

RESEARCH NOTE

Regurgitation of the koilin layer in chinstrap penguins (*Pygoscelis antarcticus*) and its association with gastric parasites

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Keywords

Cuticula gastris; host-parasite interaction; nematodes; parasitic load; regurgitation; *Stegophorus macronectes*.

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Abstract

The koilin membrane, formed by the secretions of the ventricular and pyloric glands, functions as a protective layer in the gizzards of most bird species. However, the ecological functions of koilin have never been studied in free-ranging penguins. During the two austral summers from 2012 to 2014, we observed the regurgitated koilins of chinstrap penguins (*Pygoscelis antarcticus*) at Narębski Point on King George Island, South Shetland Islands, and we detected a significant difference in the daily accumulation of regurgitated koilins between the pre-hatching and post-hatching periods in the rookery. We also found 233 gastrointestinal parasites, all *Stegophorus macronectes* (Nematoda, Acuariidae), from 26 out of 45 koilins freshly regurgitated by chinstrap penguins. We suggest that the regurgitation of koilins may benefit adult chinstrap penguins in the wild by reducing parasitic loads when they fast during incubation; it may also help decrease the risk of parasite transmission to chicks. Our results present the first observations of regurgitated koilins among breeding chinstrap penguins. How koilin regurgitation functions in penguins requires further study. Among the gentoo penguins (*P. papua*) co-occurring at the study site, we observed no regurgitated koilin layers.

The koilin membrane, also known as the cuticula gastris (Hofmann & Pregl 1907; Eglitis & Knouff 1962), is the variably developed internal lining of a carbohydrate/protein complex (probably a mucoprotein), not keratinous, secreted by the ventricular and pyloric glands in avian gizzards (Akester 1986; Baumel et al. 1993; Bels 2006; Svihus 2011). The layer protects the muscles of the gizzard from the degrading effects of gastric acids and mechanical actions of food particles (Bels 2006). The koilin layer may be relatively soft in the poorly-muscléd type of gizzards (Baumel et al. 1993).

In general, the koilin layer is replaced slowly as it wears by mechanical abrasion. McAtee (1917) suggested that the shedding of koilin layers may be observed in most species of birds. The shedding occurs as the layer gradually wears away in a few pieces or is detached from the stomach wall and digested (McAtee 1906, 1917). However, a few cases of regurgitating behaviours have been reported

in some bird species such as American kestrels (*Falco sparverius*; Vyas et al. 2009), anhingas (*Anhinga anhinga*; Bartlett & Bartlett 1899), common cuckoos (*Cuculus canrous*; Newton et al. 1896), common starlings (*Sturnus vulgaris*; Newton et al. 1896), hornbills (*Buceros* sp.; Flower 1869), little owls (*Athene noctua*; Newton et al. 1896), mistle thrushes (*Turdus viscivorus*; Newton et al. 1896) and neotropical cormorants (*Phalacrocorax brasilianus*; Bartlett & Bartlett 1899).

Although the presence of this layer has been mostly reported from post-mortem studies in various species of wild birds, most studies of koilin have focused on their structure (Akester 1986) or how abnormal koilins affect poultry health (Webb & Colvin 1964; Ono et al. 2004; Kaldhusdal et al. 2012). A recent report on chemical irritations causing regurgitation of koilin suggests that koilin layers may protect against chemical toxicity as well as providing mechanical protection (Vyas et al. 2009).

However, there is little information on the functions of koilin in wild birds and, to the best of our knowledge, no clear description of koilin regurgitation in penguins has been published.

During our study to monitor the breeding performances of chinstrap (*Pygoscelis antarcticus*) and gentoo penguins (*P. papua*) at Narębski Point during the austral summers of 2012/13 and 2013/14, we often observed koilin regurgitation in chinstrap penguins, but not in gentoo penguins, and their regurgitated koilins in and around the penguin rookery as well as along two major passages to the nearby shoreline. On the basis of these observations, we speculated that koilin regurgitation in chinstrap penguins could be related to the chick-rearing activity, which requires active foraging and delivery of foods.

In this paper, we report our observations of the regurgitated koilins and associated parasites, and the temporal change of koilin regurgitation in chinstrap penguins. We discuss the possible benefits of koilin regurgitation for free-ranging chinstrap penguins.

Methods

Study area

Narębski Point is on the south-east coast of Barton Peninsula, King George Island, in the South Shetland Islands, Antarctica. At the Narębski Point study site (62° 14' 14.3" S, 58° 46' 27.1" W), about 3000 pairs of chinstrap and 2300 pairs gentoo penguins breed every austral summer (Republic of Korea 2014). Chinstrap penguin rookeries are located on rocky slopes close to the shore and are divided into several sub-groups (colonies) by huge pillars of rocks or cliffs.

Sample collection and analysis

Temporal change in koilin regurgitation among chinstrap penguins was assessed by daily counts in six plots for 15 days from 19 December 2013 to 6 January 2014. Each plot (2.5 m × 2.5 m) was set within the same chinstrap penguin colony (1475 nests) and had 7.3 ± 0.5 breeding pairs of chinstrap penguins. Koilin was removed from the plot before the daily counts at 18 December 2013. We searched daily for freshly regurgitated koilins in the plots, and removed all found koilins from the plots to avoid double counts. When a survey was interrupted by unfavourable weather conditions (20 and 30 December 2013), we also excluded the next day's results for further analysis. We assessed the temporal change in the daily accumulation number of koilins through Spearman correlation analysis, and compared the numbers before (pre-hatching period:

5 days) and after (post-hatching period: 10 days) the mean hatching date of the colony of chinstrap penguins (25 December 2013; Kim, unpubl. data) using the Mann-Whitney rank-sum test. Although snowy sheathbills (*Chionis albus*) were occasionally observed to consume the koilin in the study area, we assumed that there was no temporal change in the effect of their scavenging activity. All statistical analyses in this study were conducted using SigmaPlot version 12.0 (SysStat 2012).

We collected the koilins on the ground in the chinstrap penguin's rookery from 7 to 30 December 2013; only freshly regurgitated layers that were intact, cylindrically shaped, and not damaged by scavengers such as snowy sheathbills nor contaminated by faeces of nearby penguins were collected (Fig. 1). At the laboratory at King Sejong Station on Barton Peninsula, the koilins were microscopically examined for gastrointestinal parasites (see Vidal et al. 2012). The number of parasites in each koilin was recorded, and they were kept in cryovials filled with 70% ethyl alcohol for further identification. Descriptive statistics, prevalence (proportion of koilins containing parasites) and the mean intensity (average number of parasites in each koilin containing parasites) with standard deviations, range and 95% confidence intervals (CIs) were calculated using the Clopper-Pearson interval (for prevalence) and 2000 bootstrap replications (for mean intensity) with QP 3.0 software (Rozsa et al. 2000; Reiczigel et al. 2013). The collected parasites, which were all nematodes, were identified later under microscopes at the laboratory at Seoul National University in the Republic of Korea. Identifications were based on taxonomic keys and morphological descriptions, in particular regarding the structure of their cephalic collars, following Mawson (1953), Zdzitowiecki & Drózd (1980) and Vidal et al. (2016).

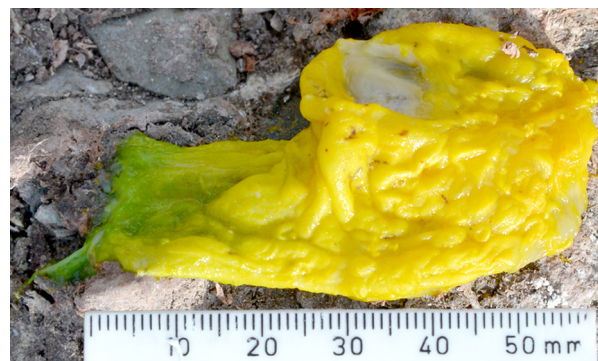


Fig. 1 A koilin layer regurgitated by an adult chinstrap penguin (*Pygoscelis antarcticus*), 21 January 2014.

Results

The daily accumulation of regurgitated koilins, which are yellow with a green tip (Fig. 1), per plot showed the temporal decline along the breeding season (Spearman's rank-order correlation; $r_s = -0.795$, $P < 0.001$). The number was higher in the pre-hatch period (mean and SD: 3.0 ± 0.3 , median: 3.0, range: 0–8, $n = 30$) than that in the post-hatching period (mean and SD: 0.7 ± 0.1 , median: 0.0, range: 0–3, $n = 60$) (Mann-Whitney $U = 181.0$, $df = 1$, $P < 0.001$; Fig. 2).

A total of 45 intact and fresh koilin layers were collected in the rookery of chinstrap penguins during the survey. We also found 233 parasites, all identified as *Stegophorus macronectes* (Nematoda, Acuariidae), in 26 out of 45 koilins. Anchored in the regurgitated koilins, the parasitic nematodes were alive at the time of collection (range: 0–61, prevalence: 57.8%, 95% CI for prevalence: 42.2–72.3%, mean intensity: 8.96, 95% CI for mean intensity: 5.96–16.70).

Discussion

Based on field observations in the austral summer of 2012/13, we confirmed that chinstrap penguins regurgitate yellow koilins, which were partially coloured green, possibly from regurgitated bile (Bels 2006), mostly before the hatching dates. Though the regurgitated koilins within the colony can be easily trampled by penguins or partially fed on by scavenging birds, the regurgitated koilins were still common in terms of distribution and quantity. The fresh koilins were cylindrically shaped when undamaged and untrampled, suggesting that free-ranging wild chinstrap penguins may replace the whole koilin layer at once

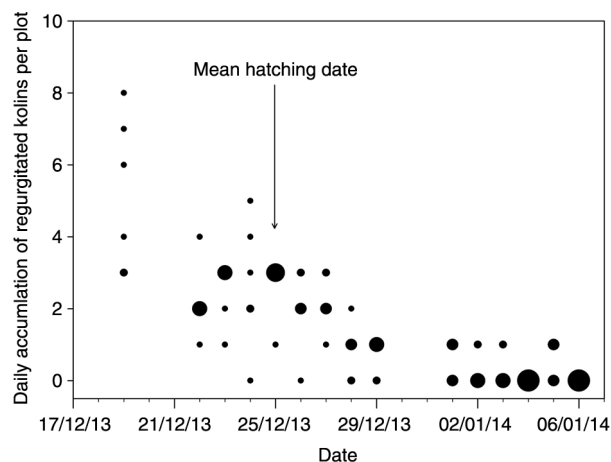


Fig. 2 Temporal change in the daily accumulation of regurgitated koilins at six plots in the breeding colony of chinstrap penguins. The size of filled circles represents the number of plots (ranging from one to six).

during the early breeding season. Considering the live parasites found in the regurgitated koilins, it may be better to regurgitate the koilin than to send it to the duodenum or further along in the intestines, to prevent pathogenic effects, such as ectopic infection.

More regurgitated koilin layers were found in the pre-hatching period, compared to the post-hatching period, indicating that chinstrap penguins are more likely to regurgitate koilin layers when they are incubating eggs compared to when they are rearing chicks. Because this layer protects the stomach wall from mechanical and chemical irritation, digestive activity is paused when this layer is regurgitated and gizzards of birds in this stage must be kept empty until the koilin is renewed (McAtee 1917; Vyas et al. 2009). Breeding parents of chinstrap penguins take turns sitting on the nest in a 5–10 day period during incubation (Lishman 1985; Borboroglu & Boersma 2013), giving them time to empty their stomachs and replace koilin layers. In contrast, when chicks hatch, parental turn-taking occurs on a daily basis (Borboroglu & Boersma 2013). Therefore, we suggest that breeding chinstrap penguins in the wild have a strategy of disposing of and renewing the whole koilin before hatching, because it cannot coincide with food delivery to chicks.

The most important role of such regurgitation must be to replace the old lining in the ventriculus for renewed gizzard protection (Bels 2006). However, as McAtee (1917) suggested, shedding koilins may help birds to reduce their parasitic load, particularly nematodes, which are usually anchored between the koilin layer and the gizzard wall. As chinstrap penguins are a marine predator that consume mainly Antarctic krill (*Euphausia superba*; Ratcliffe & Trathan 2012), their gizzards' koilin layers may not be mechanically abraded in the way that they are in granivorous birds. These penguins' gastrointestinal tracts may be under greater stress from parasites, acquired from the marine food web, than stress by mechanical abrasions. Many parasitic *Stegophorus macronectes* were observed in the regurgitated koilins in this study. This nematode was the only gastrointestinal parasite species found in the gizzard of chinstrap penguins in prior studies (Barbosa & Palacios 2009; Vidal et al. 2012; Brandao et al. 2014).

The previously reported prevalence (67%) of *S. macronectes* in three adult chinstrap penguins on Deception Island, South Shetland Islands (Vidal et al. 2012), falls into the range of the prevalence estimated from the koilin analysis in this study (42.2–72.3%). Given the mean intensity of the nematode (39.5 ± 43.9) reported from the same species in the same region (Vidal et al. 2012), getting rid of a koilin harbouring nine nematodes—the mean

intensity found in our samples—may result in a roughly 23% reduction in the gastrointestinal nematode load. However, because of the small sample size in the study by Vidal et al. (2012), this quantitative comparison is not reliable and the effect of such removal on the body condition of its host is unclear. Nonetheless, these findings are suggestive of the potential role of koilin regurgitation in reducing parasites.

Parental provisioning can transfer gastric parasites to chicks (Muzaffar & Jones 2004; Vidal et al. 2012), and reducing gastric parasites in the parent's gizzard may decrease the risk of transmission through food delivery. Although the pathogenicity and the removal effects of *S. macronectes* in adult chinstrap penguins are not well understood, increased growth rates were observed in experimentally de-wormed chinstrap nestlings compared to their control siblings, which shows the negative effects of gastrointestinal parasites on nestling body conditions, survival rate and fitness in this species (Palacios et al. 2012). Therefore, we suggest that the koilin regurgitation by fasting adults of wild chinstrap penguins may benefit their chicks to an unknown extent.

In contrast to chinstrap penguins, we never observed any regurgitated koilin layers among the gentoo penguins co-occurring at the study site. Whether, and in what manner, gentoo penguins replace their koilin layers is unknown.

To the best of our knowledge, this is the first study of koilin regurgitation in penguin species and the first report on the prevalence of parasites in regurgitated koilin layers. However, our knowledge is limited to this small study site and short time period. Further observations as well as experimental studies across other penguin species in different areas and seasons are required to better understand the details concerning the mechanisms and functions of koilin regurgitation and replacement.

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