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Case Report—

Enteric Infections by Trematodes and Nematodes in Olrog's Gull, *Larus atlanticus*

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SUMMARY. Trematodes and nematodes can be pathogenic helminths of birds. Every year during the breeding season, there is variable mortality among chicks from the largest Olrog's gull (*Larus atlanticus*) colony in Argentina. During two consecutive breeding seasons of Olrog's gull, we studied epidemiological and pathological aspects of infections by digeneans (Microphallidae) and nematodes (Acuariidae) in Olrog's gull chicks. Prevalence of nematode infection was 80.3% in 2005 and 89.2% in 2006, and mean intensity was 23.7 in 2005 and 50.8 in 2006. The risk for infection rose 34.3% and the intensity of infection 6.7% for every increase of 1 mm in head–beak length. The nematodes occupied the proventricular glands and caused disruption of their structure and mild inflammatory proventriculitis. Prevalence of digenean infection was 97.0% in 2005 and 97.3% in 2006. In 10-day-old live chicks, prevalence was 98.0% in 2006 and 95.3% in 2007. Infection was associated with severe catarrhal enteritis, lymphocyte/eosinophil-rich inflammatory responses, extensive fibroblast proliferation around the parasites, and disruption of the architecture of the adjacent crypts.

RESUMEN. *Reporte de Caso*—Infecciones entéricas por trematodos y nematodos en gaviotas cangrejeras o de Olrog, *Larus atlanticus*.

Los trematodos y nematodos son helmintos con potencial patogénico en aves. Anualmente, durante la temporada reproductiva de la gaviota cangrejera o de Olrog (*Larus atlanticus*), se observa mortalidad de magnitud variable entre los polluelos de la colonia más grande conocida para esta especie, en Argentina. Durante dos temporadas reproductivas de la especie, se estudiaron aspectos epidemiológicos y patológicos de infecciones por digeneos (Microphallidae) y nematodos (Acuariidae) en polluelos de gaviota cangrejera. La prevalencia de infección por nematodos fue del 80.3% en 2005 y del 89.2% en el 2006, con una intensidad media de 23.7% en el año 2005 y del 50.8% en el 2006. El riesgo de infección aumentó 34.3% y la intensidad de infección aumentó 6.7% por cada milímetro de aumento en la longitud de la cabeza al pico. Los nematodos ocupaban las glándulas proventriculares causando alteraciones en su estructura y proventriculitis leve. La prevalencia de infección por digeneos fue del 97.0% en el año 2005 y del 97.3% en año 2006. La infección se asoció con enteritis catarral severa, respuesta inflamatoria linfocítica/eosinofílica, proliferación de fibroblastos alrededor de los parásitos y alteración estructural de las criptas adyacentes.

Key words: helminth infections, *Larus atlanticus*, Olrog's gull

Abbreviations: CI = confidence interval; exp(β) = exponent of regression coefficient beta; GI = gastrointestinal tract; GLM = generalized linear models; OG = Olrog's gull; ZANB = zero-altered negative binomial models

Digeneic trematodes and nematodes have long been associated with pathology and mortality in wild birds. For example, *Sphaeridiotrema globulus* (Psilostomatidae) and *Cyathocotyle bushiensis* (Cyathocotylidae) were implicated in recurrent mortality of several species of wild ducks from Canada (7), whereas *S. globulus* alone was associated with mortality in Muscovy duck (*Cairina moschata*) from Australia (3), and whistling swan (*Cygnus columbianus*) in the United States (15). Regarding nematodes, acuarids have been reported to cause severe pathology in wild birds, such as *Dispharynx* spp. in brown-headed cowbird (*Molothrus ater*) (14) and African jacana (*Actophilornis africana*) (16), *Echinuria* spp. in Laysan duck (*Anas laysanensis*) and mute swan (*Cygnus olor*) (6,17), and *Streptocara* spp. in ducks (12) and in Chilean flamingo (*Phoenicopterus chilensis*) (5).

Olrog's gull (OG, *Larus atlanticus*) is a species listed as vulnerable to extinction and is endemic to the Atlantic Coast of Argentina, Uruguay, and southern Brazil (18). Yearly mortality is usually high among OG prefledged chicks in the largest known breeding colony, and it has previously been associated with infections by the acanthocephalan *Profilicollis chasmagnathi* (9). However, there is still an important gap on knowledge related with the pathology of other highly prevalent helminths in this OG population.

The objective of this article is to report epidemiological and pathological aspects of infections by nematodes Acuariidae and digeneans Microphallidae in OG chicks.

MATERIALS AND METHODS

Freshly dead Olrog's gull chicks were salvaged during two breeding seasons of the species (2005: 66 birds; 2006: 37 birds) at the Isla del Puerto breeding colony (38°48′S, 62°15′W) in the Bahía Blanca Estuary, Argentina. The total head–beak length (hereafter head–beak) of each dead chick was measured to the nearest 0.01 mm with the use of digital callipers, and this measurement was used as an indirect indicator of chick's age. Birds were necropsied within 4 hr of collection and their gastrointestinal (GI) tracts were processed for pathological analysis as described in La Sala and Martorelli (9). Also, fresh fecal samples were

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Table 1. ZANB model describing the association between chick size (head–beak length) and intensity of infection by acuarid nematodes in dead Olrog's gull chicks (data from 2005 and 2006 combined).

Count model	Coefficient (β)	SE	*P*
Intercept	−1.593	0.647	0.014
Head–beak	0.067	0.009	<0.0001
Logistic model	Coefficient (β)	SE	*P*
Intercept	−16.528	4.706	<0.001
Head–beak	0.295	0.081	<0.001

collected from 10-day-old live chicks during two consecutive seasons (143 in 2005, 124 in 2006). Fecal samples were fixed in 10% formalin and coprological analyses were conducted with the use of centrifugation (5,288 × *g*, 5 min) and Sheather solution.

Parasites were identified in a previous study (8,10) and included digeneans Microphallidae (*Maritrema bonaerense*, *Maritrema orensense*, *Odhneria odhneri*, and *Levinseniella cruzi*) and nematodes Acuariidae (*Pectinospirura argentata*, *Sciadiocara* sp., *Skrjabinoclava andersoni*). Intensity of infection was determined for the nematodes but not for the digeneans, because of the difficulties in adequately quantifying very high intensities.

For the nematodes, the outcome variable "intensity of infection" had more zeros (uninfected birds) than expected based on the Poisson or negative binomial distributions. Therefore, several types of generalized linear models (GLM) were fitted to quantify the association between infection intensity and host size while accounting for zero inflation. Based on the Akaike information criterion (1), zero-altered negative binomial models (ZANB), also known as hurdle models, provided the best fit to the data. In ZANB models, a negative binomial GLM is used to fit the count process excluding zeros (zero truncated count model), whereas a logistic GLM is used to fit the probability of measuring a positive count. For the continuous independent variable head–beak in the ZANB model, exp(β) indicated the percentage change in the dependent variable for a one-unit increase in head–beak. In the logistic model, exp(β) indicated the increase in the odds of infection for a one-unit increase in head–beak.

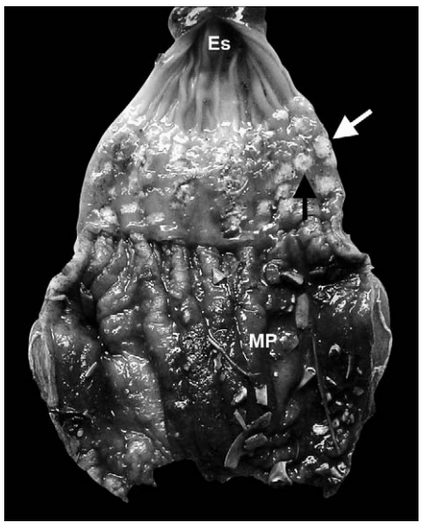

Fig. 1. Olrog's gull chick proventriculum with multiple mucosal ulcerations at points of attachment of nematodes in the glandular area of the organ (solid arrows), between the esophagus (Es) and the muscular proventriculum (MP). Crab remains are observed in the latter area.

Fig. 2. Histological section of proventriculum showing a nematode (N) infiltrating a proventricular gland (PG). Note desquamated epithelial cells (solid arrow) and mild, localized necrosis of crypts (dashed arrow) adjacent to the parasite.

RESULTS

Infection frequency and risk. In the dead chicks, prevalence of infection by Acuariidae was 80.3% (95% confidence interval [CI]: 79.5–81.1, $n = 66$) in 2005 and 89.2% (95% CI: 87.7–90.7, $n = 37$) in 2006. Mean intensity was 23.7 (range 0–147) in 2005 and 50.8 (range 0–130) in 2006. Chick size was positively associated with intensity of infection ($Z = 7.81$, df = 90, $P < 0.0001$, $n = 91$), which increased 6.7% for each millimeter of increment in head–beak (count model in Table 1). The logistic model (Table 1) showed that for every millimeter increase in head–beak, the odds of infection rose 34.3% (95% CI: 14.6–57.4, $P < 0.001$). Microphallidae prevalence was 97.0% (95% CI: 89.0–99.8, $n = 66$) in 2005, and 97.3% (95% CI: 85.0–100, $n = 37$) in 2006. In 10-day-old live chicks, Microphallidae prevalence was 98.0% (95% CI: 97.5–98.5, $n = 51$) in 2006, and 95.3% (95% CI: 94.7–95.9, $n = 64$) in 2007. Prevalence was not different (Fisher's exact test: $P > 0.05$) between dead and live chicks (years for each group combined).

Pathology. Acuariidae infections were associated with mild inflammatory proventriculitis. The nematodes were embedded in the mucosal ridges of the organ with their posterior end projecting into the organ's lumen. Clusters of nematodes occupied small areas of the organ and were associated with mucosal ulcerations (Fig. 1). The worms penetrated to the level of the muscularis mucosa or deeper into the organ, where they occupied the submucosal secretory glands and caused severe disruption of their structure, obliteration, distention, and pressure atrophy of the adjacent mucosal epithelium (Fig. 2). The glandular tissue in close contact with the parasites presented mild necrosis and desquamation (Fig. 2). Mild leukocyte infiltration was present. Cystic glands probably resulting from blockage by the parasites were observed occasionally.

Microphallidae infections were associated with compact, intraluminal mucus plugs in the duodenum, jejunum, and ileum compatible with mild to severe catarrhal enteritis (Fig. 3). Most of the chicks examined were heavily parasitized. The pathology observed was associated with *M. bonaerense*, *M. orensense*, and *O. odhneri* in the small intestine, and with *L. cruzi* in the ceca. *Maritrema* spp. could be distinguished from *O. odhneri* on histological sections of intestine stained with hematoxylin and eosin based on the shape of their vitellarium.

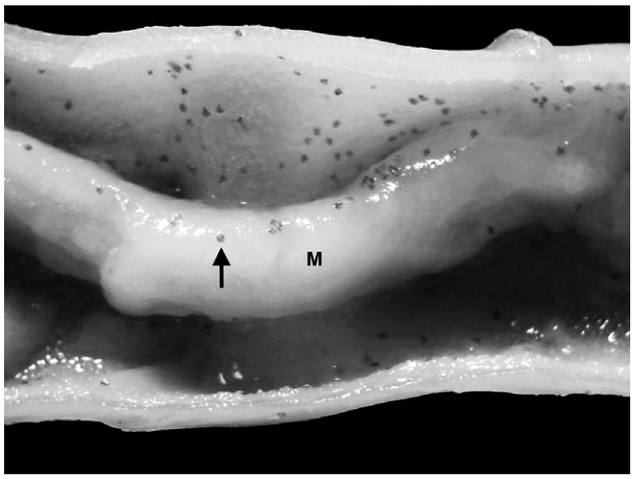

Fig. 3. Jejunum with catarrhal enteritis and a compact mucus plug (M) clogging up the intestinal lumen. Abundant microphallids can be observed embedded in the mucosa and the mucus plug (arrow).

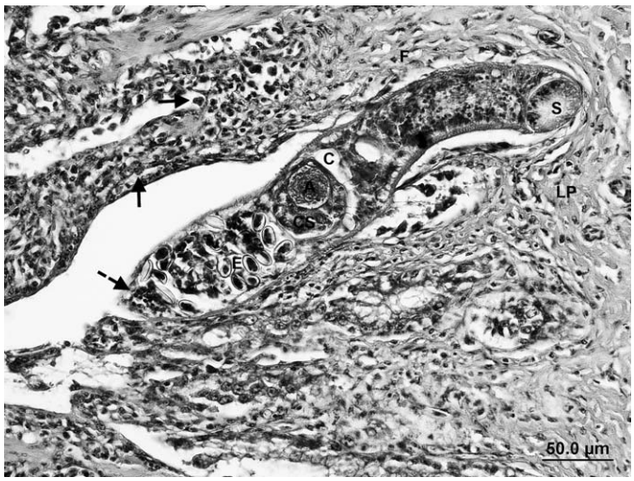

Fig. 4. *Maritrema bonaerensis* breaching through a crypt into the lamina propria (LP). A eosinophil-rich (solid arrows), mixed inflammatory response, with extensive fibroblast proliferation (F) can be observed around the parasite. The parasite's oral sucker (S), paired intestinal ceca (C), acetabulum (A), cirrus sac (CS), vitellaria (dashed arrow), and multiple eggs (E) can be observed.

The worms were either adhered superficially to the mucosal epithelium, lodged between the villi, or breaching through the crypt into the lamina propria to invade the muscularis mucosa (Fig. 4). Degeneration, necrosis, and desquamation of crypt and villi enterocytes led to blunting and flattening of the villi. Fibroplasia of the basement membrane was common. A lymphocyte/eosinophil-rich inflammatory response, with extensive fibroblast proliferation around the parasite and disrupted architecture of the adjacent crypts were present (Fig. 4). Lymphangiectasia and fibroplasia of crypts were frequently observed adjacent to the parasites. In the cecum, *L. cruzi* usually penetrated to the level of the muscularis mucosa obliterating the crypts, and it was associated with leukocyte infiltration. Histological changes in the cecum were similar to those observed in the small intestine.

DISCUSSION

This is the first report of epidemiological and pathological aspects of infections by trematodes and nematodes in OG.

Infections by acuarids of the genera *Echinuria*, *Dispharynx*, and *Streptocara* have been associated with advanced pathology in the proventriculus of species of wild birds (see introduction). Contrarily, OG chicks in our study presented only mild inflammatory responses, which could be partly explained by the fact that the nematodes were restricted to the proventicular glands and never penetrated deeper than the lamina propria. Therefore, it is possible that Acuariid parasites either are nonimmunogenic in OG or that a parasite–host balance has been achieved through coevolution over a long period. Although nematode infections seem to be mildly pathogenic, however, they may represent an added burden on their hosts.

Regarding the digenean infections, other studies (3) reported severe enteritis and ulcerations in *C. moschata* associated with *S. globulus*. Although OG chicks from this study did not present ulcerative lesions, the observed severe catarrhal enteritis may have been enough to cause the death of the birds.

Prevalence of digenean infection was very high in both dead and live chicks, and it was not different between these groups. This might suggest that, despite the severe pathology observed, infection is not negatively affecting the hosts, because in that case a substantial difference in prevalence should be evident between dead hosts and the general population (13).

During their breeding season, OG parents feed chicks from day one of hatching and themselves almost exclusively on *C. angulatus* (La Sala, pers. obs.) and *N. granulata* crabs (4), which in the Bahía Blanca Estuary are intermediate hosts of larval stages of acuarid nematodes and at least three of the species of microphallids reported here (i.e., *M. bonaerense*, *M. orensense*, and *O. odhneri*) (2). Also, *L. cruzi* has been reported parasitizing the freshwater shrimp *Palaemonetes argentinus* (11), which are frequently preyed upon by OG (La Sala, pers. obs.). This trophic specialization would be expected to increase infection intensity gradually through dietary transmission over the chick-rearing period and could explain, at least in part, the sharp increase observed among the dead chicks in the odds for acuarid infection (logistic model) and infection intensity (ZANB model) with increasing chick size (and presumably, age).

Our observations of extensive pathology associated mainly with digenean infections warrant further investigation to elucidate proximate casual relationships between infection, chick mortality, and other underlying factors such as coinfection with other pathogens in the studied population.

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