

On *Leucocytozoon* parasites of Ardeidae and description of one species

by C. PRIGIONI, E. BRAMBILLA, F. BARBIERI and M. FASOLA

Istituto di Zoologia, Università di Pavia, Piazza Botta, 9, 27100 Pavia, Italy

SUMMARY. In peripheral blood of *Nycticorax nycticorax* and *Egretta garzetta* (Aves, Ardeidae), gametocytes of the genus *Leucocytozoon* (Protozoa, Sporozoa) were found. We describe this parasite and compare it with the past records of *Leucocytozoon* in Ardeidae. While only life cycle studies will give a definitive answer to these taxonomic problems, we put forward an hypothesis about taxonomy of *Leucocytozoon* in Ardeidae.

Leucocytozoon des Ardeidae et description d'une espèce.

RESUME. Dans le sang périphérique de *Nycticorax nycticorax* et de *Egretta garzetta* (Aves, Ardeidae) ont été trouvés des gamétocytes du genre *Leucocytozoon* (Protozoa, Sporozoa). Nous décrivons ce parasite et, bien que seule l'étude de leur cycle biologique permette de résoudre les problèmes taxonomiques, nous proposons une révision de la classification des *Leucocytozoon* signalés chez les Ardeidae.

Introduction

In the order Ciconiformes, except for one record in the family Balaenicipitidae (Rodhain *et al.* 1913), only members of the family Ardeidae are recorded as hosts for species of *Leucocytozoon*. Four species have been described, but two (*L. ardeolae* and *L. iowense*) are believed by Fallis *et al.* (1974) to be junior synonyms of *L. ardeae*, so that only this latter species and *L. leboeufi* may be valid. All the records known to us of *Leucocytozoon* sp. in Ardeidae are summarized in Table I.

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Table 1. Records of species of Leucocytozoon in Ardeidae. Measurements in microns, means if available in parentheses; R = round; E = elongated.

no.	Host species	Country	Parasite species	Author	Sizes, shapes and remarks on gametocytes		
					cell	macro-	micro-
1	<i>Ardea goliath</i>	Congo	ardeae	Rodhain <i>et al.</i> 1913	14 × 17	16 × 17	E with nucleolus star shaped
2	<i>Egretta dimorpha</i>	Madagascar	»	Bennett and Blancou, 1974			
3	<i>Ardeola grayii</i>	India	ardeolae (?)	de Mello, 1937			
4	<i>Butorides virescens</i>	U.S.A.	iowense (?)	Coatney, 1938	(11.03 × 13.4)	(11.29 × 12.68)	R (2.82 × 4.24) nucleolus (1.09 × 4.92) × 1.5)
5	<i>Ixobrychus sinensis</i> = <i>Ardetta sinensis</i>	North Vietnam	leboeufi	Mathis and Leger, 1911	11.5-12	same	R 3.8 with nucleolus
6	<i>N. nycticorax</i>	Central Africa	sp.	Aubert and Heckenroth, 1911	(12-14)	(16-20)	without nucleolus
7	<i>N. nycticorax</i> hoactli	U.S.A.	»	Coatney, 1938	(10.09 × 14.04)	(9.74 × 14.77)	R (3.35 × 5.27) with nucleolus (0.91 × 1.24)
8	<i>N. nycticorax</i>	Italy	»	Franchini, 1924	Slightly bigger than in no. 9		
9	<i>Ardea purpurea</i>	»	»	»	6 × 16, 9 × 14-18	5 — 12 6 × 13	E with nucleolus
10	<i>Butorides striatus</i> atricapillus = <i>Ardea atricapilla</i>	Senegal Niger	»	Leger and Leger, 1914	12	10	with nucleolus
11	<i>Butorides striatus</i> atricapillus = B. atricapilla	Congo	»	Rohdain <i>et al.</i> , 1913	10 × 12	(10)	with nucleolus

Blood smears collected during a study of nesting habits of herons in Northern Italy contained parasites of the genus *Leucocytozoon*.

In the present paper, we describe this material and attempt to review *Leucocytozoon* parasites of Ardeidae.

Methods

Blood smears were obtained during May and June 1974 to 1978, from chicks in the nests of colonies of herons. Smears were air dried, fixed in 100 % methanol, and stained in Giemsa. Blood smears were photographed using Orthoplan with Orthomat and parasites were studied using a Visopan Reichert (1 mm. = 0.8 μ). Only mature gametocytes with regular shape were studied. Area measurements were made by drawing the cells on the Visopan and counting squares in a superimposed millimeter grid. Indices defined by Bennett *et al.* (1974) were calculated. Gametocytaemia was expressed as number of parasites per 1000 erythrocytes. Names of birds are from Peters (1931).

Results

From 1974 to 1978 blood smears were taken from 102 birds (72 night herons *Nycticorax nycticorax*, 11 little egrets *Egretta garzetta* and 19 purple herons *Ardea purpurea*) in nine heronries spaced 10 or 20 km apart near Pavia, Northern Italy. Of these birds, 12 were infected by *Leucocytozoon*: 8 of 11 night herons examined and 3 of 4 little egrets in 1974 in one heronry, and 1 of 4 night herons in another heronry in 1976 (1).

Mean gametocytaemia in infected night herons was 4 parasites per 1000 erythrocytes (range 2-30), and in little egrets it was 1. Sex ratio of the gametocytes in night herons was in favour of macrogametocytes with a maximum of 1:4, in three little egrets it was 1:1, 1:3, and 1:5.

Although we did not sample every heronry each year, we believe that the distribution of the parasites is uneven in space and time. For instance in the heronry with chicks heavily infected in 1974 we found no parasites in 1976; in other heronries around the infected ones we found no evidence of infection in the same year. Of the two heronries with infected birds one was in a wood of medium size willows and the other was in a poplar plantation. Both locations were beside ponds and irrigation canals (described as heronry no. 1 and 3 by Fasola and Barbieri 1975).

(1) In 1979, further blood smears were collected from 77 night herons and 5 little egrets in 4 heronries. Of these, 9 night herons (2 out of 27 examined in one heronry and 7 out of 40 in another one) were infected by *Leucocytozoon*. This material was not used in the following description.

Our observations are too limited to allow conclusions about the incidence of Leucocytozoonosis in wild populations. However, in the heronry where we found 11 infected chicks out of 15 in 1974, the reproductive success was 2.2 young night herons fledged per nest (Fasola and Barbieri 1975). This value is only a little below normality for this species (pers. obs. and Hafner 1978), indicating that a significant mortality due to this parasite did not occur. In the literature, high patogenicity is sometimes attributed to species of *Leucocytozoon* in poultry and in wild birds, but cases of parasitized birds which are apparently healthy are also reported (Fallis *et al.* 1974).

In canals near the heronries with infected birds, we observed larvae and pupae of *Eusimulium angustitarse* (Lundstrom 1911) and *E. latigonium* (Rubrov 1956), Diptera Simuliidae. The latter species, which is widespread in Italy, is thought by Rivosecchi (1978) a probable vector of Leucocytozoonosis in this zone. Larvae and pupae of the same species of Simuliidae also were present near non-infected heronries.

Description of the parasite.

Host: *Nycticorax nycticorax* and *Egretta garzetta*.

LOCALITY: Pavia, northern Italy.

Immature gametocytes (fig. 1, 2). Youngest parasites round with pale pink nucleus and deep red nucleolus. Mean diameter 4.8 μ in night heron, no immature gametocytes found in egrets. Host cell nucleus very little distorted and placed peripherally.

Macrogametocytes (fig. 3, 4 and table II). Round, cytoplasm with a few vacuoles and many granulations (pseudopigmentation) stained deep blue; nucleus elongated, about 4 by 2.4 μ in both hosts, placed eccentrically and stained pale pink; nucleolus, about 1.2 μ in both hosts, stained deep red. Host cell nucleus elongated, stained brown red with clearer belt extending up to 1/3 of parasite circumference. A significant proportion of the host cell cytoplasm remains attached to the parasite.

Microgametocytes (fig. 4, 5 and table II). Smaller than macrogametocytes and irregular in shape, cytoplasm stained slightly blue with pale pink chromatic granulations; diffused nucleus, and nucleolus sometimes evident.

SCHIZOGONY: unknown.

VECTOR: unknown.

MATERIAL: deposited in the International Reference Centre for Avian Haematozoa, Newfoundland, Canada, no. 67589.

In both hosts, the parasite is morphologically identical, except for dimensions that are significantly larger in the little egret (table II).

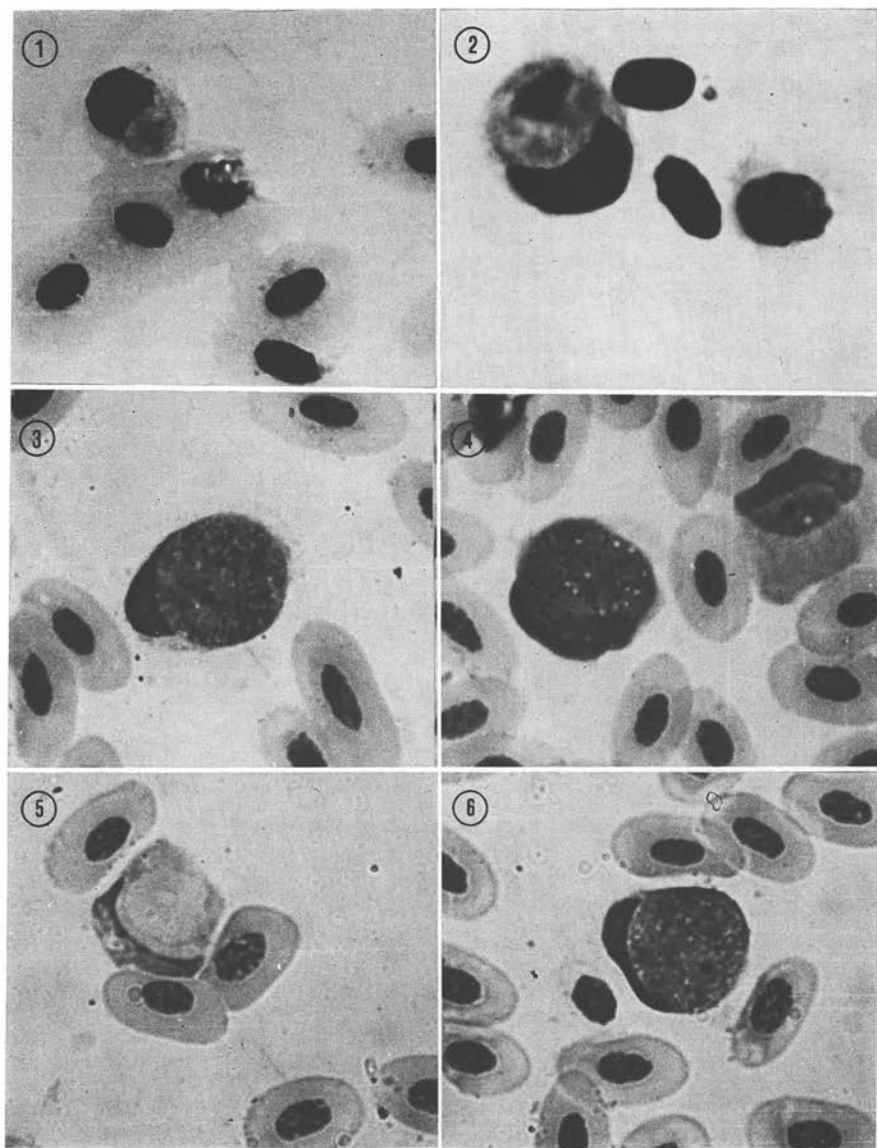


Fig. 1-5. *Leucocytozoon* sp. from *Nycticorax nycticorax*. 1, 2. Immature gametocytes. 3. Macrogametocyte. 4. Macrogametocyte (left) and microgametocyte (right) with evident nucleolus. 5. Microgametocyte without evident nucleolus.

Fig. 6. *Leucocytozoon* sp. from *Egretta garzetta*. Macrogametocyte.

Table II. Dimensions of the parasite and of blood cells of host birds, presented as means in microns with standard deviations in parentheses.. All data based on 10 parasites and 10 normal erythrocytes from each of 9 *N. nycticorax* and 3 *Egretta garzetta*, except data on microgametocytes of *Egretta garzetta*, based on 17 measurements.

	<i>N. nycticorax</i>			<i>Egretta garzetta</i>		
	length	width	area	length	width	area
Macrogametocyte	12.3 (0.9)	11.0 (1.0)	108.5 (11.2) **	12.8 (0.7)	11.3 (1.0)	119.8 (8.8) **
Host cell nucleus	9.3 (1.3)	2.9 (0.7)	20.9 (4.8) *	10.7 (1.8)	2.9 (0.9)	23.8 (5.5) *
Microgametocyte	10.3 (1.4)	8.6 (0.9)	64.4 (12.3) **	11.1 (1.2)	9.0 (1.3)	80.5 (10.9) **
Normal erythrocyte	12.8 (0.4)	7.1 (0.3)	71.8 (7.8) n.s.	13.1 (0.4)	7.1 (0.5)	75.8 (4.4) n.s.
Nucleus of normal erythrocyte	5.5 (0.4)	2.6 (0.3)	11.7 (1.4) *	5.6 (0.4)	2.8 (0.3)	12.3 (1.3) *
Parasite index		1.5			1.6	
Host nuclear index		1.8			1.9	
Host-parasite index		1.8			1.9	

Parasite index = area of macrogametocyte/area of normal erythrocyte; Host nuclear index = area of the host cell nucleus of the macrogametocyte/area of the nucleus of normal erythrocyte; Host-parasite index = area of the host cell-parasite complex/area of normal erythrocyte.

Statistical comparison of mean areas of cells and nuclei between the two bird hosts:

** p 0.02; * p 0.05; n.s. not significant.

We calculated for our material the indexes (*table II*) proposed by Bennett *et al.* (1974) finding a small difference between the parasites in night herons and little egrets. This difference is due to the fact that gametocytes area larger in the egret, suggesting that these indexes should be considered together with the other morphological criteria.

Comments

Most species of *Leucocytozoon* are known and named only by gametocytes in peripheral blood of birds, without data on life cycle. Taxonomic criteria include morphology, shape and size of gametocytes of the parasite and distortion of the host cell. However the uncertain evaluation of these characters has resulted in many records of unidentified species and in cases of probable synonymy (Fallis *et al.* 1974). More difficulties arise because a species of *Leucocytozoon* may show variation in dimensions within the same or between different host species (Bennett and Campbell 1975, and *table II*) and because the host family specificity of the parasite is now in doubt after Bennett and Cameron (1975) found a single species parasitizing different families of *Passeriformes*.

These difficulties apply also to the comparison of our material with the description of *Leucocytozoon* recorder from Ardeidae (*table I*). Moreover some of these descriptions were made from small samples of parasites, they do not include illustrations, and the dimensions given cannot be compared by statistical analysis.

Bearing in mind these difficulties, we believe that our material:

— differs markedly from the description of *L. ardeae* (Rodhain *et al.* 1913), which has larger gametocytes which are equal in size, a host cell nucleus that may encircle the parasite and a recognisable star shaped nucleus in microgametocytes;

— differs slightly from the description of *L. leboeufi* (Mathis and Leger 1911), which has the nucleus of the microgametocyte round and both gametocytes equal in size and rather regular in shape;

— differs slightly from descriptions of *L. sp.* in night herons (Aubert and Heckenroth 1911) (nucleus of the macrogametocyte without nucleolus) and in *N. nycticorax hoactli* (Coatney 1938) (nucleus of the macrogametocyte round, and both gametocytes larger);

— is identical to the description of *L. sp.* (Franchini 1924) in night herons and in purple herons in northern Italy (except for the lack of granulations in macrogametocytes, and identical to the description of *L. sp.* in *Butorides striatus atricapillus* by Leger and Leger (1914).

The description of *L. sp.* in *Butorides striatus* (Rodhain *et al.* 1913) is insufficient for comparison.

In summary, while only life cycle studies will answer taxonomic problems about *Leucocytozoon* spp., we provisionally suggest that the parasite observed by us:

- is distinct from *L. ardeae* and the synonyms of this species as proposed by Fallis *et al.* (1974) (records no. 1, 2, 3, 4 in table I);
- is probably distinct from *L. leboeufi* (no. 5 in table I) and from *L. sp.* of records no. 6, 7;
- is conspecific with *L. sp.* of records no. 8, 9, 10.

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References

- Aubert P., Heckenroth F.: Sur trois *Leucocytozoon* des oiseaux du Congo français. *C.R. Soc. Biol.*, 1911, 70, 958-959.
- Bennett G. F., Blancou J.: A note on the blood parasites of some birds from the Republic of Madagascar. *J. Wildl. Dis.*, 1974, 10, 239-240.
- Bennett G. F., Cameron M. F.: Mixed infection of species of *Leucocytozoon* in individual birds from Atlantic Canada. *J. Parasitol.*, 1975, 61, 1091-1095.
- Bennett G. F., Campbell A. G.: Avian Leucocytozoidae. 1. Morphometric variation in three species of *Leucocytozoon* and some taxonomic implication. *Can. J. Zool.*, 1975, 53, 800-812.
- Bennett G. F., Khan R. A., Campbell A. G.: *Leucocytozoon gruzi* sp. n. (Sporozoa: Leucocytozoidae) from a sandhill crane, *Grus canadensis* (L.). *J. Parasitol.*, 1974, 60, 359-363.
- Coatney G. R.: Some blood parasites from birds of the Lake Okaboji Region. *Am. Midl. Nat.*, 1938, 20, 336-340.
- Fallis A. M., Desser S. S., Khan R. A.: On species of *Leucocytozoon*. *Adv. Parasitol.*, 1974, 12, 1-67.
- Fasola M., Barbieri F.: Aspetti della biologia riproduttiva degli Ardeidi gregari. *Ricerche Biol. Selvaggina*, 1975, 62, 1-59. Istituto Nazionale Biologia della Selvaggina, Bologna.
- Franchini G.: Observations sur les hématozoaires des oiseaux d'Italie. *Ann. Inst. Pasteur, Paris*, 1924, 58, 470-515.
- Hafner H.: Le succès de reproduction de quatre espèces d'Ardeides *Egretta g. garzetta* L., *Ardeola r. ralloides* Scop., *Ardeola i. ibis* L., *Nycticorax n. nycticorax* L. en Camargue. *Terre Vie*, 1978, 52, 279-289.
- Léger A., Léger M.: *Leucocytozoon* d'oiseaux du Haut-Sénégal et Niger. *Bull. Soc. Pathol. Exot.*, 1914, 7, 391-395.
- Mathis C., Léger M.: *Leucocytozoon* d'un paon, d'un crabier et d'un bengali du Tonkin. *C.R. Soc. Biol.*, 1911, 70, 211-212.
- de Mello I. F.: On Haematozoa of Indian birds. *C.R. 12^e Cong. Internat. Zool.*, Lisbon, 1937, 2, 1391-1445.
- Peters J. L.: Checklist of birds of the world. *Museum of Comparative Zoology*, 1931, Cambridge, Mass., 1, pp. 97-142.
- Rivosecchi L.: Simuliidae. Diptera Nematocera. In « Fauna d'Italia ». *Calderini*, édit. Bologna, 1978.
- Rodhain J., Pons C., Vandenbranden F., Bequaert J.: Notes sur quelques hématozoaires du Congo belge. *Arch. Protistenk.*, 1913, 29, 259-278.