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Short communication

Intestinal and haematic parasitism in the birds of the Almuñecar (Granada, Spain) ornithological garden

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ABSTRACT

Birds from the Almuñecar ornithological garden (Granada, Spain) were surveyed from June 2006 to May 2007 to establish programmes to prevent, control, and treat intestinal and haematic parasites. A total of 984 faecal samples and 41 samples of blood were collected from Psittacidae, Cacatuidae, Phasianidae, and Anatidae. One or more intestinal parasites were identified in 51.6% of the samples. Blood parasites were found in 26.8% of the birds examined. The most frequent pathogenic endoparasites were coccidians, such as *Cyclospora* sp. (4.5%), *Eimeria* sp. (4.1%) and *Isospora* sp. (2%) and helminths such as *Capillaria* sp. (10. 1%), *Ascaridia* sp. (4.9%) and *Heterakis gallinarum* (4.9%). All the parasites varied with season but the most were found year round. Multiple parasitic infections by intestinal parasites were common, with 196 of 984 faecal samples having 2–5 intestinal parasites. The most frequent cases of multiple parasitism were *Blastocystis* plus *Entamoeba* sp. and *Blastocystis* plus *Cyclospora* sp. The haematic protozoa detected were *Haemoproteus* sp. (17%) and *Plasmodium* sp. (7.3%). Multiple parasitism by *Haemoproteus* sp. and *Plasmodium* sp. was detected in 1 sample of *Gallus gallus*. After each sampling, some of the affected animals were treated according to our results, and the corresponding programmes of prevention and control were designed.

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1. Introduction

Zoological aviary gardens involve the housing and moving of birds for which the case history of exposure to diseases is completely unknown. The parasites inevitably form a load on the host bird and therefore may affect its ability to grow, survive, and reproduce; parasites can even alter behaviour, abundance, and distribution of an entire species (Varghese, 1987). Birds that are kept outdoors or in flocks are more likely to have a problem with parasites because of their increased risk of exposure. Workers in charge of feeding and cage cleaning should take care against parasite transmission from one cage to another with their footwear and utensils.

Avian haematozoa infection has been related to behavioural changes, sexual selection, and the reduction and extinction of native bird populations (Basto et al., 2006). However, the paucity of research concerning the distribution, prevalence, and pathogenicity of these parasites has limited recognition of their importance in tropical-bird demography and veterinary wildlife management.

Prevalence data remain scarce for endoparasites in birds of zoological gardens. However, in these studies, haematozoa such as *Atoxoplasma*, *Leucocytozoon*, *Hepatozoon*, *Haemoproteus*, *Plasmodium*, *Trypanosoma* and microfilariae were frequent (Valkinas et al., 2005; Basto et al., 2006). Common gastrointestinal parasites included *Ascaris*, *Capillaria*, *Ascaridia*, *Strongyloides*, *Heterakis*, Strongyloidea,

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Spiruroidea, trematode and cestode eggs, *Balantidium coli*, *Entamoeba coli*, and *Entamoeba histolytica* (Figueiroa Lyra de Freitas et al., 2001). In Spain, gastrointestinal parasites in the birds of the zoological garden Peña Escrita (Almuñecar, Granada) were studied and *Struthio camelus*, *Anser cygnoides*, *Gallus gallus*, and *Phasianus colchicus* were found to be parasitized by *Entamoeba* sp., *Iodamoeba buetschlii*, *Endolimax nana*, *Eimeria* sp., *Cryptosporidium* sp., *Blastocystis* sp., *Hetarakis gallinarum*, and *Capillaria* sp. (Pérez Cordón et al., 2008).

The present study reflects the prevalence of gastrointestinal and haematic parasites in the birds of the ornithological park Loro Sexi (Almuñecar, Granada), aimed at establishing programmes to prevent, control, and treat intestinal parasitism in this zoo.

2. Materials and methods

This study was conducted in the bird park Loro Sexi in Almuñecar (Granada, Spain), an ornithological zoo of over 12,000 m^2. The park consists of 39 cages and 2 ponds where autochthonous as well as exotic species are found. There are 67 species and in many cases several species of Psittacidae, Cacatuidae, Phasianidae and Anatidae are kept together.

From June 2006 to May 2007, the species in the zoo were faecal sampled every 2 months, by fresh deposit. On each occasion, 4 samples were taken from each cage. One sample was stored without preservative while the other 3 were preserved in potassium dichromate at 2.5%. Samples were transported to the laboratory and preserved at 4 °C until examined. First, a macroscopic study was made of all the samples to determine their characteristics (consistency, colour, odour, mucosity, or macroscopic blood) and the possible presence of nematodes and/or cestodes, as well as the fragments of parasites. For this, a portion of the faecal sample was taken without preservative and was diluted uniformly in saline solution (0.9%) in a Petri dish for direct examination with the naked eye or under a binocular microscope (Olympus CKX31, Olympus Life Science Europa GMBH, Spain). After this, a light microscope was used with Lugol's (iodine). The Faust concentration (Faust et al., 1939) was used with all the samples: faecal suspensions were filtered through gauze, and the material on the filter was diluted to 10 ml with sterile water. This suspension was centrifuged at 500 × *g* for 2 min in conical propylene tubes, followed by supernatant decantation. Sterile water was then added to reach 10 ml of total volume, and the process was repeated until a clear supernatant was obtained. Each sample was examined at 400× magnification for the presence of eggs, larvae, trophozoites or cysts, and some samples were stained with Ziehl-Neelsen and Giemsa. The final pellet was resuspended in 10 ml of $ZnSO_4{\cdot}7H_2O$ (703 g/l, 33%, specific gravity 1.118) for flotation. The tube was inverted six times and the fluid removed with a pipette to fill both chambers of the McMaster slide to quantify the cysts, oocysts, and eggs 10 min after loading the slide (MAFF, 1977). The immature oocysts of coccidia were incubated in 2.5% potassium at 37 °C with continuous stirring until the complete maturation of the oocysts.

The Harada-Mori technique (Beaver and Orihel, 1965) was used to recover larvae (L1–L3) from the stool samples for identification of helminth species. A 20 cm × 13 cm filter paper strip was smeared with 1 g of faeces and inserted into a 15 ml centrifuge tube containing 4 ml distilled water. The tube was maintained in a slanted position at room temperature for 10 days. The filter paper strip was kept moist by capillary flow. Distilled water was added when necessary to maintain the original fluid level. After 10 days, a small amount of fluid was withdrawn from the bottom of the tube and examined for larvae. Specimens were examined daily under a dissection microscope at 10× magnification. Larvae were identified according to their morphology using the description of Beaver et al. (1964).

Blood from some birds was collected in all four seasons of the year. Blood was collected either from the branchial artery or by clipping the toenail of the birds and preserving the blood in containers in anticoagulant (EDTA) at 4 °C. Part of the blood was used for smears (0.5 ml) that were air-dried, fixed in 100% ethanol, and stained in Giemsa. A minimum of four slides per bird were examined; 1 ml of the blood of each birds was passed through 3-μm polycarbon filters that were washed afterwards and filters were examined under a light microscope. Another ml of the blood was used for Knott's technique (Knott, 1939) to detect microfilariae. The sample examined under a Nomarski light microscope (Olympus TH4-200 Olympus Life Science Europa GMBH, Spain). The parasites were identified based on our broad teaching and research experience as well as with parasitology atlases and books (Sullivan, 2004; Foreyt, 2001).

Zoological nomenclature of the birds was taken from Howard and Moore (1980) and the International Commission of Zoological Nomenclature (1999).

We studied seasonality, analysing the general prevalence of each parasite and the prevalence over the four samplings. The Friedman rank sum test was used to look for seasonal differences. The significance level was set at $P < 0.05$.

3. Results

A total of 984 faecal samples and 41 samples of blood were collected from Psittacidae, Cacatuidae, Phasianidae, and Anatidae. 508 of the 984 faecal samples studied (51.6%), presented from 1 to 5 intestinal parasites. Blood parasites were frequent also, and 11 of the 41 blood samples (26.8%), showed haemoparasites. The results are summarized in Tables 1–3.

In the coprological study, protozoa were more prevalent than helminths (Table 1). *Blastocystis* was the most frequent (23.6%), a great quantity of parasites appearing in each sample. The prevalence of coccidians was of 18.7%. *Cyclospora* sp. and *Eimeria* sp. were the most prevalent (4.5% and 4.1%, respectively), although some oocysts (7.3%) were not sporulated and after incubation at 37 °C they failed to mature, and thus their identification was not possible. The prevalence of *Cryptosporidium* was low (0.8%), oocysts being detected in only two samplings. The helminths detected are shown in Table 2. The most prevalent was *Capillaria* sp. (10.1%), while *Ascaridia* sp. and

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