THE RADIOGRAPHIC AND ENDOSCOPIC ANATOMY AND DIGESTIVE MECHANISMS OF CAPTIVE AFRICAN PENGUINS (SPHENISCUS DEMERSUS)

Authors: Kline, Sarah, Kottyan, Jennifer, Phillips, Jess, Wack, Allison, Pate, Nathan, et al.

Source: Journal of Zoo and Wildlife Medicine, 51(2) : 371-378

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2019-0076

DOI: 10.1638/2019-0076 *Journal of Zoo and Wildlife Medicine* 51(2): 371–378, 2020

THE RADIOGRAPHIC AND ENDOSCOPIC ANATOMY AND DIGESTIVE MECHANISMS OF CAPTIVE AFRICAN PENGUINS (*SPHENISCUS DEMERSUS*)

Sarah Kline, DVM, MS, Jennifer Kottyan, BS, Jess Phillips, BS, Allison Wack, DVM, Nathan Pate, DVM, Dipl ACVP, and Ellen Bronson, Med Vet, Dipl ACZM

Abstract: The anatomy of the avian gastrointestinal (GI) tract is uniquely suited to each species' dietary requirements. African penguins (*Spheniscus demersus*) are charismatic and popular exhibit animals. As their prevalence grows, there is a need to understand their unique digestive tract to diagnose abnormalities. Reference material specific to the digestive tract of piscivores is scant, and knowledge of the GI tract of a healthy penguin is based on information from other birds. The purpose of this study is to determine the normal gross anatomy, transit time, and histopathologic structures of the penguin GI tract. Twelve clinically healthy penguins were selected for this study from the colony at the Maryland Zoo in Baltimore, which, at the time of this study, consisted of 55 birds. All penguins underwent a barium contrast study, and radiographic images were obtained until the entire GI tract was empty. Approximately 2 wk later, each penguin was anesthetized, and an endoscopic evaluation of the anterior GI tract was performed. Time from barium administration to defecation ranged from 17 to 70 min, and on average, barium clearance was 17.6 hr (range, 5–36 hr). Fluid from the ventriculus had an average pH of 2.75 and contained a mixed bacterial population. Koilin presence and thickness appreciated on endoscopy did not correspond with the thickness determined on histopathology. The results of this study provide a comparative baseline to use during diagnostic workups and help guide treatment decisions.

Key words: African penguin, Contrast study, Endoscopy, Gastrointestinal tract, Koilin, *Spheniscus demersus.*

INTRODUCTION

The avian gastrointestinal (GI) tract is unique both in its anatomical and physiologic makeup. Although the basic components, esophagus, stomach, and intestines, are consistent with other taxa, birds have evolved to maximize nutrient absorption and efficiently reduce GI content weight by increasing their GI transit time. Piscivorous birds, such as penguins, lack a crop, allowing them to swallow whole live prey. Food entering the esophagus travels immediately into the stomach, which is subdivided both grossly and by function into the proventriculus and ventriculus. The proventriculus secretes gastric enzymes to initiate chemical digestion, and hydrochloric acid production causes the pH to plummet. The ventriculus mixes and pummels food through muscular contractions. Digesta then pass through the pyloric sphincter into the shortened small intestine for nutrient absorption. Interruptions in any part of the GI tract can inhibit digestion and decrease nutrient absorption, resulting in a variety of clinical signs.

From 2012 to 2014, the Maryland Zoo diagnosed four African penguins (*Spheniscus demersus*) with ventriculitis based on histopathologic examination of endoscopic biopsies of the ventriculus. One additional penguin in the collection was found deceased, and the diagnosis was made at necropsy. These penguins presented with inappetence, protracted regurgitation, and lethargy. Known causes of ventriculitis are diverse and include infectious agents, heavy metal intoxication, foreign body ingestion, increased grit consumption, and neoplasia; however, to the authors' knowledge, there are no reports of ventriculitis in penguin species.[6]

A thorough diagnostic workup was performed with each of the four penguins; however, reference material specific to the digestive tract of this species is scant.[5,15] Any understanding of a normal penguin GI tract is based on information from other fish-eating birds or general avian sources.[1,2,5,8,9,12,15] Information on normal penguin-specific GI anatomy or transit times is lacking in the literature as well. The purpose of this study was to determine the normal anatomy, transit time, clearance time, and both gross and histopatho-

From the Maryland Zoo in Baltimore, 1876 Mansion House Drive, Baltimore, Maryland 21217, USA (Kline, Kottyan, Phillips, Wack, Bronson); and the Department of Molecular and Comparative Pathobiology, The Johns Hopkins University School of Medicine, 733 N. Broadway, Baltimore, Maryland 21205, USA (Pate). Present address (Kline): Arkansas Veterinary Emergency and Specialists, 11619 Pleasant Ridge Road, Little Rock, Arkansas 72212, USA; (Pate): Center for Advanced Preclinical Research (CAPR), Fredrick National Laboratory for Cancer Research, Frederick, Maryland 21702, USA. Correspondence should be directed to Dr. Kline (sarahvet2012@gmail.com).

logic structures of the penguin GI tract to create a baseline for further ventriculitis investigation.

MATERIALS AND METHODS

Twelve clinically normal penguins were selected from the colony at the Maryland Zoo in Baltimore, which consisted of 55 birds at the time of the study. The penguins were housed together and received the same diet, enrichment, and environmental stimulation. Penguins were offered a variety of hand-fed or tossed fish including Pacific herring (*Clupea pallasii*), capelin (*Mallotus villosus*), rainbow trout (*Oncorhynchus mykiss*), and lake smelt (*Osmerus mordax*). The enclosure consisted of a large, round freshwater dump-and-fill pool, which was drained and cleaned weekly, and a central island that provided indoor access at all times and included a nest box area. The study group consisted of six males and six females, with ages ranging from 3 to 24 yr. Complete blood work and fecal pathogen screening were performed on all study penguins before the study, and a weight was obtained for drug calculations. Blood work included a complete blood count, plasma biochemistry panel, protein electrophoresis, and malaria (*Plasmodium* sp.) screening via blood smear. All procedures were reviewed and approved by the Maryland Zoo's Institutional Animal Care and Use Committee.

Barium contrast radiographs

All study penguins underwent an awake barium contrast study, and radiographic images were obtained until the entire gastrointestinal tract was empty and no contrast material could be identified radiographically. A Fujifilm FCR CX-Z (Fujifilm Medical Systems USA, Inc, Stamford, CT 06902, USA) stationary digital setup was used to obtain radiographs. Penguins were restrained using one of three methods to reduce stress. Some birds responded best to no restraint, one preferred a penguin restraint device fashioned from a cat litter box with an attached acrylic glass face, and the rest were most at ease in a solid-sided recycling bin. Animal staff familiar with the birds positioned the standing penguins and monitored them throughout the procedure.

Previous avian studies used a 30% weight-by-volume barium sulfate suspension administered at 10–30 ml/kg.[3,4,6,13,14] For this study, and to minimize regurgitation, each penguin was gavage-fed 12.5 ml/kg barium sulfate (Liquid E-Z-Paque 60% w/v, E-Z-EM Canada, Inc, Lake Success, NY 11042, USA), diluted to 30% w/v with tap water, using a 12-Fr red rubber catheter and a 60-ml syringe. Penguins were fasted overnight before this portion of the study. Before administration, the barium was warmed to 32.2°C using a warm-water bath to reduce its impact on normal peristalsis.[11] Each morning, two new study penguins were chosen, and their radiograph times staggered so that one started at 0900 hours and the other at 0945 hours. All study penguins underwent the same procedure: initial scanning two-view radiographs, barium administration, elevation of the penguin's head for 5 sec, and an immediate second set of radiographs for a timepoint of zero. Radiographs were repeated at 5, 10, 15, 20, 45, 90, 120, 240, and 300 min. If there was motion artifact or a positioning issue, another radiograph was repeated immediately. Both images were saved for evaluation, and the study was continued using the original timepoint schedule. Animal staff noted the time of first barium defecation, which marked transit time. If the barium had not completely passed through the GI tract by 300 min, the penguin was returned to the group, the fast was continued overnight, and radiographs were repeated at 0900, 1200, and 1500 hours the next day. The study ended at 1500 hours on day 2 even if the GI tract was not completely empty on radiographs.

Endoscopic evaluation

Each penguin was anesthetized approximately 14–21 days after the contrast study, and an endoscopic evaluation of the anterior GI tract was performed. Food was withheld overnight before the procedure. Penguins were manually restrained and induced using a face mask and 5% isoflurane (IsoFlo, Zoetis Inc, Kalamazoo, MI 49007, USA). Once the penguin was relaxed, it was intubated and maintained on gas anesthesia. The area around the endotracheal tube and glottis was packed with gauze (secured to a hemostat) to prevent aspiration in the event of reflux, and the penguin was positioned in sternal recumbency with its head slightly elevated. Penguins were monitored using continuous Doppler readings from the ulnar artery or heart (Ultrasonic Doppler Flow Detector, Parks Medical Electronics, Inc, Aloha, OR 97078, USA), pulse oximetry (SurgiVet, Smiths Medical ASD, Inc, St. Paul, MN 55112, USA), end tidal CO_2 (SurgiVet), heart rate auscultation, and visual observation of respirations with intermittent positive pressure ventilation performed when indicated. Anesthetic monitoring parameters were recorded every 5 min from induction to recovery and standing.

An Olympus CF 60-in. flexible endoscope (CF P20L scope and EVIS CLV-U20 light source, Olympus, Lake Success, NY 11042, USA) was slowly introduced, and a thorough visual examination with photographs (Veterinary Video Camera III, Karl Storz Veterinary Endoscopy America Inc, Goleta, CA 93117, USA) was performed extending from the oropharynx to the pyloric sphincter of the ventriculus. Koilin distribution, subjective thickness, and color were noted. Gastric fluid was collected using a 5-Fr red rubber tube and a 30-ml syringe. The sample pH was acquired immediately (3VDT4 Test Kit, Micro Essential, Brooklyn, NY 11210, USA), and slides were made within 15–30 minutes of sampling for biodiversity evaluation using a Gram stain. Refrigerated ventricular fluid was sent to Idexx Laboratories (Memphis, TN 38141, USA) for aerobic, anaerobic, and fungal culture with identification. Three representative biopsy samples from the ventriculus, proventriculus, and esophagus (for a total of nine samples) were obtained: two samples from each region were placed in 10% buffered formalin for histopathology and koilin thickness determination, and the third was placed in 2.5% buffered glutaraldehyde for future study use.

All penguins were standing within 5 min and, once recovered, were administered 10 ml kaolin pectin (Kaolin Pectin 4, VetOne, Boise, ID 83705, USA) as a gastroprotectant using a red rubber tube and syringe.

RESULTS

Barium radiographs

Radiographic anatomy: Radiographs were evaluated by a single veterinarian (Fig. 1). The coelomic cavity was radiographically divided into four regions (Fig. 2). The esophagus extends from the oropharynx, wraps along the left side of the neck, and extends to the mid-coelomic cavity (regions 1, 2, and 3). It is identifiable by its longitudinal folds, highlighted best by barium residue clinging to the esophageal lumen. In all penguins, the barium had exited the esophagus within 15–30 minutes. In most penguins, the proventriculus appeared as the last identifiable dilation between the esophagus and the ventriculus. Additional dilations were seen intermittently in the esophagus, but the lumen returned to a consistent size, 10–15 mm in diameter, once the barium passed through. The proventriculus lacks distinct longitudinal folds and extends from the lower middle to the caudal fourth of the coelomic cavity, still to the left of midline (regions 3 and 4). The ventriculus appearance varied based on the phase of muscle contractions; the most common presentations were a bilobed or rounded appearance. In all penguins, it was found in the caudal fourth of the coelomic cavity (region 4). Radiographically, the intestines sit caudal and to the right of the ventriculus (regions 2, 3, and 4). When both are filled with barium, the diameter of the small intestine (mean, 2.4 ± 0.51 mm SD) is approximately half the diameter of the large intestine (mean, 6.2 ± 0.94 mm SD).

Gastrointestinal transit time: Time from barium ingestion to barium defecation ranged from 17 to 70 min, with a mean of 43.58 ± 28.44 min (SD). Mean clearance time was calculated at 17.6 ± 11.57 hr (SD); however, the exact time was difficult to determine as some penguins evacuated it overnight. One penguin retained barium in the ventriculus for more than 36 hr. This was thought to be caused by excessive adhesion of the barium to the GI mucosa because barium was not present in the intestines.

Endoscopic evaluation

Anesthetic induction time ranged from 2 to 4 min, and mean procedure length was 52.6 ± 11 min (SD). During endoscopy, koilin was observed in all but one penguin, and no lesions or abnormalities were noted visually. In most penguins, the koilin appeared patchy or uneven; therefore, the three biopsy samples obtained were subjectively representative of what the researchers saw. Also, researchers noted that reflux between the small intestine, proventriculus, and ventriculus occurred in all penguins during endoscopic evaluation. Gastric fluid pH averaged 2.75 ± 0.5 (SD). No parasitic organisms were seen on light microscopy of gastric fluid, and the fluid contained a mixed bacterial population. Most gastric fluid culture samples had no isolated aerobic, anaerobic, or fungal growth, although one had a *Clostridium* sp. identified and three had *Paecilomyces* sp. growth.

Esophageal, proventricular, and ventricular samples submitted for histopathology were all within normal appearance. Koilin thickness was determined by measuring from the distal tip of the gastric pits to the distal edge of the koilin layer (Fig. 3). Three measurements were obtained, and the lengths averaged for each individual animal. Thickness was not determined for three penguins as significant processing artifact (characterized by koilin fragmentation greater than 0.1 mm or koilin compression) was appreciated. Mean koilin thick-

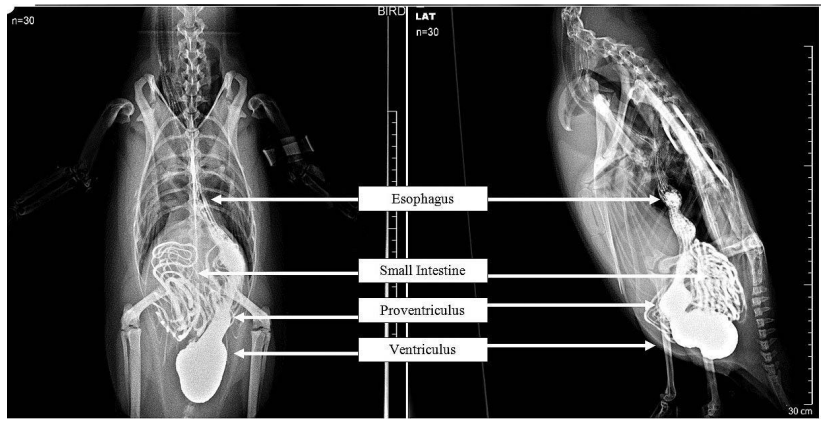

Figure 1. Lateral and ventrodorsal radiographs with identified GI components. Overview of the gastrointestinal tract of an African Black-footed penguin (*S. demersus*). Barium contrast is used to highlight prominent features of each portion of the GI tract.

ness was 0.82 ± 0.22 mm (SD). There was no association between endoscopic koilin appearance and koilin thickness observed on histopathology (Table 1). All samples exhibited mucosal cellular infiltrates ranging from 2 to 50 per 40× field, consisting of primarily lymphocytes along with low numbers of plasma cells (Fig. 4).

DISCUSSION

In carnivorous and piscivorous birds lacking crops, food passes from the oropharynx into the esophagus, which is grossly characterized by longitudinal lumenal folds that allow the esophagus to expand during swallowing. Microscopically, the esophagus is lined by an incomplete stratified squamous epithelium with underlying mucous glands, consistent with other avian species.[8] In this study, barium contrast passed into the proventriculus within 15–30 min. Barium clinging to the wall of the esophagus highlighted the longitudinal folds, making this anatomical region easily identifiable as extending from the oropharynx, down the left side of the neck, and ending mid-coelomic cavity (Fig. 5).

From the esophagus, food enters the proventriculus, in which uniformly distributed macroscopic papillae lead to gastric glands containing oxynticopeptic cells that produce hydrochloric acid and pepsin to break down food into more digestible metabolic products.[8] In most avian species, the proventriculus has no other grossly distinguishable features; however, in piscivorous

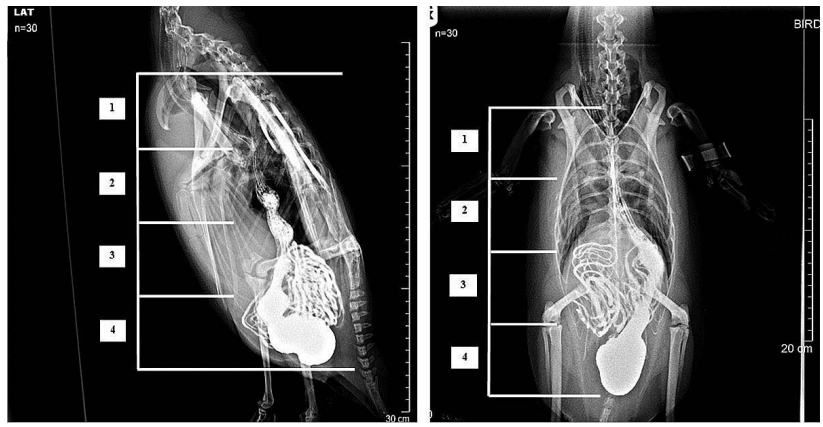

Figure 2. Lateral and ventrodorsal radiographs with referenced regions 1–4. The gastrointestinal tract was divided into four equal portions using the top of the shoulder and the cranial articulating surface of the seventh caudal vertebrae as measuring points. From there, researchers determined where each GI component (esophagus, proventriculus, ventriculus, and small intestines) routinely occurred.

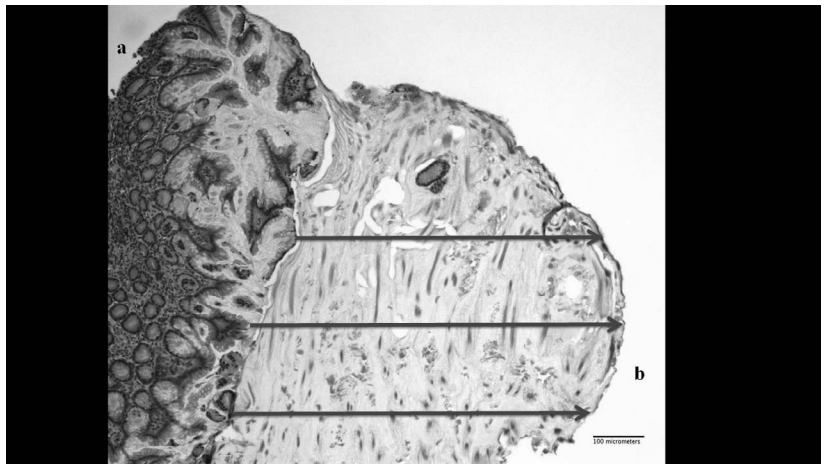

Figure 3. Ventriculus. Histopathology of a koilin biopsy and determination of koilin length. Ventriculus. Koilin was measured from distal tip of the gastric pits (a) to the distal edge of the koilin layer (b). Three measurements were obtained, and the lengths were averaged (hematoxylin and eosin [H&E]).

and carnivorous birds, longitudinal folds may be seen.[8] These allow the proventriculus to become more sac-like and stretch to accommodate the incoming meal. However, these folds can complicate identification of the proventriculus in contrast films. In this study, the proventriculus was identifiable as the last dilation before the ventriculus and longitudinal folds were not readily appreciated on radiographs or endoscopically. This may be because of a decrease in the number or size of the folds compared with other avian species. Endoscopically, the presence of gastric papillae was the most distinguishing feature of the proventriculus.

Between the proventriculus and ventriculus is a small intermediate zone with microscopic structures similar to both major stomach compartments.[8] In this study, it was demarcated by a slight constriction, appreciated both radiographically and during endoscopy.

Food then moves into the ventriculus, where the combination of chemical and mechanical forces further break it down. Consistent with other piscivorous species, the ventriculus in penguins is round, soft, and distensible. The major and minor ventricular muscles are significantly less hypertrophied than in other nonpiscivore species.[8] These muscles are asymmetri-

Table 1. Comparison of gross koilin appearance vs thickness determined during histopathology.

Penguin no.	Visible koilin description	Thickness (mm)
1	Koilin thin and patchy	ND[a]
2	No visible koilin	ND
3	Koilin thin and patchy	0.71
4	Sparse koilin	0.78
5	Diffusely thick koilin	1.21
6	Moderately patchy koilin	0.71
7	Diffusely thick koilin	0.73
8	Diffusely thick koilin	0.74
9	Sparse koilin	0.78
10	Koilin thin and patchy	1.19
11	Diffusely thick koilin	0.61
12	Diffusely thick koilin	ND

[a] ND: Not determined due to significant processing artifact.

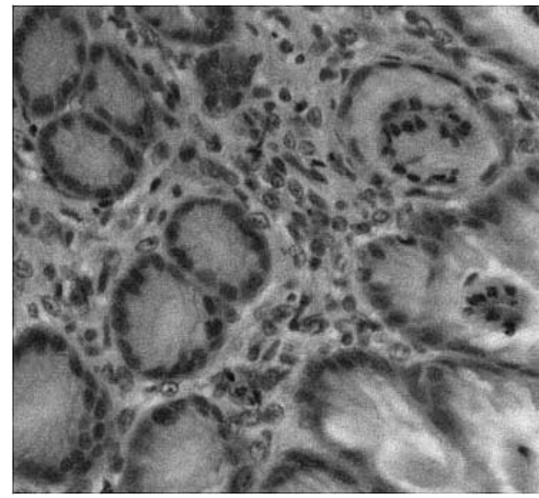

Figure 4. Ventriculus. Inflammatory cells within the lamina propria ventriculus. Ventriculus. Small numbers of mononuclear inflammatory infiltrates are present within the lamina propria of the ventriculus (H&E).

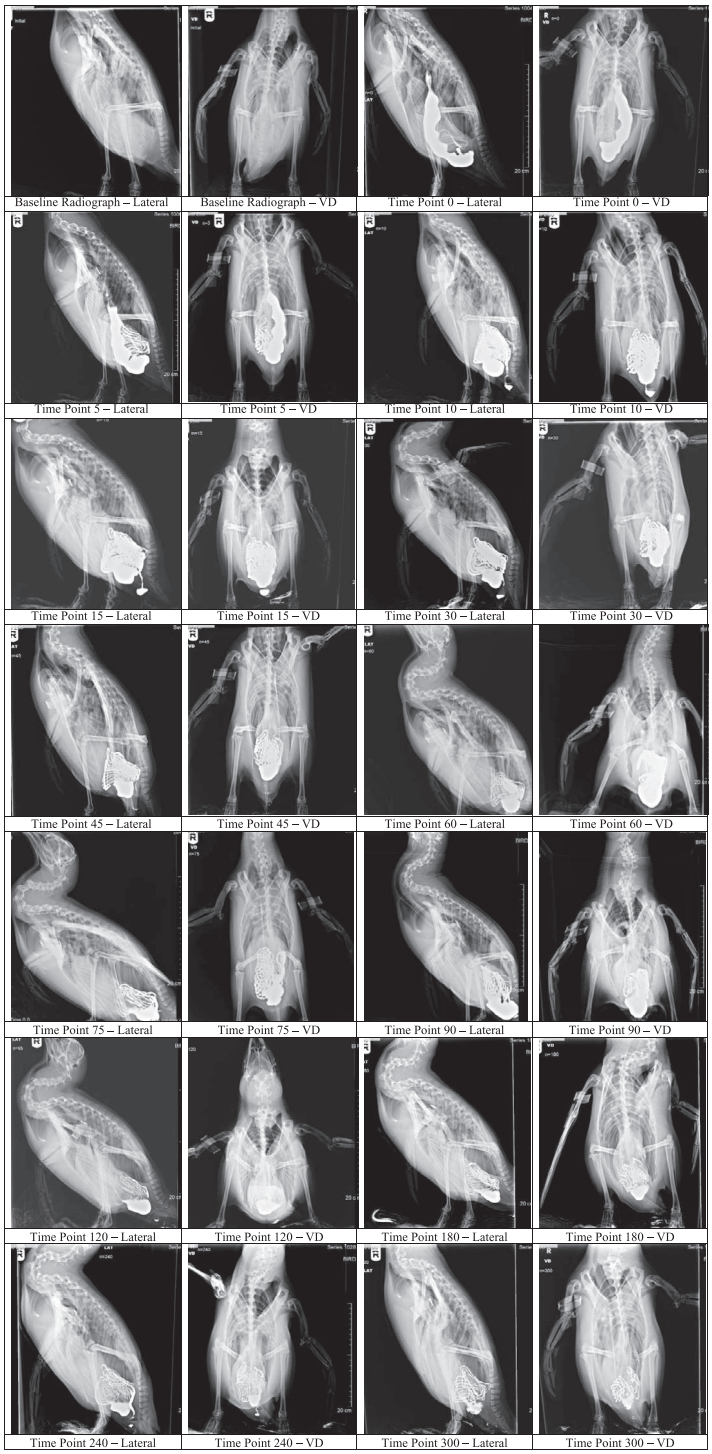

Figure 5. Radiographic series to illustrate barium movement through the GI tract. Timepoints from prebarium administration to 300 hr to show barium movement through the GI tract. Individual penguins varied; however, this is a representative series.

cally arranged to allow for simultaneous rotational and crushing movements. To protect the mucosal layer, the ventriculus is lined by a tough, green polysaccharide–protein–phospholipid complex called koilin.[1] The koilin layer is thickest over the cranioventral and caudodorsal thick ventricular muscles in galliformes, which exhibit the most mechanical force during digestion. However, in some piscivorous birds, the muscles are significantly less developed.[8] Koilin structure resembles that of reinforced concrete: vertical rodlets of hard koilin are interspersed within a horizontal matrix of softer koilin and surface epithelium.[1] The soft horizontal koilin is hardened by the influx of hydrochloric acid from the proventriculus. Koilin's yellow-green color is from bile pigment reflux from the small intestine.[10] Because of the abrasive nature of the ventriculus, the luminal surface of the koilin is naturally worn away with each successive meal. Additional koilin is produced by glands in the underlying gastric mucosa.

In this study, endoscopic gross characterization of koilin did not correlate with measurements taken during histopathology. This could be partially because of variable orientation of the tissue within the paraffin blocks as opposed to an actual presence or lack of koilin. It is worth noting that all penguins had some degree of lymphoplasmacytic cellular infiltrate within the mucosal layer without histologic evidence of any underlying infectious etiologies. All penguins had white blood cell counts within normal parameters, and none were exhibiting symptoms of gastrointestinal distress. At this time, the significance and etiology of these infiltrates is unknown. The authors have been unable to find any information regarding levels of cellular infiltrates in other avian species; however, small numbers of lymphoplasmacytic cellular infiltrates have been reported in a number of mammal species without a concurrent identifiable disease process. Microarchitectural features of inflammation, such as fibrosis, necrosis, degeneration, atrophy, and/or glandular dilation or distortion were not observed in this study, and the lymphocytes and plasma cells noted could represent normal GI-associated lymphoid tissue rather than true inflammatory infiltrates. *Paecilomyces* isolated from the GI fluid is a commonly occurring fungal organism isolated from food, soil, air, and wood. It has been associated with opportunistic mycoses in immunocompromised human patients, but its significance has not been determined in nonhuman animals.[7] Bacteria consistent with a clostridial organism and *Paecilomyces* were not found on histopathology of any ventriculus biopsies nor in the direct fecal microscopic examinations, so ingestion and a transient presence was suspected.

Mean fasted gastric pH was low (2.75), consistent with other carnivorous species, and bacterial assessment of the fluid was unremarkable. Radiographic studies in turkeys show a complex cycling of ventricular contractions, in which food is alternately propelled orally into the proventriculus and aborally into the small intestine.[8] This retropulsion was suspected during radiographs in several penguins in this study because of variable filling of the ventriculus from one timepoint to the next and supported during endoscopy when reflux between the two compartments was observed in all penguins by researchers.

Transit time (time from barium ingestion to barium defecation) varied with a range of 17–70 min. Time did not subjectively appear to be associated with stress level and activity or objectively with age and sex. It was also noted that GI clearance time (time from ingestion to no barium remaining in the GI tract) varied between individual penguins (mean, 17.6 hr; range, 5–36 hr). Similar variability has been reported in other avian studies and may be caused by differences in ventricular emptying.[4]

While this study provides new and useful information, there are several limitations and avenues for further investigation. Barium contrast studies provide brief glimpses of anatomy at specific timepoints: what happens between the timepoints is unknown. In addition, radiographs show contrast studies in shades of gray; it is impossible to determine fluid movement unless the barium moves as a bulk mass. To overcome these limitations, ideally fluoroscopy would be used to have a constant visualization of barium movement; however, this is not practical in most zoo settings. Most research using fluoroscopy during contrast studies in awake birds use either raptors or psittacines, which use perching.[2,13] Perching limits the amount of rotational movement a bird has, and therefore quality imaging can be performed with an awake bird. Most of the penguins in this study required extensive manipulation by keepers to keep them oriented correctly or even standing upright. Furthermore, this study was performed with liquid barium after a typical overnight fast period, allowing the measurement of essentially a fasting transit time. Penguins consume solid whole food items; therefore, barium beads, either within food items or alone, may aid in measuring transit time and GI emptying

time when the penguin has a meal. Finally, the diet provided for penguins varies slightly between institutions. The number of penguins was selected to achieve an adequate representation of the 55 birds within the colony; however, the data extracted may not represent *Spheniscus* penguins as a whole.

The results of this study provide a comparative baseline to use during diagnostic workups and help guide treatment decisions.

Acknowledgments: The authors thank the penguin keepers at the Maryland Zoo, as well as registered veterinary technicians Jennifer Sohl, Kaitlin Studer, and Heather Brittingham for their assistance, time, and expertise, which made this study possible.

LITERATURE CITED

1. Akester AR. Structure of the glandular layer and koilin membrane in the gizzard of the adult domestic fowl (*Gallus gallus domesticus*). J Anat 1986;147(4):1–25.

2. Beaufrere H, Nevarez J, Taylor WM, Jankowski G, Rademacher N, Gaschen L, Pariaut R, Tully TN. Fluoroscopic study of the normal gastrointestinal motility and measurements in the Hispaniolan Amazon Parrot (*Amazona ventralis*). Vet Radiol Ultrasound 2010;51(4):441–446.

3. Bloch RA, Cronin K, Hoover JP, Pechman RD, Payton ME. Evaluation of gastrointestinal tract transit times using barium-impregnated polyethylene spheres and barium sulfate suspension in a domestic pigeon (*Columba livia*) model. J Avian Med Surg 2010;24(1):1–8.

4. Doss GA, Williams JM, Mans C. Contrast fluoroscopic evaluation of gastrointestinal transit times with and without the use of falconry hoods in red-tailed hawks (*Buteo jamaicensis*). JAVMA 2017;251(9):1064–1069.

5. Fowler GS, Fowler ME. Order Sphenisciformes (penguins). In: Fowler ME, Cubas ZS (eds.). Biology, medicine, and surgery of South American wild animals. Ames (IA): Iowa State University Press; 2001. p. 53–65.

6. Gelis S. Evaluation and treating the gastrointestinal system. In: Harrison GJ, Lightfoot TL (eds.). Clinical avian medicine, Volume 1. Palm Beach (FL): Spix Publishing; 2006. p. 429–432.

7. Houbraken J, Verweij PE. Identification of *Paecilomyces variotii* in clinical samples and settings. J Clin Microbiol 2010;48(8):2754–2760.

8. King AS, McLelland J. Birds—their structure and function, 2nd Ed. Eastbourne (UK): Bailliere Tindall; 1984. p. 84–109.

9. Langlois I. The anatomy, physiology, and diseases of the avian proventriculus and ventriculus. Vet Clin Exot Anim 2003;6(1):85–111.

10. Macwhirter P. Basic anatomy, physiology and nutrition. In: Tully TN, Dorrestein GM, Jones AK (eds.). Handbook of avian medicine. 2nd ed. New York (NY): Saunders; 2009. p. 25–55.

11. Silverman S, Tell LA. Radiology equipment and positioning techniques. In: Radiology of birds: an atlas of normal anatomy and physiology. St. Louis (MO): Elsevier; 2010. p. 1–15.

12. Silverman S, Tell LA. Mallard duck (*Anas platyrhynchos*). In: Radiology of birds: an atlas of normal anatomy and physiology. St. Louis (MO): Elsevier; 2010. p. 204–226.

13. Vink-Nooteboom M, Lumeij JT, Wolvekamp WTC. Radiography and image-intensified fluoroscopy of barium passage through the gastrointestinal tract in six healthy Amazon Parrots (*Amazona aestiva*). Vet Radiol Ultrasound 2003;44(1):43–48.

14. Wagner WM, Kirberger RM. Radiographic gastrointestinal contrast study in the ostrich (*Struthio camelus*). Vet Radiol Ultrasound 2003;44(5):546–552.

15. Wallace RS. Sphenisciformes (penguins). In: Miller ER, Fowler ME (eds.). Zoo and wild animal medicine, Volume 8. St. Louis (MO): Elsevier; 2015. p. 82–88.

Accepted for publication 21 February 2020