisiana)	2.0	3	20	8
	2.5	23	30	9
	3.0	31	45	6
	3.5	31	60	6
	4.0	10	90	21
	4.5	2	120	7
			150	18
			180	25
Mean or total	3.14±0.04	100	109.9	100
Heart blood (Louisiana)	2.0	9	20	3
	2.5	44	30	4
	3.0	36	45	6
	3.5	9	60	7
	4.0	2	90	14
			120	7
			150	20
			180	39
Mean or total	2.75±0.03	100	129.9	100

TABLE 1.—*Length of specimens of Babesia and magnitude of angle of the intraglobular couple*—Continued

BABESIA ARGENTINA (SYN., BABESIELLA MINOR)

Kind of smear and source	Length		Magnitude of angle	
	Microns	Frequency	Degrees	Frequency
Peripheral blood (Argentina)	2.0	3	20	1
	2.5	8	30	3
	3.0	10	90	6
	3.5	3	120	1
	4.0	1	150	7
			180	7
Mean or total	2.82±0.07	25	123.2	25

BABESIA BERBERA (SYN., BABESIELLA BERBERA)

Peripheral blood (Algeria)	2.0	9	20	6
	2.5	14	30	9
	3.0	15	45	1
	3.5	6	60	3
	4.0	4	90	13
	4.5	1	120	6
	5.0	1	180	12
Mean or total	2.89±0.06	50	93.3	50

BABESIA BOVIS

Peripheral blood (Germany)	2.0	13	20	1
	2.5	11	30	1
	3.0	1	45	2
			90	4
			120	1
			150	3
			180	13
Mean or total	2.26±0.04	25	136.4	25

With respect to the angle occurring between the longitudinal axes of the members of the couple in piroplasms, the writer's data show two main groups as follows: (1) *B. bigemina*, with a mean angle of 57° in the Texas strain and 32.8° in the Argentine strain; and (2) *B. argentina* from Louisiana and Argentina, *B. berbera* (syn., *Babesiella berbera*), and *Babesia bovis*, in which species the range of the mean angle was from 93.3° for *B. berbera* to 136.4° for *B. bovis*. It will be noted from table 1 that for all the species in the second group, except *B. berbera*, the mode was about 180°. The latter species was bimodal, i.e., at 90° and 180°. A difference of 20° between the means of *B. argentina* in the peripheral blood and in the heart blood shows that in a given species this character is subject to wide variations. In living couples of *B. argentina*, the writer has observed rapid rotations from an angle of 90° to one of 270°. In fixed material, the maximum figure that can be read is 180° and, therefore, the statistics may not show the real differences between the angles of the two species. Table 1 shows that in the four species of piroplasms the range of variation was from 20° to 180°, i.e., there is complete overlapping. Although in the smaller *Babesia*, i.e., *B. argentina*, *B. berbera*, and *B. bovis*, the mean angle is greater than in *B. bigemina*, the limits of variation in all four species are the same so far as the angle is concerned.

Drawings, all on the same scale, of *Babesia bigemina* from Texas, *B. argentina* from Louisiana and Argentina, *B. berbera* from Algeria, and *B. bovis* from Germany are presented in figure 1. These drawings

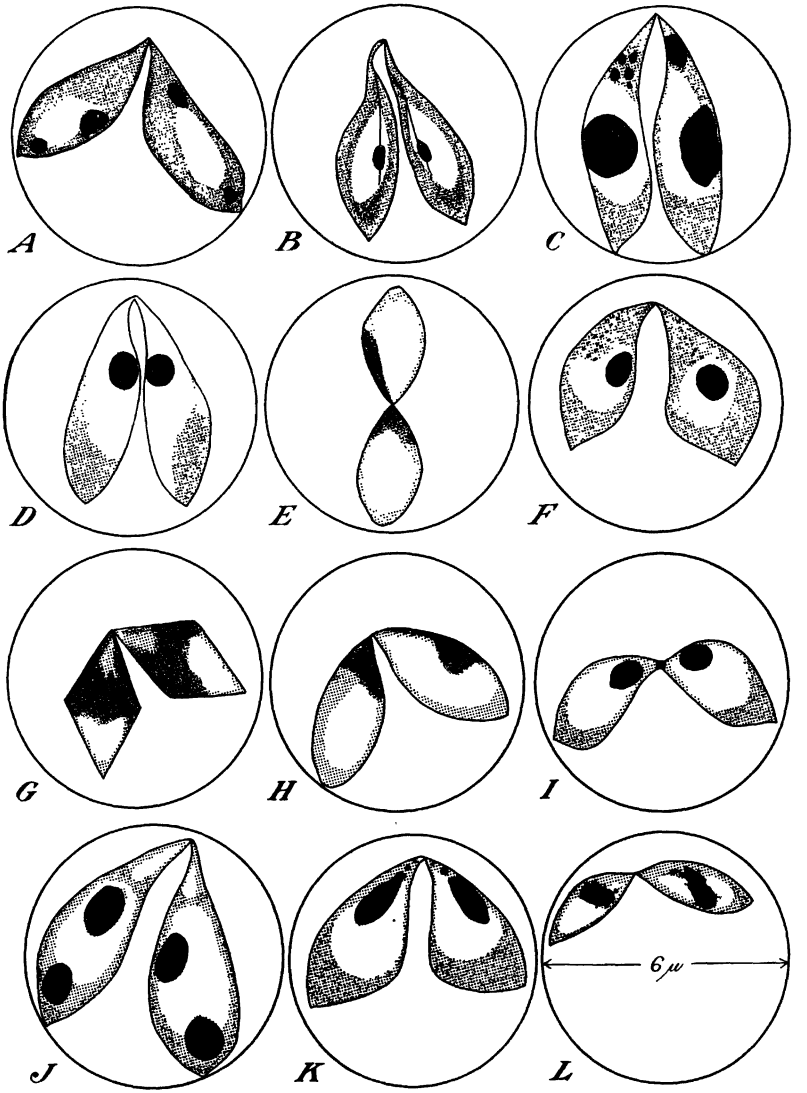

FIGURE 1.—Camera lucida drawings of four species of *Babesia: A, B, C, B. bigemina* from peripheral blood; *D, B. bigemina* from heart blood; *E, F, B. argentina* from peripheral blood; *G, H, B. argentina* from heart blood; *I, B. argentina* (syn., *Babesiella minor*) from peripheral blood; *J, K, Babesia berbera* (syn., *Babesiella berbera*) from peripheral blood; *L, Babesia bovis* from peripheral blood. *B*, from a preparation stained with iron haematoxylin; all others with Giemsa's stain.

show in general the same features as those of previous investigators; there are no morphological criteria other than size, magnitude of angle, and position within the erythrocyte for the determination of species.

Typical forms of *Babesia bigemina* are shown in figure 1, *A*, *B*, and *C*. Figure 1, *B*, from a preparation which was stained in iron haematoxylin, shows a fine fibril such as was described by Dennis (*4*) as the rhizoplast. It could not be found in preparations in which other stains were used. The writer was unable to differentiate the structure which Dennis (*4*) described as the nuclear membrane. However, there was usually a halo about the large chromatin granule.

Figure 1, *D*, illustrating the appearance of *Babesia bigemina* from the heart, shows 1 mass of chromatin in the nucleus, not 2 or 3 masses such as occur in parasites from peripheral blood; this characteristic was general in the writer's material.

Figure 1, *E* to *H*, illustrates *Babesia argentina* from Louisiana, *E* and *F* being from peripheral blood and *G* and *H* from heart blood. *G* is similar to the couple of spindle-shaped parasites shown in text figure 3 of Smith and Kilborne's (*13*) paper. These authors mentioned spindle forms as being typical of *B. bigemina*. The writer has found spindle forms consistently in *B. argentina* but never in *B. bigemina*.

I, in figure 1, is *Babesia argentina* (syn., *Babesiella minor*) from Argentina. *J* and *K* are *B. berbera* (syn., *Babesiella berbera*) from a preparation by the Institut Pasteur d' Algérie. The size of *B. berbera* in *J* is similar to that of *B. bigemina;* although large parasites were rare in *B. berbera*, their occurrence was demonstrated by statistics also. *B. berbera* (*K*) is indistinguishable from *B. argentina* (*F*). Figure 1, *L*, shows that *B. bovis*, which was received from Hannover, Germany, was easily distinguishable from the three other species both by its smaller size and by its marginal position within the erythrocyte.

PHYSIOLOGICAL CHARACTERS OF THE PIROPLASMS

NUMBER DURING LIFE IN THE PERIPHERAL BLOOD AND POST MORTEM IN THE ORGANS

In the writer's cases of *Babesia argentina* infection, the parasites were scanty in the peripheral blood, agreeing in this respect with descriptions of infection produced by this species in other parts of the world. On September 18, 1931, blood was drawn from a "carrier" at Kaplan, La., defibrinated, and then injected intravenously into two Brahman-Hereford cows, nos. 77 and 78, at Jeanerette, La. Both cows reacted on the sixth day, no. 77 died on the eleventh day and no. 78 on the twelfth day; each case had haemoglobinuria for 3 days prior to death. During this time infected corpuscles were scanty and hard to demonstrate in the smears. In occasional smears, however, they were plentiful; when plentiful they occurred in groups. Figure 2 shows the occurrence of *B. argentina* in 15 out of 16 corpuscles in the blood of cow 77.

It has been stated elsewhere in this paper that whereas *Babesia argentina* was scanty in the peripheral blood it occurred abundantly in smears made from the organs. Figure 3 represents 16 corpuscles in a capillary of the brain of cow 77; 15 of the corpuscles were parasitized; in one case there was a quadruple infection, in another a triple infection, and there was one couple of extraglobular piroplasms.

In *Babesia bigemina* infections the parasites were numerous in the peripheral blood and were uniformly distributed, never occurring in groups as in the case of infection produced by *B. argentina*. Capillaries which were congested with infected erythrocytes were not found

post mortem in the organs, not even in the brain. This was in marked contrast to the condition occurring in fatal cases produced by *B. argentina.*

The foregoing data indicate the following things: (1) The principal seat of multiplication of *Babesia argentina* is in the capillaries, par-

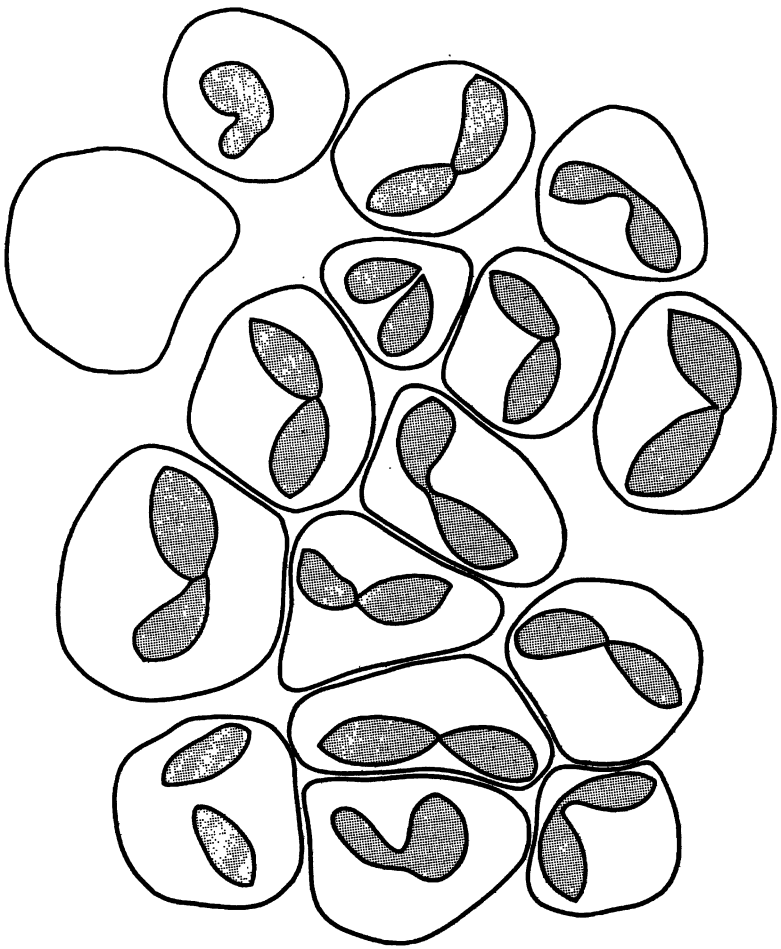

FIGURE 2.—Camera lucida drawings showing the occurrence of *Babesia argentina* in 15 out of 16 corpuscles of the peripheral blood; live blood from cow 77.

ticularly in those of the internal organs, and when released into the peripheral circulation *B. argentina* occurs in clusters and is not uniformly distributed; (2) infections with *B. bigemina* are heavy in the peripheral blood; (3) in the peripheral blood *B. bigemina* does not occur in groups as does *B. argentina;* (4) the infections with *B. bigemina* in the capillaries of the internal organs are light as compared with those of *B. argentina.*

BEHAVIOR IN CULTURE

By the method of Bass and Johns (*1*) the writer was able to cultivate *Babesia argentina* for 96 hours, but he did not succeed in

establishing subcultures. *B. bigemina* could not be cultivated, the parasites becoming rounded up within several hours after transfer to the culture tubes and disappearing within 36 hours.

REACTIONS TO TRYPAN BLUE

A case of infection with *Babesia bigemina* was discovered in bull 70 on April 2, 1932, at the experimental barn. Rees (*9*) has reported this case as one of accidental transmission by an infected lancet. A count on April 2 showed 120 infected erythrocytes per 1,000. This bull weighed 650 pounds and was treated by intravenous injection with 600 mg of trypan blue in 500 cc of physiological saline. In smears which were taken 4 hours after treatment there was only an occasional parasite, and on the following morning there were none. The prognosis on the morning of April 4 was favorable, but the bull was accidently killed by a drenching procedure. Smears from the heart, brain, spleen, and liver were all negative for piroplasms.

Bull 71 had a very severe case of infection with *Babesia bigemina* on August 2, 1932, the urine being colored a deep blood red, and although no counts were made, the infection in the peripheral blood was heavy. This bull weighed about 800 pounds. It was injected with 1 g of trypan blue at 4 p.m. by the same method as with bull 70. At 10 p.m. there were very few parasites in the smears and these appeared to be poorly defined and did not stain as do normal piroplasms. The smears that were made the next morning were negative. Within 24 hours after treatment haemoglobinuria disappeared; the bull made a rapid recovery.

The writer has been favored with the cooperation of H. Laughlin, a veterinarian, in treating nine cases of infection with *Babesia argentina* that occurred in his local practice from March 2 to August 31, 1932, microscopic diagnoses having been made by the writer. Details concerning two of these cases were as follows:

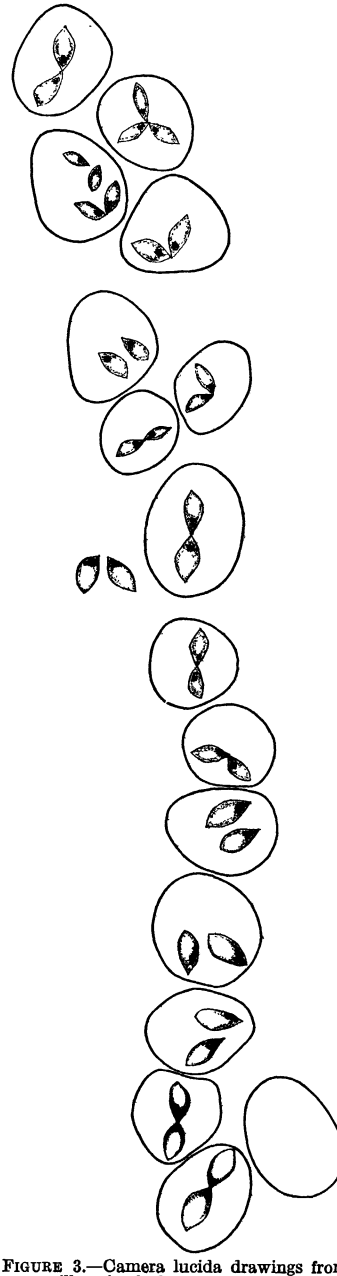

FIGURE 3.—Camera lucida drawings from a capillary in the brain of cow 77, showing 16 corpuscles, 15 of which were parasitized with *Babesia argentina;* also 1 pair of extra-globular corpuscles.

A purebred Jersey bull weighing about 1,500 pounds had haemoglobinuria and a temperature of 107° F. on July 13, 1932. Five smears showed *Babesia argentina;* in one smear the

parasites were fairly numerous. At 9 a.m. the bull was injected intravenously with about 2 g of trypan blue; at 9 p.m. it was in extremis. At 5:30 a.m. on July 14, it was dead. While the body temperature was still near normal, smears of the heart, brain, and spleen were made, and these showed very heavy infections with *Babesia argentina*. The organisms were apparently rounded up but stained normally.

A grade Jersey steer weighing about 600 pounds was observed on August 31, 1932, at 11 a.m. and showed the same symptoms and parasites as the Jersey bull. It was injected intravenously at 12 m. with 3 g of quinine hydrochloride in 500 cc of normal saline, and at 1 p.m. with 1 g of trypan blue. On September 1, its condition was unchanged. On September 2, at 8 a.m., it was dead. Necropsy results were the same as in the case of the Jersey bull.

All seven of the remaining cases of *Babesia argentina* were treated with trypan blue only. One was examined post mortem on August 27, about 1 hour after death. This case had received two intravenous injections of trypan blue, one 72 hours before death and the other 48 hours before death. Smears of the organs showed numerous parasites apparently normal when compared with those in untreated fatal cases. Of the other six cases, not examined post mortem, three died within 24 hours, another within 48 hours, and the remaining two within 96 hours after treatment.

SUMMARY

A species of *Babesia* occurring in the United States has the following characters: (1) Minute size, (2) scanty occurrence in the peripheral blood although occasional occurrence in the field of the microscope in groups, (3) heavy infection in the internal organs, where quadruple infections of erythrocytes are common, and (4) the common occurrence of an angle of about 180° between the longitudinal axes of the two members of the intraglobular couple. On the basis of the above characters this species has been determined as *Babesia argentina* Lignières, 1903. The author's investigation has confirmed the above-mentioned characteristics.

Babesia bigemina is larger than *B. argentina*, infections are heavy in the peripheral blood and scanty in the internal organs, and the angle between the longitudinal axes of the members of the intraglobular couple is usually less than 60°.

The present investigations indicate that the piroplasms described and illustrated by Smith and Kilborne in 1893 included *B. argentina* as well as *B. bigemina*, although these workers did not differentiate the two species.

The mean length of a Texas strain of *B. bigemina* was $4.02 \pm 0.04\mu$ and that of an Argentine strain was $5.00 \pm 0.05\mu$; the mean lengths of a Louisiana strain and an Argentine strain of *B. argentina* were $3.14 \pm 0.04\mu$ and $2.82 \pm 0.07\mu$, respectively; that of an Algerian strain of *B. berbera* was $2.89 \pm 0.06\mu$; and that of a German strain of *B. bovis* was $2.26 \pm 0.04\mu$.

B. bigemina and *B. argentina* in the peripheral blood were larger than in the heart blood, but the difference in each case was smaller than 1μ, and less than that shown between peripheral blood forms and heart forms in the figures of Smith and Kilborne.

The magnitude of the mean angle formed by the intraglobular couple in piroplasms was as follows: (1) Argentine strain of *B. bigemina*

32.8°, (2) Texas strain of *B. bigemina*, 57°, (3) Argentine strain of *B. argentina* (syn., *Babesiella minor*), 123.2°, (4) Louisiana strain of *B. argentina*, 109.9°, (5) Algerian strain of *B. berbera*, 93.3°, and (6) German strain of *B. bovis*, 136.4°.

The writer's drawings of *B. bigemina* show this piroplasm in the heart blood with only one mass of chromatin but in the peripheral blood with more than one mass.

Spindle-shaped forms have been shown in the present paper to characterize *B. argentina* and not *B. bigemina*, contrary to the findings of Smith and Kilborne (*13*).

No significant morphological differences were detected between *B. argentina* and *B. berbera*, nor between a Louisiana strain and an Argentine strain of *B. argentina* (syn., *Babesiella minor*), but *B. bovis* was distinguishable from both of the above-named species by its smaller size and its marginal position within the erythrocyte.

B. argentina was cultivated for 96 hours in vitro; *B. bigemina* could not be cultivated.

In agreement with the results of previous investigators it was found that *B. bigemina* was killed by intravenous injections of trypan blue, but *B. argentina* was not demonstrably affected.

LITERATURE CITED

(1) Bass, C. C., and Johns, F. M.
1912. The cultivation of malarial plasmodia (plasmodium vivax and plasmodium falciparum) in vitro. Jour. Expt. Med. 16: 567–579.

(2) Becker, E. R., and Rees, C. W.
1932. The occurrence of more than one species of piroplasma in the United States. Jour. Parasitol. 19: 92.

(3) Brumpt, E.
1920. Les piroplasmes des bovidés et leurs hôtes vecteurs. Bull. Soc. Path. Exot. 13: 416–460, illus.

(4) Dennis, E. W.
1930. The morphology and binary fission of babesia bigemina of Texas cattle-fever. Calif. Univ. Pubs., Zool. 33: [179]–192, illus.

(5) Lignières, J.
1903. La piroplasmose bovine. Nouvelles recherches et observations sur la multiplicité des parasites, leur évolution, la transmission naturelle de la maladie et la vaccination. Arch. Parasitol. 7: [398]–407, illus.

(6) Nuttall, G. H. F., and Graham-Smith, G. S.
1908. The mode of multiplication of piroplasma bovis, and p. pitheci in the circulating blood compared with that of p. canis, with notes on other species of piroplasma. Parasitology 1: 134–142, illus.

(7) ——— and Hadwen, S.
1909. The successful drug treatment of canine piroplasmosis, together with observations upon the effect of drugs on piroplasma canis. Parasitology 2: 156–191, illus.

(8) Rees, C. W.
1930. Experimental transmission of bovine anaplasmosis and piroplasmosis by means of an infected lancet. North Amer. Vet. 11 (10): 17–20, illus.

(9) ———
1932. The transmission of piroplasmosis by means of a lancet. Jour. Parasitol. 19: 92.

(10) Reichenow, E.
1929. Lehrbuch der protozoenkunde, eine darstellung der naturgeschichte der protozoen mit berücksichtigung der parasitischen und pathogenen formen... Aufl. 5, 3 pts., illus. Jena.

(11) SCHILLING, C., and MEYER, K. F.
1927. PIROPLASMOSEN. *In* Kolle, W., and Wassermann, A. V., Handbuch der Pathogenen Mikroorganismen, Aufl. 3, Bd. 8, lfg. 9, pp. 1–94, illus. Jena.

(12) SERGENT, E., DONATIEN, A., PARROT, L., LESTOQUARD, F., PLANTUREUX, E., and ROUGEBIEF, H.
1924. LES PIROPLASMOSES BOVINES D'ALGÉRIE. Arch. Inst. Pasteur Algérie 2: 1–146, illus.

(13) SMITH, T., and KILBORNE, F. L.
1893. INVESTIGATIONS INTO THE NATURE, CAUSATION, AND PREVENTION OF SOUTHERN CATTLE FEVER. U.S.Dept.Agr., Bur. Anim. Indus. Bull. 1, 301 pp., illus. (Also published under title of Investigations into the Nature, Causation, and Prevention of Southern Cattle Fever in U.S. Dept. Agr. Ann. Rpts. 8–9: 177–304, illus. 1891–92.)

(14) THEILER, A.
1912. THE TREATMENT OF REDWATER IN CATTLE WITH TRYPAN BLUE. Vet. Jour. 68: 64–73.

(15) WENYON, C. M.
1926. PROTOZOOLOGY; A MANUAL FOR MEDICAL MEN, VETERINARIANS AND ZOOLOGISTS. 2 v., illus., London.

(16) YAKIMOFF, W. L., and BELAWINE, W.
1926. LA NOUVELLE ÉSPECE DU SOUS-GENRE BABESIELLA MESNIL, EN RUSSIE (USSR). Centbl. Bakt. [etc.] Originale (I) 103: 315–320, illus.

(17) ——— and BOURZEFF, W. J.
1927. DIE RINDERPIROPLASMOSE UND IHRE ERREGER IN WEISSRUSSLAND (BELORUSSIE). Arch. Protistenk. 59: [339]–349, illus.

(18) ——— SUDZILOWSKY, M., and ROSTEGAIEFF, E. F.
1930. ZUR FRAGE DER PIROPLASMENARTEN DES RINDES IN WEISSRUSSLAND. Ztschr. Infektionskrank. u. Hyg. Haustiere 37: 70–81, illus.

(19) ——— and WASSILEWSKY, W. J.
1926. CONTRIBUTIONS À L'ÉTUDE DES PIROPLASMES BOVINE EN RUSSIE. Centbl. Bakt. [etc.] Originale (I) 97: 192–210, illus.