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DESCRIPTION AND EPIZOOTIOLOGY OF *BABESIA POELEA* N. SP. IN BROWN BOOBIES (*SULA LEUCOGASTER* (BODDAERT)) ON SAND ISLAND, JOHNSTON ATOLL, CENTRAL PACIFIC

Thierry M. Work and Robert A. Rameyer

U.S. Geological Survey, Biological Resources Division, National Wildlife Health Center, Honolulu Field Station, P.O. Box 50167, Honolulu, Hawaii 96850

ABSTRACT: We describe a new species of piroplasm from brown boobies (*Sula leucogaster*) on Sand Island, Johnston Atoll National Wildlife Refuge, central Pacific. Mean parasitemia in adults and chicks was less than 1%, with the parasitemia in chicks significantly greater than in adults. There was no significant relation between the age of chicks and the degree of parasitemia. Parasitized red cells and red cell nuclei were significantly smaller than those of unparasitized cells, and infected birds appeared clinically normal. Prevalence of the parasite in chicks (54%) was significantly greater than in adults (13%), and the geographic distribution of parasitized chicks was skewed toward the eastern end of Sand Island. On the basis of morphologic characteristics, we named it *Babesia poelea*. The specific name is a concatenation of the Hawaiian names for dark (po'ele) and booby ('a). This is the second documentation of an endemic avian hemoparasite in seabirds from the central Pacific.

Numerous reports of avian hemoparasites exist in the literature; however, accounts of avian piroplasms are relatively few (Bishop and Bennett, 1992). Schurenkova (1938) was the first to describe an avian piroplasm, *Sogdianella moshkovskii,* from an eagle in Tadjikistan, although Laird and Lari (1957) later reassigned it to the genus *Babesia* Starkovici, 1893. Since Schurenkova's (1938) description, piroplasms have been documented from terrestrial birds on most continents, including Africa (Mohammed, 1958), Southeast Asia (Toumanoff, 1940), central Asia (Laird and Lari, 1957; Yakunin and Krivkova, 1971), Europe (Peirce, 1973), and North America (Croft and Kingston, 1975).

Piroplasms in seabirds have been documented even more rarely. Peirce and Feare (1978) mentioned an undescribed piroplasm from a masked booby (*Sula dactylatra* Lesson) in the Indian Ocean. Earle et al. (1993) described *Babesia peircei* from the jackass penguin (*Sphenicus demersus* Linnaeus) in South Africa. This paper describes a piroplasm we encountered while routinely examining blood smears of brown boobies (*Sula leucogaster* (Boddaert)).

MATERIALS AND METHODS

Johnston Atoll (16°45′N, 169°31′W) is a national wildlife refuge (NWR) located ~1,154 km southwest of Honolulu, Hawaii. The atoll does not form part of the Hawaiian Islands; rather, Johnston Atoll is believed to be most closely associated with a Pacific mountain range called the Marcus-Necker Rise (Amerson and Shelton, 1976). Johnston Atoll is composed of 4 islands. Birds in this study came from Sand Island. Sand Island is composed of 2 segments; the western segment is man-made dredge coral connected to the eastern segment (the original Sand Island) by a causeway of dredged coral (Fig. 1). On Johnston Atoll, brown boobies nest exclusively on Sand and East (Hikina) Islands, which together support a population of about 500 pairs.

We captured 35 brown booby adult females and 35 adult males in April 1995 and 35 chicks in July 1995 with hand nets. Birds were classified as adult females, adult males, or chicks based on facial features, characteristics of their call, plumage, and body size (Pratt et al., 1987). Nesting sites of chicks were recorded as was date of hatching when available. The age of chicks was calculated in days using date of hatching and date of sampling. We weighed boobies to the nearest 50 g with a 2.5-kg spring scale. Birds were restrained manually and bled from the cutaneous ulnar vein using 2.5-cm 20-gauge needles and 5-ml sterile syringes. One-half milliliter of blood was stored at 4 C in 500-μl ethylenediaminetetraacetatic acid (EDTA) tubes for 8–10 hr.

Blood smears were made in duplicate from EDTA blood, allowed to air dry, stored in slide boxes, and returned to the laboratory for staining and microscopic examination. Smears were stained with Wright's giemsa (Difquick; Fisher Scientific, Pittsburgh, Pennsylvania) according to manufacturer's instructions. Prior to examination, slides from all birds were randomized and subsequently read blind. Parasitemia in each bird was measured by counting 10,000 red cells under oil immersion (1,000×) for each smear and calculating the percentage of red cells infected.

Using a calibrated ocular micrometer, we measured cell length and width and nuclear length and width of 142 parasitized and 30 unparasitized red cells. We also measured length and width of 1,198 parasites. Parasites were classified into 3 morphologic categories according to a simplified scheme adapted from Laird and Lari (1957). Ring and schizont forms conformed to those described by Laird and Lari (1957); the remainder were assigned to the amoeboid category. Chromatin granules were counted or classified as "clumped" if so observed. The location of the parasite in the host red blood cell was classified as "polar" if it was situated near or at the end of the elliptical nucleus or "lateral" if adjacent to the major axis of the nucleus. We recorded presence or absence of intracytoplasmic red pigment in the parasite. The area of parasitized and unparasitized host cell and nucleus was calculated using the formula for an ellipse, $\pi \times 0.5a \times 0.5b$; where a = major axis (length) and b = minor axis (breadth).

Parasitemia, parasite length, parasite width, number of chromatin granules greater than zero, red blood cell area, and red blood cell nucleus area were summarized using means, standard deviations, medians, and ranges. Prevalences of parasitized birds and parasitemia were calculated separately for adults (pooled males and females) and chicks. Parasitemia of adults versus chicks was compared, as was area of parasitized versus unparasitized red blood cells and red blood cell nucleus. Pairwise comparisons were done using Student's *t*-test. In cases where assumption of normality or equal variance was violated, we used the Mann-Whitney *U*-test. Association between prevalence and age was evaluated using the chi-square test. Simple linear regression was used to assess the relationship between age of chicks and parasitemia (Daniel, 1987). Level of significance for all tests was 0.05. Nest locations of parasitized and unparasitized chicks were plotted on a map of Sand Island to assess geographic distribution of infection.

RESULTS

Babesia poelea was noted only in red blood cells of infected boobies; no stages were detected in white cells. Prevalence of *B. poelea* in brown booby chicks (19/35 [54%]) was significantly greater ($\chi = 18.4$, df = 1, $P < 0.01$) than in adults (9/70 [13%]). Parasitized host cells had a significantly smaller area than unparasitized cells ($t = 3.923$, $P < 0.01$). Nuclei of par-

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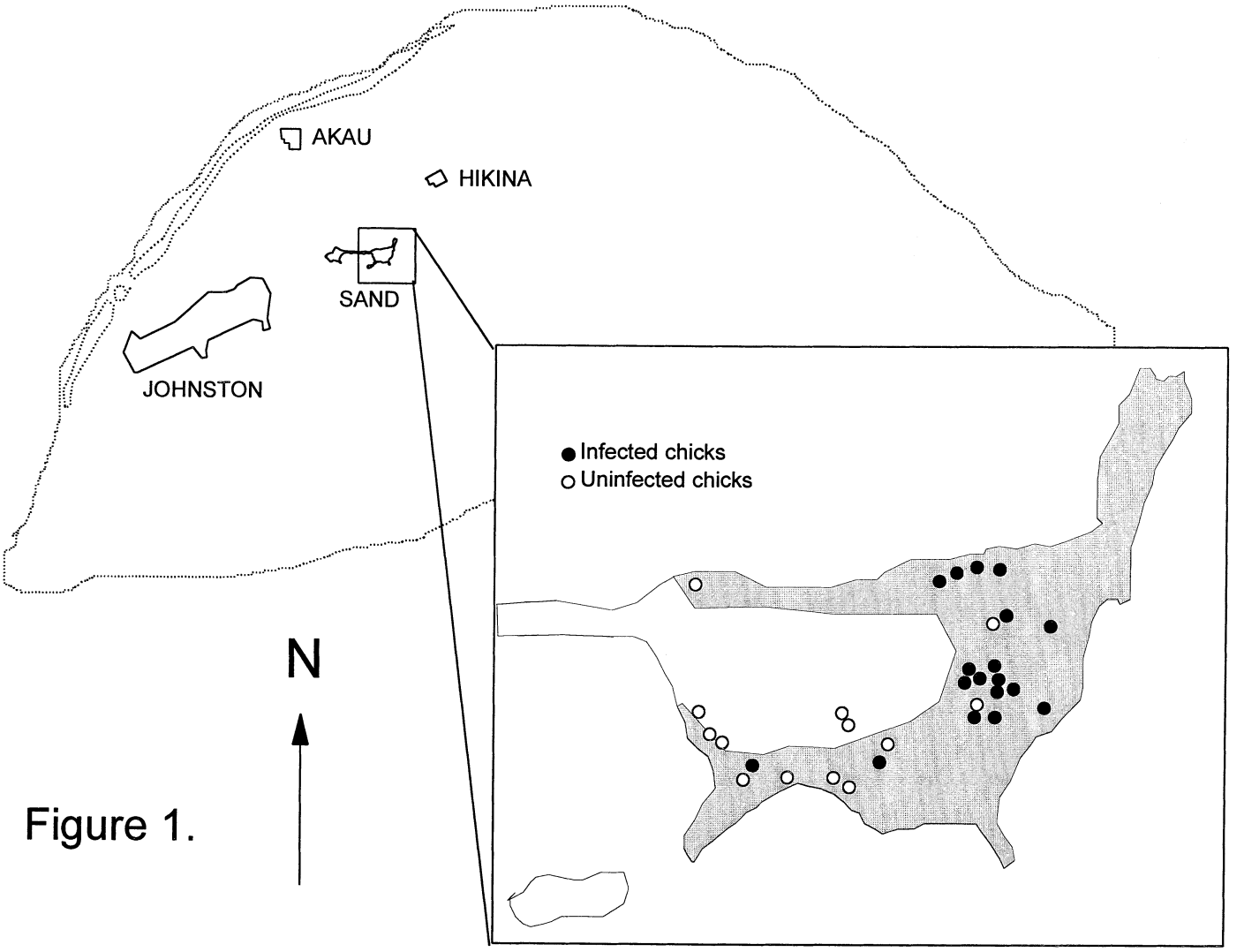

Figure 1.

FIGURE 1. Johnston Atoll with the eastern segment of Sand Island magnified (inset). Shaded area depicts general nesting area of brown boobies on Sand Island.

asitized host cells had a significantly smaller area than unparasitized cells (t = 3635, $P < 0.01$; Table I). Mean (SD) parasitemia in 19 chicks was 0.61% (0.65) and ranged from 0.01% to 2.07%. Mean (SD) parasitemia in 9 adults was 0.02% (0.02) and ranged from 0.01% to 0.06%. Chicks had significantly higher parasitemias than adults (t = 65, $P < 0.01$). Although there was a significant downward trend in parasitemia when plotted against age of chicks (r^2 = 0.17, df = 17, $P < 0.01$), the low R value indicated that age was a minor factor in explaining parasitemia in chicks; age of parasitized chicks at time of sampling ranged from 77 to 95 days. Parasitized chicks appeared clustered near the eastern end of Sand Island (Fig. 1).

TABLE I. Morphometrics (mean ± SD, range in parentheses) of *Babesia poelea*-parasitized (n = 142) and -unparasitized (n = 30) brown booby red blood cell (RBC) and nucleus of RBC.

	Breadth (μm)	Length (μm)	Area (μm²)
Parasitized RBC	7.5 ± 0.6 (6.4–8.8)	13.6 ± 1 (11.2–16)	80.8 ± 9.8 (60.3–110.6)
Nucleus of parasitized RBC	3.1 ± 0.3 (2.4–4)	6 ± 0.6 (4.8–8)	14.9 ± 2.1 (10.6–22.6)
Unparasitized RBC	8.1 ± 0.6 (6.4–8.8)	14.6 ± 1 (12.8–16)	92.6 ± 9.1 (68.4–110.6)
Nucleus of unparasitized RBC	3.2 ± 0.4 (2.4–4)	6.7 ± 0.7 (5.6–8)	17 ± 2.2 (13.6–20.1)

DESCRIPTION

Babesia poelea n. sp.

Amoeboid: Parasites were pleomorphic with a pale blue homogenous cytoplasm with well defined borders (Fig. 2A–D). Parasites were away from, or closely apposed to, the host cell nucleus, with no evident displacement of the host cell nucleus. Most amoeboid forms were situated lateral to the host cell nucleus. Chromatin granules were usually present near the edge of the parasite and clumped granules were observed occasionally. The shape varied from amorphous to pyriform or lanceolate. Parasites were generally 1 per cell although rare dual infections were noted. Some parasites appeared to have either a bud forming off the main body or 2 closely apposed identical pyriform or lanceolate structures. No hemozoin pigment

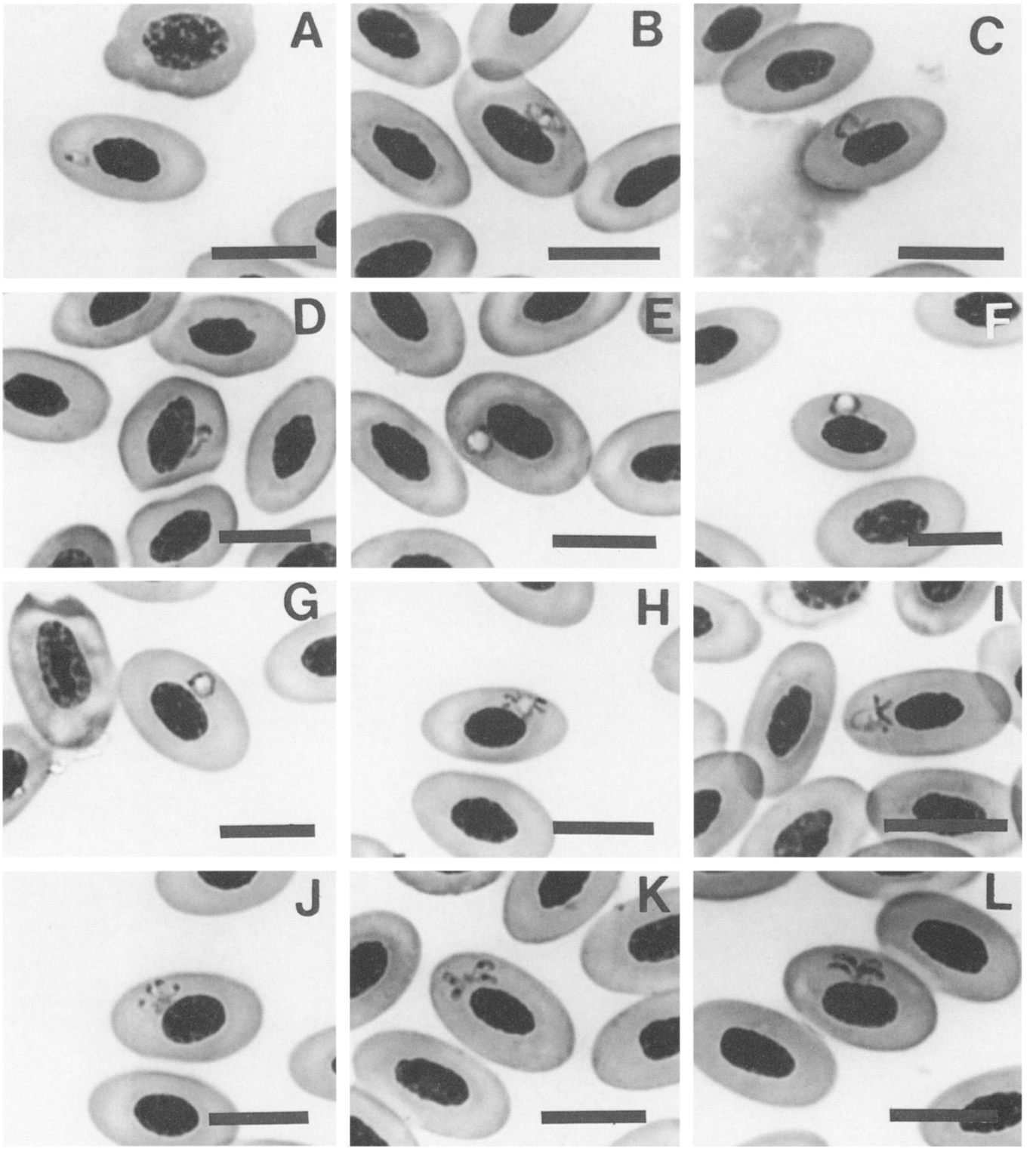

FIGURE 2. Various morphs of *Babesia poelea* in brown booby red blood cells. (**A–D**) Amoeboid. (**E–G**) Ring. (**H–L**) Schizont. Bar = 10 μm.

was observed, and intracytoplasmic red pigment was seen occasionally. Amoeboid forms made up 78% (932/1,198) of the total parasites and comprised 78% (912/1,173) and 80% (20/25) of total forms seen in chicks and adults, respectively (Table II).

Ring: Parasites were round to oblong. Cytoplasm was pale blue with an ill-defined, lighter staining, centrally placed, refractile vacuole occupying a majority of the organism's volume (Fig. 2E–G). This form was away from the nucleus and was usually lateral to and did not displace the host cell nucleus. Chromatin granules were present and situated near 1 edge of the parasite, often forming a "signet ring" appearance. Intracytoplasmic red pigment was rare, and hemozoin was absent. Ring forms made up 19% (230/1,198) of the total parasites and comprised 20% (226/1,173) and 16% (4/25) of total forms seen in chicks and adults, respectively (Table II).

TABLE II. Length and width (μm) of amoeboid, ring, and schizont forms of *Babesia poelea,* position in brown booby red cells, and presence of intracytoplasmic chromatin and pigment.

	Amoeboid	Ring	Schizont
Total	932	230	36
Chromatin (n > 1)	710	131	30
Clumped	122	76	5
None	112	23	1
Mean ± SD*	1.1 ± 0.4	1.1 ± 0.40	3.3 ± 0.9
Range	0–6	0–3	0–4
No. lateral	667	145	24
No. with red pigment	70	21	1
Length (n)	92	30	20
Mean ± SD	2.5 ± 0.7	2.0 ± 0.8	3.8 ± 0.8
Range	0.8–4.8	0.8–4	4–4.8
Width (n)	92	30	20
Mean ± SD	1.4 ± 0.6	1.6 ± 0.6	2.4 ± 0.6
Range	0.8–2.4	0.8–3.2	1.6–4

* Mean includes numbers of chromatin granules greater than zero.

Schizonts: These ranged from fan-shaped to cruciform. Apparent early stages were characterized by unipolar budding of 4 chromatin granules that seemed to develop into lanceolate forms (merozoites) joined at the pointed end (Fig. 2H–L). Each merozoite was pale blue, well defined, and contained a chromatin granule either in the mid-body or near the blunt end. This stage was away from, usually lateral to, and did not displace the host cell nucleus. No hemozoin pigment was observed, and red intracytoplasmic pigment was extremely rare. Schizonts made up 3% (36/1,198) of the total parasites observed and comprised 2% (35/1,173) and 4% (1/25) of total forms seen in chicks and adults, respectively (Table II).

Avian host: Sulidae: brown booby (*Sula leucogaster* (Boddaert)).

Locality: The type locality is Sand Island, Johnston Atoll, Central Pacific, U.S.A. (16°45′N, 169°31′W).

Type specimens: Babesia poelea is described from parasites in an immature brown booby (*Sula leucogaster*) from Sand Island, Johnston Atoll NWR, collected on 25 July 1995. Paratype material includes 18 slides from brown booby chicks collected on 25 July 1995 and 9 slides from brown booby adults collected on 15–16 April 1995 from Sand Island, Johnston Atoll. The hapantotype from a single chick (accession no. G462374) and parahapantotypes, also from chicks (accession nos. G462375 and G462376) were deposited at the International Reference Center for Avian Haematozoa (Queensland Museum, Grey Street, South Brisbane, Queensland 4101, Australia).

Etymology: The specific name is a concatenation of the Hawaiian names for dark (po'ele) and booby ('a).

Remarks

The parasite described in our study is compatible with a piroplasm on the basis of lack of intracytoplasmic hemozoin pigment and apparent reproduction in red blood cells by shizogony with production of merozoites. We have assigned this organism to the genus *Babesia* on the basis of lack of observed schizogony in host lymphocytes (Levine, 1985).

The systematics of piroplasms are in a state of flux. Laird and Lari (1957) acknowledged as much with avian piroplasms when describing a *Babesia* from a crow in Pakistan. Since their discovery, avian *Babesia* have been assigned various genera, a topic reviewed by Mohammed (1958). Laird and Lari (1957) assigned the name *Babesia moshkovskii* to many previously described avian piroplasms, including the parasite discovered by Schurenkova (1938). Laird and Lari (1957) based their decision on the fact that the organism was morphologically indistinct from that described by Schurenkova (1938), that the hosts were geographically contiguous with other hosts of previously described avian piroplasms, and that infected hosts were probably all exposed to the putative acarine vector *Argas* sp. This precedent was followed by Levine (1971), who grouped 5 species of avian piroplasms into the name *B. moshkovskii* based on similar rationale.

Peirce (1973) classified avian piroplasms into 3 genera on the basis of geographic discontinuity of infected hosts and an assumed common exposure of infected hosts to the tick *Hyalomma* sp. Subsequently, Peirce (1975), like others before him (Laird and Lari, 1957; Levine, 1971), grouped all avian piroplasms in the genus *Babesia* and classified species presumably based on host family.

Recent genetic evidence in mammalian piroplasms suggests that morphologic similarity may not suffice to classify these organisms (Persing and Conrad, 1995). To date, no molecular or life-cycle studies on avian piroplasms have been published, so we are currently left with a combination of morphology, host type, and geographic distribution to describe species of avian piroplasms. These criteria are imperfect (Laird and Lari, 1957; Peirce, 1973), and more objective standards to classify avian piroplasms are needed.

Because of the absence of described piroplasm species from Sulidae, and according to the classification proposed by Laird and Lari (1957) and Levine (1971), the parasite described herein would presumably be named *B. moshkovskii* based on similar morphology to other avian piroplasms. However, we have opted to assign a unique specific name to this taxon. Our rationale for this is 2-fold. First, the parasite was found in a pelagic Sulidae, a host phylogenetically, geographically, and ecologically isolated from terrestrial avian hosts of described piroplasms such as Ardeidae (Toumanoff, 1940), Falconidae (Mohammed, 1958), Corvidae (Laird and Lari, 1957; Yakunin and Krivkova, 1971), and Sphenicidae (Earle et al., 1993). Second, the suspected acarine vectors for avian piroplasms proposed by Laird and Lari (1957), Yakunin and Krivkova (1971), Peirce (1973), and Croft and Kingston (1975) have not been documented on Johnston Atoll (Amerson and Shelton, 1976). In the absence of transmission studies and genetic data, we believe the naming of *B. poelea* is justified based on these 2 criteria.

With the exception of size and the presence of anaplasmoid and large red solid forms observed by Laird and Lari (1957) and Croft and Kingston (1975), the different forms of *B. poelea* were similar to comparable morphs of avian piroplasms described by Schurenkova (1938), Toumanoff (1940), Laird and Lari (1957), Mohammed (1958), Yakunin and Krivkova (1971), Peirce (1973), Croft and Kingston (1975), and Earle et al. (1993). The different schemes proposed by these authors, and by us, to assign names to morphologic stages of avian piroplasms suggest that confusion exists in piroplasm taxonomy. Like Peirce (1973), we opted to classify the morphs of *B. poelea* into 3 basic categories because we judged that we had insufficient objective criteria to differentiate them into the morphs outlined by other authors cited in this paper. The definitive classification of developmental stages of avian piroplasms awaits objective evaluation.

The size of *B. poelea* was within the range of that reported by Toumanoff (1940), Laird and Lari (1957), Mohammed (1958), Corradetti and Scanga (1964), Yakunin and Krivkova (1971), Peirce (1973), Croft and Kingston (1975), and Earle et al. (1993) but was somewhat smaller than sizes reported by Schurenkova (1938). Lack of morphometrics and figures precluded an objective comparison with a piroplasm described by Peirce and Feare (1978). Solid red dividing forms (Laird and Lari, 1957; Croft and Kingston, 1975) were not seen here. This could be because of variation in parasite morphology between hosts or variation in fixation and staining technique. Unlike Laird and Lari (1957), we did not assign an "anaplasmoid form" because we had no objective criteria to differentiate these from red blood cell nuclear fragments. Hence, the parasitemia reported here may be conservative.

The low parasitemia and absence of clinical signs in infected birds indicate that *B. poelea* exists in brown boobies as an asymptomatic infection. Indeed, in contrast to our study, others have found markedly higher parasitemias ranging from lows of 2–5% (Yakunin and Krivkova, 1971) up to 8–40% (Mohammed, 1958; Croft and Kingston (1975). However, in only 1 case (Mohammed, 1958) did these authors elaborate on how parasitemia was calculated. Mohammed (1958) encountered some mortality in experimentally infected falcons, and Croft and Kingston (1975) noted anemia in young falcons infected with *B. moshkovskii.* We did not observe anemia in infected boobies (data not shown).

The smaller area of infected red blood cell and red blood cell nuclei was intriguing. This smaller area cannot be attributed to a microcytic anemia because anemia was not seen in infected birds. Bias is also an

unlikely reason because we randomly selected slides to measure uninfected cells. Other avian hemoparasites, such as *Haemoproteus* Kruse, 1890, can increase or decrease red cell size (Bennett and Campbell, 1972). Other investigators cited here have not documented significant effects of avian piroplasms on red cell morphology. The phenomenon of decreased cell size in *B. poelea*-infected cells may merit further investigation.

The higher prevalence of infection in chicks was not surprising and has been noted in other seabirds (Work and Rameyer, 1996). Although there are few accounts of prevalence of infection in other studies of avian piroplasms, Croft and Kingston (1975) observed that approximately 20% of young falcons and no adults were infected with *B. moshkovskii.* It is likely that chicks are more susceptible to piroplasm infections, which decrease in intensity or disappear with increasing age and acquisition of immunity.

The identity of the vector of *B. poelea* remains speculative. The few hematophagous arthropods that exist on Sand Island include hippoboscids like *Olfersia spinifera* (Maa, 1968) and the argasid ticks *Ornithodoros capensis* Neumann and *Ornithodoros denmarki* Kohls, Sonenshine and Clifford (Garrett and Haramoto, 1967). The most likely vectors of *B. poelea* are the argasid ticks. Although the life cycle of avian piroplasms has not been documented, mammalian piroplasms are typically transmitted by ticks (Friedhoff, 1988). Amerson and Shelton (1976) found both *O. capensis* and *O. denmarki* widely distributed on Sand Island and most densely near the eastern and northeastern portions of the eastern segment. Distribution of this tick may explain the predominance of infected booby chicks on the eastern portion of Sand Island. Historically, brown boobies have nested predominantly on the eastern portion of the eastern segment of Sand Island (Amerson and Shelton (1976). The eastern portion of Sand Island has a dense cover of grasses that potentially provides more shelter for resting ticks. Recently, boobies have been moving toward the western margins of the eastern segment; however, no brown boobies currently nest on the western segment of Sand Island.

The findings of this study prompt the question of whether *B. poelea* is specific to brown boobies. A possibly related piroplasm was noted by Peirce and Feare (1978) in masked boobies from the Indian Ocean, but its specific status remains uncertain. Mohammed (1958) and Corradetti and Scanga (1964) were unable to successfully infect avian hosts other than falcons with *Babesia shortti,* suggesting that avian piroplasms may be species specific. Seventy sooty tern (*Sterna fuscata* Linnaeus) blood samples from Sand Island were negative for blood parasites (Work, 1996). However, sooty terns may not be an ideal alternate host for *B. poelea.* Sooty terns nest and reside in less vegetated portions of Sand Island and could be less exposed to ticks seeking sheltered resting sites. It would be interesting to determine if other avian species nesting closely with brown boobies on Johnston Atoll, such as wedge tailed shearwaters (*Puffinus pacificus* (Gmelin)), are also infected or if brown boobies on the nearby northwestern Hawaiian Islands, including French Frigate Shoals, Laysan Island, Necker Island, Lisianski Island, and Kure Atoll (Fefer et al., 1984), are infected. On the basis of available evidence, we suspect that *B. poelea* is a species-specific, subclinical infection that exists in brown boobies throughout the central Pacific.

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