

FIRST RECORD OF *HAEMOPROTEUS* SP. PARASITING RED-LEGGED PARTRIDGES (*ALECTORIS RUFA*)

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Abstract

During a health status survey in a Spanish red-legged partridge (*Alectoris rufa*) breeding facility, blood smears were obtained from 40 juvenile birds (26 males and 14 females). In four of the males (15.4 %) typical elongate and sausage-shaped, partial or totally circling host cell nucleus gametocytes were observed in red blood cells. It was identified as an *Haemoproteus* sp. gametocyte. The intensity of infection in males was 5.0 ± 24.0 gametocytes/1000 erythrocytes. No females were infected. To the best of our knowledge, this is the first description of an *Haemoproteus* sp. infection in this species. Morphometric characteristics did not allow us to identify the parasite species, since very few is known on this topic in European phasianids. Parasited partridges showed a marked splenomegaly. Infected erythrocytes presented smaller nuclei, with a nuclear displacement ratio of 0.80. The only heavily parasited individual (122.8 gametocytes/1000 erythrocytes) showed in addition lower serum urea and erythrocyte and leukocyte counts, and a lighter liver.

Zusammenfassung

Während einer Untersuchung über den Gesundheitszustand einer spanischen Rothuhn (*Alectoris rufa*) Zucht, wurden von 40 jungtieren (26 männlich und 14 weiblich) Blutaufstriche gewonnen. In vier der männlichen Vögel (15.4 %) wurden innerhalb der roten Blutzellen typische längliche, wurstförmige Gametozyten beobachtet, die zum Teil oder ganz den Kern der Wirtszelle umringen. Diese Blutparasiten wurden als *Haemoproteus* sp. Gametozyten identifiziert. Die Infektionsintensität war 5.0 ± 24.0 Gametozyten/1000 Erythrozyten. Kein weibliches Rothuhn war infiziert. Nach unseren Daten, handelt es sich um den ersten Bericht über einen *Haemoproteus* sp. Blutparasiten in dieser Vogelart. Die morphometrischen Eigenheiten erlaubten die Spezies-identifizierung nicht, da noch wenig über Blutparasiten der Europäischen Phasianiden bekannt ist. Die infizierten Erythrozyten hatten kleinere Kerne, mit einem Kernverschiebungsverhältnis von 0.80. Das einzige stark parasitierte Rothuhn (122.8 Gametozyten/1000 Erythrozyten) hatte einen niedrigen Harnstoffwert und geringere Erythrozyten und Leukozyten, sowie eine leichtere Leber.

Résumé

Key words: *Alectoris rufa*, blood parasite, *Haemoproteus*, splenomegaly, red-legged partridge, Spain,

Introduction

Haemoproteus sp. is an obligate heteroxenous blood protozoan parasite of birds which uses blood-sucking dipterans of the families Hippoboscidae and Ceratopogonidae for its

transmission. The infections are characterised by schyzogony (merogony) in visceral endothelial cells, gametocyte development in circulating erythrocytes, and presence of pigment in granules in infected erythrocytes (12). Haemoproteids are not commonly associated with disease, and occur widely in avian populations, including Phasianidae (2). In the Iberian peninsula, 8 *Haemoproteus* species have been reported in 18 avian hosts (4).

The red-legged partridge (*Alectoris rufa*) is a medium-sized Phasianidae, subfamily Phasianinae, native from the Iberian peninsula. There are about 1,7-3,6 millions of reproductive pairs (8). In addition, more than 4 million farm-reared juveniles are released yearly to supplement stocks for shooting (9). Despite the ecological and economical importance of this species, few workers have studied the prevalence and intensity of haemoparasites in these birds, only *Plasmodium relictum* having previously been reported (4).

Material and Methods

In November, 1999, 40 apparently healthy young farm-reared red-legged partridges (26 males and 14 females) were surveyed in order to study the sanitary status of a breeding facility (Lugarnuevo, Jaén, southern Spain). Blood samples for the preparation of blood smears were obtained from the brachial vein. The air-dried blood smears were subsequently fixed in absolute methanol and stained with Giemsa's solution (10). The slides were examined microscopically at a magnification of 1000x under immersion oil by the same person (JM). 10,000 red blood cells (RBC) were counted from 5 distant fields of the smear. *Haemoproteus* linear dimensions were obtained as described in (1). Measurements are expressed as means followed by standard deviations. The number of specimens measured is indicated by *n* and the nuclear displacement ratio by NDR.

Packed cell volume (PCV), was measured in heparinized capillary tubes. The RBC count was carried out in a Neubauer chamber, and the number of white blood cells (WBC) was estimated counting the number of leukocytes per 10,000 erythrocytes in the blood smear. Serum proteins (SP) were measured with a refractometer, and serum urea (SU) by spectrophotometry. Spleen weight (SW) and liver weight (LW) were recorded to the nearest milligram.

A Mann-Whitney U test was used to compare erythrocyte morphometric characteristics and haematological and physiological values depending on the presence or absence of the parasite.

Results

During RBC counting, an deep blue elongate and sausage-shaped body was observed into the cytoplasm of erythrocytes. It had golden brown pigment granules and circled partial or totally the host cell nucleus, which was displaced from the central position in most of the cases (NDR=0.80±0.18, table 1). Despite the RBC dimensions were not altered, the nuclei from infected erythrocytes were smaller than those from uninfected ones (U test, z=5.08, p<0.001 for nucleus length, and z=4.93, p<0.001 for nucleus width, table 1). Some of the gametocytes were immature, usually in lateral position and with an ameboid outline.

These bodies were identified as a *Haemoproteus* sp. gametocytes (see picture 1). Their morphometric characteristics are reflected in table 1. The absence of measures of one of the samples is due to the fact that this partridge only hosts immature gametocytes. However, the measurements did not allow us to identify the species (see below). To the best of our knowledge, this is the first description of an *Haemoproteus* species parasiting red-legged partridges.

The prevalence of infection was 0.1 (I.C. 95%= 0.028-0.237) in the whole sample, and 0.154 (I.C. 95%=0.027-0.410) in the case of males. No females were infected. Intensity of the parasitaemia was 5.0±24.0 infected cells per 1000 RBC (range: 0-122.8).

Splenomegaly was the most common finding among parasited partridges. In fact, those parasited showed a heavier spleen than those non-parasited (U test, z=-2.41, p<0.05, table 2). Only one of the affected partridges suffered from a markedly heavily parasitaemia (122.8 gametocytes per 1000 erythrocytes). This individual showed not only a marked splenomegaly,

but RBC and WBC counts, SU and LW were markedly lower than in uninfected males (see table 2).

Discussion

Despite the importance of red-legged partridge as ecological and economic resource, *Haemoproteus* sp. had not been previously described. This could be due to i) few research on this topic, ii) absence of sanitary control of partridge breeding facilities, iii) low prevalence of this parasite in partridges or iv) absence of clinical signs associated to its presence which may keep the parasite undiagnosed. Another possibility for the absence of previous records could be that gametocytes of most species of *Haemoproteus* disappear from the blood in autumn (13), when red-legged partridges are released and/or hunted, and therefore when most of the researches are carried out.

Many authors agree that this parasite is not commonly associated with clinical signs in its hosts (3). However, despite the parasited birds were not apparently sick, we found splenomegaly, which is one of the commonly cited lesions in haemoproteosis (5).

The current classification of *Haemoproteus* spp. is based primarily on host taxonomy since gametocytes have few distinctive features. The philosophy of familiar specificity among the haemoproteids is nowadays adopted (2). There are currently six haemoproteids accepted for the Phasianidae, two of them inhabiting Phasianinae from North America and Asia (2). However, there is a lack of research on this topic in Palearctic Galliformes (8). The only *Haemoproteus* species accepted in Europe is *H. mansonii*, parasite of the red grouse (*Lagopus lagopus scoticus*) in Scotland (U.K.) (14), but Fallis and Bennett (7) demonstrated that it could not be transmitted to members of other subfamilies of Phasianidae. Therefore, the haemoproteid we describe here could be a new species.

To Bennett et al. (3) species of *Haemoproteus* are apparently relatively benign. However, there are some reports of major mortality among flocks of galliform and anseriform birds due to this species (e.g. 11). In fact, we did find some clinical changes associated with the parasite. Thus, we believe more research should be carried out to study the prevalence and effect of this parasite in red-legged partridge populations. The identification of the *Haemoproteus* species with molecular techniques would also help in this topic.

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