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Piroplasms *(Protozoa: Sarcodina)* of Wild Mammals in California[1]

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ABSTRACT

Piroplasms were found in the blood of *Neotoma lepida, Peromyscus californicus, Microtus californicus, Spermophilus beecheyi* and *Sylvilagus audubonii* during wildlife disease surveys in Southern California. Although naturally infected animals appeared healthy, splenectomy of naturally or experimentally infected animals invariably resulted in severe parasitemias and anemias, often terminating fatally. Limited attempts to infect different mammal species were made with varying success. All of the piroplasms were indistinguishable morphologically and were considered to belong to the same species: *Babesia microti* (Franca, 1912).

INTRODUCTION

Piroplasms are small intra-erythrocytic protozoal parasites, morphologically resembling *Plasmodium* trophozoites, which are widely distributed in mammals. Although of considerable economic importance in some parts of the world as causes of hemolytic diseases in domestic animals, until recently they had not been considered of importance to humans. In 1957, however, a fatal case of human piroplasmosis, probably caused by *Babesia bovis,* a parasite of cattle, occurred in a splenectomized Yugoslav.[10] More recently, a nonfatal human case was diagnosed in California.[13] This second case also occurred in a splenectomized individual, but unlike the first was thought caused by a piroplasm normally infecting wild, rather than domestic animals.[8]

It therefore seemed appropriate to review our knowledge of the nature and distribution in the United States of piroplasms in wild mammals. For California, we could find just 2 reports on this subject: a research note by Wood[17] reporting a piroplasm in blood of a raccoon from Los Angeles County, and our own abstract[15] of the data presented below.

We conducted surveys of small wild mammals on military bases in Southern California during 1963-65. The surveys included examination of blood smears with primary emphasis on detection of hematozoa. Unfortunately, our studies were interrupted because of military orders, and are therefore incomplete; nevertheless, it was considered apropos to report the piroplasm findings at this time.

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MATERIALS AND METHODS

Trapping was done at the following military bases in Southern California: Marine Corps Base, Twenty-nine Palms, San Bernardino County; San Clemente Island; Camp Elliot Training Base, San Diego County; and Camp Pendleton Marine Corps Base, San Diego County. The terrain and fauna of Camp Elliot were quite similar to those of Camp Pendleton, so these areas are considered together. The 2 rabbits from San Diego suburbia were caught in the yards of colleagues. Animals were caught in box traps, anesthetized with chloroform, and bled out from the heart. Thin heart-blood smears were stained with Giemsa and searched for hematozoa using the oil immersion objective of a compound brightfield microscope. Slides were searched for 10 minutes before being considered negative.

When screening of blood smears revealed the presence of piroplasms, efforts were made to trap more individuals of the same species from the same area. These animals were splenectomized, kept alive in the laboratory, and bled by toe or tail clippings at regular intervals. When possible, cross-infection experiments were attempted by intraperitoneal inoculation of ¼ to ½ ml (approx. 5.0 x 10^6 parasites) of infected heparinized blood into uninfected animals. Wild-caught animals were considered uninfected if they had no detectable parasitemias after holding for one month or if they were negative for 7 days following splenectomy.

Except for *Sylvilagus audubonii* and *Spermophilus beecheyi,* on which there was no experimental work other than screening of blood smears, every infected animal was autopsied, either at death or when killed (usually after the peak parasitemia) and smears and sections of heart, lung, liver and kidney examined. Spleens removed for splenectomy were similarly examined.

RESULTS

Survey results: Four hundred and fourteen animals of 24 species were studied.

The following animals were examined with negative results:

(1) San Clemente Island: *Urocyon littoralis,* gray fox, (3); *Mus musculus,* house mouse, (5); *Peromyscus maniculatus,* deer mouse (15).

(2) Twenty-nine Palms: *Ammospermophilus leucurus,* antelope squirrel, (4); *Spermophilus tereticaudus,* round-tailed ground squirrel, (4); *Dipodomys merriami,* kangaroo rat, (6); *D. deserti,* desert kangaroo rat, (1); *Peromyscus crinitus,* canyon mouse, (12); *P. eremicus,* cactus mouse, (2); *Sylvilagus audubonii—Sylvilagus audubonii arizonae* (J. A. Allen, 1877)—desert cotton tail, (2).

(3) Camp Pendleton and Camp Elliot: *Perognathus fallax,* San Diego pocket mouse, (1); *P. californicus,* California pocket mouse, (4); *Reithrodontomys megalotis,* Western harvest mouse, (6); *Peromyscus eremicus,* cactus mouse, (30); *P. maniculatus,* deer mouse, (78); *P. boylii,* brush mouse, (27); *P. truei,* pinon mouse, (8); *Neotoma lepida—Neotoma lepida intermedia* Rhoads, 1894—desert woodrat, (35); *N. fuscipes,* dusky-footed woodrat, (60); *Mus musculus,* house mouse, (4); *Mustela frenata,* weasel, (1); *Didelphis marsupialis,* opossum, (1); *Lepus californicus,* black-tailed jack rabbit, (1).

The following were found infected with piroplasms:

(1) Marine Corps Base, Twenty-nine Palms: *Neotoma lepida lepida* Thomas, 1893, desert woodrat, (4/23).

(2) Camp Pendleton: *Peromyscus californicus insignis* Rhoads, 1895, California mouse, (1/17); *Microtus californicus sanctidiegi* Kellogg, 1922, California vole, (9/28); *Spermophilus beecheyi* subsp., California ground squirrel, (3/25).

(3) Private homes in San Diego suburbs: *Sylvilagus audubonii sanctidiegi* (Miller, 1899), desert cottontail, (2/2).

Description of parasites: Piroplasms from all 5 hosts were similar in appearance (Fig. 1): we could find no morphological characteristics which permitted differentiation under the microscope. The following descriptions therefore apply to all of the California piroplasms.

In naturally infected intact animals, piroplasms were as shown in Fig. 1, D. There was usually a single parasite per infected red blood cell (RBC). When stained

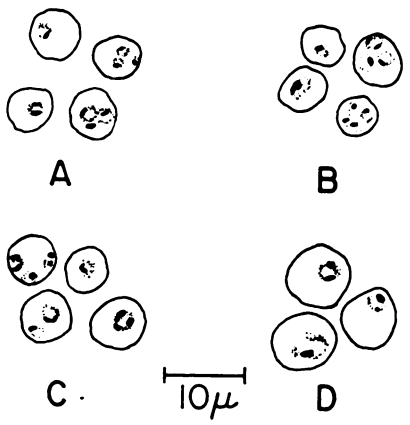

FIGURE 1. *Camera lucida drawings of piroplasms in mammalian erythrocytes. Hosts were as follows: A,* Peromyscus californicus, 8 *days after splenenectomy; B.* Microtus californicus, 10 *days after splenectomy; C,* Neotoma lepida, 13 *days after splenectomy; D,* Spermophilus beecheyi, *intact animal.*

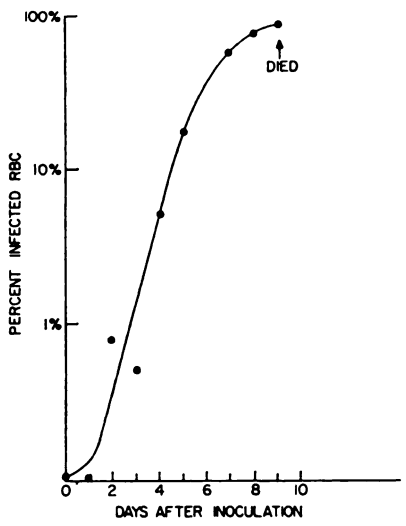

FIGURE 2. *Parasitemia after splenctomy in* Microtus californicus *naturally infected with piroplasms.*

with Giemsa, parasites had a single or double dot of purplish chromatin (nucleus) surrounded by a light bluish strand (cytoplasm) enclosing a central whitish area (vacuole). The overall appearance was that of a tiny purple satellite in an elliptical bluish orbit. Not uncommonly, the cytoplasm was not closed, but appeared to trail off into the host RBC. The diameter of the cytoplasmic mass was usually 1-2 microns, but when trailing off might measure the length of the RBC.

In splenectomized animals, most infected host RBC contained more than one parasite (Fig. 1, B and C). Parasites were usually discreet; in rare cases where they were joined together, the arrangemnt did not resemble a Maltese cross. Parasites with trailing cytoplasm were more common in splenectomized than in intact animals. In animals successfully infected with piroplasms from a different host, there were no constant changes in appearance of the organism.

Microscopic examination of Giemsa stained smears and sections of internal organs, and of the spleens, from infected animals never revealed exo-erythrocytic stages of piroplasms. Nothing suggestive of Koch's bodies was ever seen.

Pathogenicity: Animals which were infected in nature appeared healthy at the time of capture and after being held for several days. The number of infected RBC in such animals did not exceed 2 percent and was usually considerably less. Splenectomy, however, invariably resulted in heavy parasitemias, and, not uncommonly, death. It is presumed that deaths were due to piroplasmosis since splenectomy alone in the wild animals was never fatal. In animals which had piroplasmosis when captured, the single splenectomized *Peromyscus californicus,* 2 of 4 splenectomized *Neotoma lepida* and 3 of 5 splenectomized *Microtus californicus* all died. There was usually a "lag" of 3-7 days following splenectomy, followed by a short period

TABLE 1. *Results of attempts to infect intact and splenectomized animals with Piroplasms from* Neotoma lepida *and from* Microtus californicus.

Animals Experimentally Infected	Piroplasms from N. lepida		Piroplasms from M. californicus	
	No. tested	Infection*	No. tested	Infection*
Microtus californicus				
Intact	1	Neg	2	++
Splenectomized	1	+	11	++ to ++++
Peromyscus californicus				
Intact	1	Neg	1	Neg
Splenectomized	1	Neg	2	++
Peromyscus maniculatus				
Intact	—	—	3	Neg to ++
Splenectomized	—	—	5	Neg to ++
Neotoma lepida				
Intact	2	+++	1	Neg
Splenectomized	4	++++	1	Neg
Neotoma fuscipes				
Intact	2	++	—	—
Splenectomized	2	++++	—	—

*Neg = no parasitemia; + = dividing forms seen, 0-2 per cent RBC infected; ++ = 0-5 per cent RBC infected, polychromasia and anisocytosis of hosts RBC; +++ = 5-50 per cent RBC infected; ++++ = more than 50 per cent RBC infected, at least one third of infections fatal.

(2-4 days) of intense parasite multiplication (Fig. 2). A similar phenomenon was seen in different species which were not refractory to infection (Fig. 3). In animals which survived, the number of infected RBC gradually declined, usually to pre-operative levels, within 3-4 weeks.

Although detailed pathological studies were not done, the heavily infected animals had obvious anemia: the blood was icteric and watery, with extreme anisocytosis, poikilocytosis, and polychromatophilia. Microscopic examination of tissue sections revealed cloudy swelling of liver parenchyma cells and tubular necrosis of the kidney in animals which died within 7-10 days after splenectomy. In survivors killed several weeks after peak infection, hematin-like pigment was widely distributed throughout the reticulo-endothelial system.

A few attempts were made to infect one mammal species with piroplasms from another. Results in the case of *Microtus californicus* and *Neotoma lepida* are shown in Table I (See also Fig. 3). Attempts to infect intact and splenectomized laboratory mice and rats with piroplasms from *Microtus californicus, Neotoma lepida* and *Peromyscus californicus* were unsuccessful: circulating intraerythrocytic parasites could be detected for several days after intraperitoneal injection of infected blood, but there was no active multiplication. Some success was obtained with hamsters inoculated with piroplasms from *N. lepida.* Five hamsters were injected: 3 intact animals developed slight parasitemias with dividing forms present, and one splenectomized hamster did not become infected; but a second splenectomized hamster developed a heavy parasitemia, with 55 percent erythrocytes infected after 6 days.

DISCUSSION

Name for parasites: Small piroplasms of different wild mammals are almost indistinguishable[16] morphologically. Nevertheless, in many cases a certain degree of host specificity has either been assumed[5] or proven[11] so that piroplasms from new

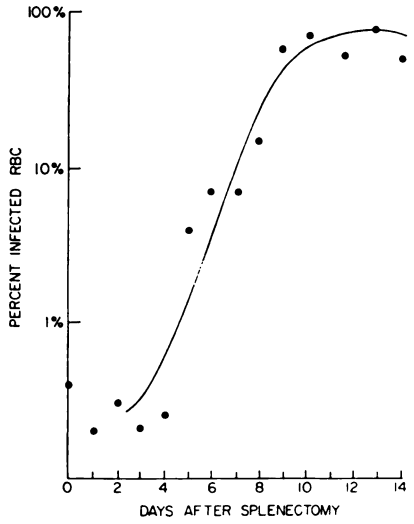

FIGURE 3. *Parasitemia in splenectomized* Neotoma fuscipes *inoculated intraperitoneally with piroplasms from* Neotoma lepida.

Twenty-nine Palms did. The limited survey of *Sylvilagus audubonii* revealed 2 hosts have often been described as new species. This practice was condemned by Rodhain[7] who noted the wide host range of *Babesia rodhaini* and by Shortt and Blackie[9] who considered piroplasms from such varied hosts as moles (*Talpa europaea*), field voles (*Microtus agrestis*) and bank voles (*Clethrionomys glareolus*) to all belong to the same species.

On the basis of our limited experience with piroplasms of *Microtus californicus, Neotoma lepida* and *Peromyscus californicus,* we concluded (as did Shortt and Blackie[9]) that all of these piroplasms best fit the original description by Franca[2] of a piroplasm from "*Microtus incertus* Lelys" (=*Microtus arvalis incertus*). The correct name for the three piroplasms would therefore be *Babesia microti* (Franca, 1912), since the generic name proposed by Franca, *Smithia,* is no longer recognized.[6] In the United States, *Babesia microti* has been previously reported from *Microtus pennsylvanicus* in Massachusetts[12] and in New York[4].

We are less confident in the case of piroplasms from *Spermophilus beecheyi* and *Sylvilagus audubonii,* since no experimental work was done with them, and we cannot be certain they did not have exo-erythrocytic stages. Two previously reported ground squirrel piroplasms in the United States from *Spermophilus variegatus* in Texas[11] and from *S. tridecemlineatus* in Iowa[1] were morphologically similar to the California parasites and neither had exo-erythrocytic stages. In the case of piroplasms from lagomorphs, however, such stages have been reported for hares (*Lepus europaeus*) in Italy.[14] We are unaware of reports of piroplasms in rabbits in the United States.

Epidemiological factors: Although any discussion of the possible relation of the piroplasms herein described to human disease is speculative, there were several findings of possible epidemiological significance. The remarkable change in pathogenicity of the piroplasms following splenectomy, whether of the normal or of a different host species, certainly suggests that the host range could be increased by splenectomy. In addition, infections occurred only in certain areas: *Neotoma lepida*

and *N. fuscipes* at Camp Pendleton did not have piroplasms, whereas *N. lepida* at Twenty-nine Palms did. The limited survey of *Sylvilagus audubonni* revealed 2 of 2 infected in San Diego, and neither of 2 infected at Twenty-nine Palms. It may be of interest that although the species of animals involved were the same, there are slight (subspecific) taxonomic differences between *N. lepida* and *S. audubonii* from the 2 areas.

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