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Pathology of enteric infections induced by the acanthocephalan *Profilicollis chasmagnathi* in Olrog's gull, *Larus atlanticus*, from Argentina

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Abstract

Acanthocephalans can be pathogenic helminths of marine birds. Every year during the breeding season, there is variable mortality among prefledged chicks from the largest known Olrog's gull (*Larus atlanticus*) colony. Mortality has been associated with infection by the acanthocephalan *Profilicollis chasmagnathi*. Our aim was to study the role of chicks' size as a risk factor for intensity of infection and severe pathology, and to expand upon previous pathological findings reported in acanthocephalan-infected chicks. Size of the chick was associated with intensity of infection and number of intestinal perforations, which increased by 6.9% and 4.1%, respectively, for each millimetre increment in chick size. Infection was associated with inflammatory enteritis and granulomatous peritonitis. Complete intestinal perforations were observed in 85% and 97.3% of the studied chicks in 2005 and 2006, respectively, and they were observed very early during the post-hatching period. Our results show: (1) the presence of advanced pathology associated with acanthocephalan infections in chicks, beginning very early in the post-hatching period; and (2) significant increases in the intensity of infection and the associated pathology as a function of size of chicks, in dead chicks during this period.

Introduction

Acanthocephalans are helminths that may be pathogenic to birds (Taraschewski, 2005; Atkinson *et al.*, 2008), and they have been associated with varying levels of mortality, mainly in coastal birds. For example, mass mortalities have been reported in common eiders, *Somateria mollissima*, in the Dutch Wadden Sea (Camphuysen *et al.*, 2002) and mute swans, *Cygnus olor*, in Canada and Scotland (Sanford, 1978; Pennycott, 1998).

Olrog's gull is a species listed as vulnerable to extinction (IUCN, 2010) and is endemic to the Atlantic Coast of Argentina, Uruguay and southern Brazil (Yorio *et al.*, 2005). Variable mortality has been reported among Olrog's gull prefledged chicks since 2003 in the largest known breeding colony for this species, and the acanthocephalan *Profilicollis chasmagnathi* was postulated as one of the possible causes of death (La Sala &

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Martorelli, 2007). Although this first study provided baseline information on disease pathology, there is still an important gap in knowledge related to pathological and epidemiological aspects of acanthocephalan infections in this species.

The objective of this work is to extend previous findings in acanthocephalan-infected Olrog's gulls by: (1) assessing the role of chicks' size/age as risk factors for higher intensity of infection and increased pathology; (2) studying the association between intensity of infection and pathology; and (3) further characterizing pathological features of infection.

Materials and methods

Source of the data

The study incorporated data from necropsies of freshly dead, prefledging chicks salvaged during four breeding seasons of Olrog's gull (2003: 6 birds; 2005: 66 birds; 2006: 37 birds; 2007: 1 bird) at the Isla del Puerto breeding colony (38°48′S, 62°15′W) in the Bahía Blanca Estuary, Argentina.

The total head–beak (hereafter head–beak) and tarsus–metatarsus (hereafter TMT) lengths of each dead chick were measured to the nearest 0.01 mm using digital callipers, and this metric was used as a proxy for each chick's age. Chicks were necropsied within 4 h of collection, and each gastrointestinal tract (hereafter GI) was removed and fixed in 10% formalin until it could be examined. At the laboratory, each GI was opened and specimens of *P. chasmagnathi* present in the GI were counted. Complete, acanthocephalan-associated perforations were recorded and their intensity was used as an estimate of severity of infection. Acanthocephalans found in the coelomic cavity, which had perforated the GI, were quantified. Affected regions of the gut were prepared for histopathological examination; samples were dehydrated in ethanol, embedded in paraffin, sectioned at 5 μm, stained with haematoxylin/eosin, periodic acid–Schiff (PAS) or Masson's trichrome, and examined under light microscopy.

Statistical analyses

The analyses included combined sets of data from 2005 and 2006. Prevalence of infection and its 95% confidence interval (95% CI) and mean intensity (number of parasites/number of infected hosts) were computed and interpreted as measures of frequency and intensity of infection, respectively. The degree of clustering of parasites in the host population (dead prefledging chicks) was calculated using the variance-to-mean ratio (Elliot, 1977).

Because the dependent variables 'intensity of infection' and 'number of perforations' approximated a theoretical negative binomial distribution, their association with the explanatory variable 'chick size' (approximated by head–beak and TMT) in the subpopulation studied here (dead chicks) was assessed using generalized linear models (GLM) with a negative binomial response using the function 'glm.nb' in the package MASS (Vernables & Ripley, 2002) of the statistical program R (R Development Core Team, 2010). Because head–beak and TMT were highly correlated (>70%), they were examined separately to avoid collinearity, and the Akaike information criterion (AIC; Akaike, 1974) was used to select the most parsimonious, final model, as suggested by the lowest AIC value. The association between 'intensity of infection' and 'number of perforations' was assessed using the same approach described above.

For continuous independent variables, such as head–beak, the predicted percentage of change in the dependent variable for every one-unit increase in the value of the independent variable was computed as $[\exp(\beta) - 1] \times 100$, where β is the regression coefficient of the dependent variable. Simulations were used to fit curves of the evolution of infection intensity and associated intestinal perforations as a function of head–beak. The relative goodness of fit of the models was assessed based on the AIC criterion. Terms (variables) were considered to improve the model fit if their exclusion increased AIC by more than two units (Burnham & Anderson, 2002).

Results

Parasite identification

Based on morphological characteristics and measurements of 10 males and 10 females (table 1), the parasite was identified as *P. chasmagnathi* (Holcman-Spector *et al.*, 1977). The body consisted of an elongated trunk with abundant spines on its anterior third, a slender neck and a

Table 1. Measurements of *P. chasmagnathi* from Olrog's gull, *Larus atlanticus*.

Organ	Males	Females
Trunk	2.50–14.80 × 0.79–3.70	3.25–29.00 × 0.35–3.20
Proboscis	0.66–2.00 × 0.40–2.20	0.61–1.60 × 0.80–1.76
Proboscis receptacle	4.62–4.75 × 0.10–0.41	5.05 × 0.24
Neck	1.22–4.17 × 0.20–0.40	1.75–4.30 × 0.24–0.41
Lemniscus	3.10–5.49 × 0.12–0.32	5.50–7.00 × 0.25–0.52
Testis (ant.)	0.80–1.81 × 0.50–1.41	na
Testis (post.)	0.88–1.19 × 0.59–0.63	na
Ovaries	na	0.17–0.68 × 0.10–0.41
Cement glands	1.47–1.69 × 0.04–0.05	na
Eggs	na	52.5 × 23.0*

*Measurements in μm; na, not applicable.

Table 2. Infection levels of *P. chasmagnathi* in dead chicks of Olrog's gull.

	2005 ($n = 37$)	2006 ($n = 66$)
Prevalence (95% CI)	90.9 (90.1–91.7)	97.3 (97.3–98.1)
Mean intensity	38.9	53.4
Range	0–234	0–159
Coefficient of dispersion ($\delta^2/\overline{\chi}$)	67.27	23.07

large spherical proboscis. Proboscis hooks were arranged in 16–20 longitudinal rows with 8–9 hooks each. The proboscis receptacle was cylindrical, double-walled, extending between the proboscis base and the first third of the trunk. Lemnisci were larger than the receptacle. Males had spherical or oval testes, four paired cement glands and a prominent copulatory bursa with numerous cuticular rays and two muscular sphincters. Females had a uterine bell, uterine duct, uterus, muscular vagina, vulva and subterminal genital pore. Eggs were ovoid without filaments.

Parasite aggregation, distribution and accumulation over time

The degree of parasite aggregation in the studied subpopulation (i.e. the dead chicks) is presented along with frequency estimates in table 2. The intensity of infection in different regions of the GI was consistent between years (2005: jejunum = 51.4%, ileum = 39.9%, caeca = 1.2%, rectum = 0.8%, coelom = 6.7%; 2006: jejunum = 36.6%, ileum = 50.7%, caeca = 1.3%, rectum = 0.7%, coelom = 10.8%), with the exception that the proportions were inverted between the jejunum and ileum between years. Complete perforation of the intestines was observed in 85% (34/40) and 97.3% (36/37) of the chicks studied in 2005 and 2006, respectively.

Based on the AIC, the probabilities of infection intensity and number of GI perforations were better described by head–beak than by TMT, so only the former variable was included in the models. Head–beak was positively associated with intensity of infection ($Z = 11.29$, df = 90, $P < 0.001$), which increased by 6.9% for each millimetre of increment in head–beak (table 3, fig. 1). Similarly, head–beak was positively associated with the number of intestinal perforations ($Z = 5.29$, df = 65, $P < 0.001$), which increased by 4.1% for each millimetre of increment in head–beak (table 4, fig. 1). The number of perforations increased by 2.2% ($Z = 9.82$, df = 76, $P < 0.001$) for each unit of increment in intensity.

Table 3. Negative binomial model describing the association between head–beak and intensity of infection by *P. chasmagnathi* in dead chicks of Olrog's gull.

Model = intensity of infection ~ head–beak
$n = 91$

Term	Coefficient	Std error	P	ΔAIC*
Intercept	−1.221	0.435	<0.01	–
Head–beak (mm)	0.067	0.006	<0.0001	167.8

*AIC value increment if the single term is dropped.

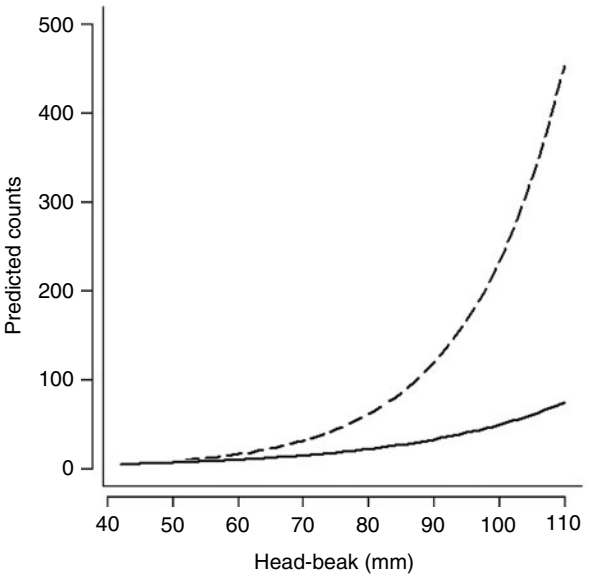

Fig. 1. Simulation of *P. chasmagnathi* intensity (dashed line) and associated intestinal perforations (solid line) as a function of chick size (head–beak length) in dead Olrog's gull chicks.

Gross pathology

Many of the worms perforated the intestinal wall with their trunk and attached with their proboscis to the serosa of adjacent intestinal loops, causing intense peritoneal reactions observed as strong fibrous adhesions. Some extraintestinal worms perforated from the serosal towards mucosal surfaces of the intestinal wall (both completely and incompletely). Intestinal rupture was observed in one bird where the entire metasomae of five adult acanthocephalans protruded into the peritoneal cavity. Many of the worms found free in the coelom were encapsulated by a thick layer of fibrotic tissue which often contained the decomposed parasite. Some worms were found with their proboscis embedded into the parenchyma of the pancreas. The majority of the large, intra-intestinal female worms found in the largest chicks were gravid.

Histopathology

In the earliest stages of parasite penetration into the GI wall, a band of necrotic debris was observed around the leading edge of the presoma. An intense proliferation of fibroblasts was present throughout the thickness of the muscularis externa, and a mixed inflammatory infiltrate composed of macrophages, lymphocytes and eosinophils surrounded the parasites' presomae. With deeper invasion into the muscularis externa, abundant multinucleated giant cells were observed surrounding the parasite. In the most advanced stage of penetration, the presoma was surrounded by a granulomatous reaction composed of giant cells, macrophages, eosinophils and

Table 4. Negative binomial model describing the association between head–beak and the number of GI perforations associated with *P. chasmagnathi* in dead chicks of Olrog's gull.

Model = GI perforations ~ head–beak $n = 66$				
Term	Coefficient	Std error	*P*	ΔAIC*
Intercept	−0.161	0.549	0.77	–
Head–beak (mm)	0.041	0.008	<0.0001	88.6

* AIC value increment if the single term is dropped.

lymphocytes (figs 2, 3 and 4). Grossly, these appeared on the serosal surface of the GI as prominent nodules containing the parasite's proboscis. Occasionally, *P. chasmagnathi* perforated the intestinal wall with minimal evidence of granuloma formation.

Profilicollis chasmagnathi was also present in the caecum and tended to occupy most of its lumen. The degree of penetration was variable and ranged from superficial to complete perforations of the organ's blind end. The intensity of infection was limited to one worm per caecum. There were profound architectural changes of the crypts, acute and chronic cellular infiltrates in the lamina propria, granuloma formation and intense hyperplasia of mucosa-associated lymphoid tissue.

Discussion

This study shows: (1) the presence of advanced pathology associated with *P. chasmagnathi* infections in Olrog's gull chicks from very early in the post-hatching period; (2) that the intensity and severity of infection increase significantly as a function of chick size during that period; and (3) that the severity of infection increases with intensity of infection. Also, the present work elaborates on previous findings describing the pathological features of acanthocephalan infections in this vulnerable species.

In general, the pathological findings presented here agree with descriptions made in domestic and wild birds (Nath & Pande, 1963; Bishop & Threlfall, 1974; Taraschewski & Hofmann, 1991), mammals (Nelson & Nickol, 1986; Richardson & Barnawell, 1995) and fish (de Buron & Nickol, 1994; Sanil *et al.* 2011) infected with different species of acanthocephalans. Fibrous nodules similar to those observed in Olrog's gull chicks have been reported in other avian species, such as *Melanerpes carolinus* (Picidae) infected by *Mediorhynchus centurorum* (Nickol, 1969), domestic ducks infected by *Polymorphus minutus* and *Filicollis anatis* (Nicholas & Hynes, 1958; Taraschewski & Hofmann, 1991), and *Somateria mollissima* (Anatidae) infected by *Profilicollis botulus* (Bishop & Threlfall, 1974). Also, the composition of inflammatory cells surrounding the parasites and comprising the granulomas resembles those described by Gonzales-Viera *et al.* (2009) in *Leucocephalus modestus* (Laridae) infected by *Profilicollis altmani*, as well as by Taraschewski & Hofmann (1991) in domestic ducks infected by *Filicollis anatis*.

Granulomas are an important protective innate response which becomes stronger as the bird matures (Juul-Madsen *et al.*, 2008). Complete perforations by small, immature *P. chasmagnathi* were observed in some

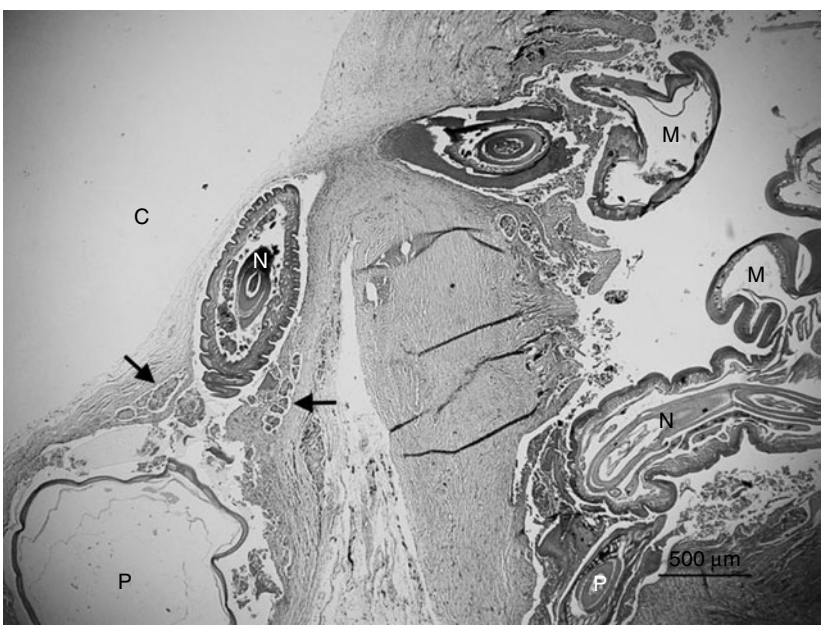

Fig. 2. *Profilicollis chasmagnathi* perforating the intestinal wall to the serosa level. Two adults of *P. chasmagnathi* can be observed with their metasoma (M) in the intestinal lumen, and their presoma (neck = N; proboscis = P) penetrating the intestinal wall and contained in a granuloma with abundant giant cells (arrows).

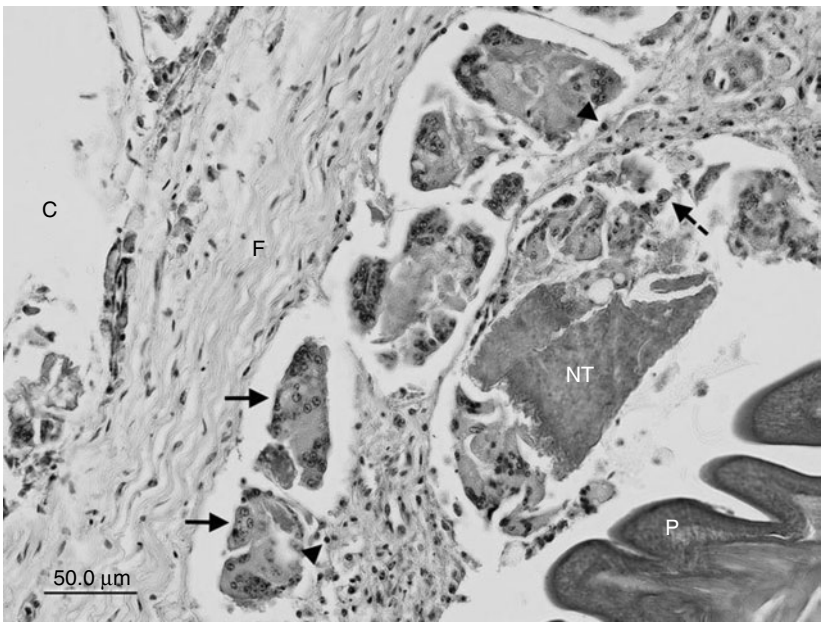

Fig. 3. Granuloma surrounding *P. chasmagnathi*. Multinucleated giant cell (solid arrows), macrophage (dashed arrow), lymphocytes (arrowheads), and host necrotic tissue (NT) are observed adjacent to *P. chasmagnathi* (P). Abnormal fibroblast proliferation and fibrosis (F) surrounds the parasite-containing granuloma isolating it from the coelomic cavity (C).

very small chicks (*c*. 5 days of age) without the nodule formation typically observed in larger birds, and may thus be explained by a lack of competent innate responses to *P. chasmagnathi* in the smallest chicks that died.

Here, *P. chasmagnathi* was identified at very high prevalence and high intensities in the subpopulation of dead chicks, with full-thickness intestinal perforations being observed in most of the birds. The intensity and severity of infection (number of GI perforations) increased sharply as a function of chick's size/age, whereas infection became more severe with increasing intensity.

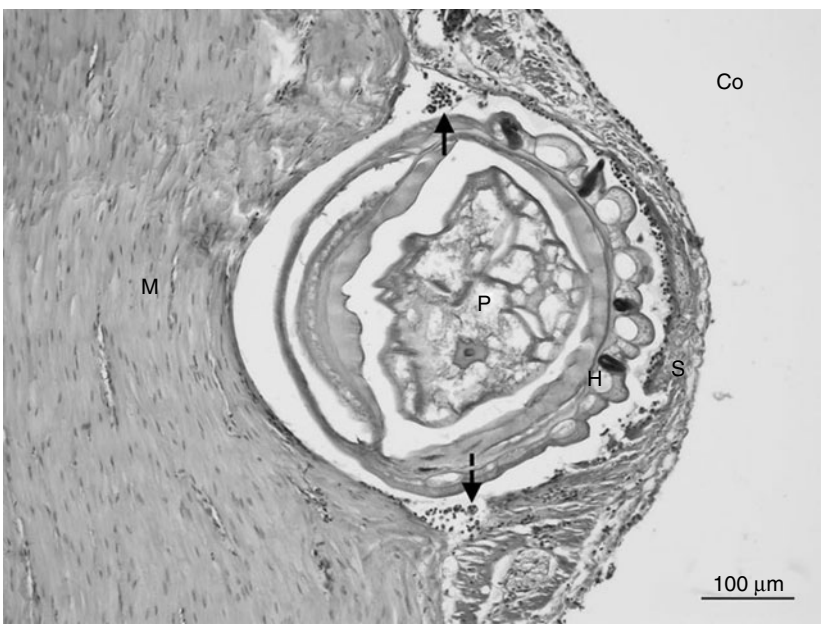

Fig. 4. Interface between *P. chasmagnathi* (P) and the thickened GI serosa (S). Intense mononuclear cell infiltration with lymphocytes (solid arrow) and macrophages (dashed arrow) are observed at the interface between *P. chasmagnathi* and host tissue. Co, coelomic cavity; M, muscularis externa; H, proboscis hooks.

During their breeding season, Olrog's gull parents feed chicks from day one of hatching and themselves almost exclusively on *Cyrtograpsus angulatus* (pers. obs.) and *Neohelice granulata* (Delhey *et al.*, 2001) crabs, which are intermediate hosts of *P. chasmagnathi* larvae (Martorelli, 1989). This would be expected to increase infection intensity over time through constant re-infections, with chicks in good condition being capable of fighting off infection but feeble birds succumbing to it.

Our study population was composed only of dead chicks, whereas no data were available from live, healthy birds. Therefore, the chicks studied here were likely to have been in poor condition prior to death and are expected to have had weakened immune responses. This could explain, at least in part, the significant increase in infection intensity and severity as a function of chick size/age, as well as the increase in severity associated with parasite intensity.

At this early stage there is no evidence to support a causal association between *P. chasmagnathi* and death. Then, one may speculate that poor condition may have increased infection-associated pathology, which further reduced condition and so on, or, alternatively, that infection and the subsequent pathology may have led to poor condition. Regardless of the directionality of events leading to the death of these birds, and despite the lack of data from live chicks, our results: (1) show that before death, intensity and severity of infection build up gradually over time as a function of size; and (2) further support previous findings suggesting that the pathology associated with *P. chasmagnathi* may be regarded as sufficient to cause death.

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