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Case Report

Anemia and Hyperplastic Gastritis in a Giant Anteater (*Myrmecophaga tridactyla*) due to *Physaloptera magnipapilla* Parasitism

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Abstract

A free living female adult of *Myrmecophaga tridactyla* was captured in an area of deforestation in Salta Province of northwest Argentina, for subsequent relocation in Iberá Park. Three days after entering the quarantine it died with clinical signs of anorexia and ascites. It presented severe hypochromic microcytic anemia, hypoproteinemia and increase of CPK, AST and LDH on blood analysis. Grossly, there were ascites, hydrothorax, pulmonary congestion and severe catarrhal hyperplasic gastritis. The gastric mucosa was diffusely hyperemic with two clusters of numerous *Physaloptera magnipapilla* attached in the fundic region. Fundic mucosa was irregular with convoluted surface and covered with abundant brown-yellow catarrhal exudates. Microscopically, the gastric mucosa had well described punctate areas (pit-like structures) at points of *P. magnipapilla* attachment. Additionally, there were mucous hyperplasia of gastric glands and focal fibrosis with scarce infiltration of plasma cells, lymphocytes, multinucleated giant cells and eosinophils in the underlying lamina propria. This report describes a case of anemia and hyperplastic gastritis due to *Physaloptera magnipapilla* parasitism in a free-living *Myrmecophaga tridactyla*.

Key words: wildlife diseases, *Physaloptera magnipapilla*, hypochromic microcytic anemia, hyperplastic catarrhal gastritis.

Introduction

Physaloptera (Nematoda: *Physalopteridae*) are parasites found in the stomach of amphibians, reptiles, birds, and a wide range of mammals throughout the world (12). These parasites are hematophagous and, after attaching to the gastric mucosa, they can cause gastritis and anemia (3). The clinical signs are anorexia, vomiting, weight loss, weakness, dyspepsia and diarrhea with tarry feces (3, 6, 10). The life cycle of *Physaloptera* spp. is heteroxenous with carnivores (e.g. dogs, cats, lions, pumas, fox, linx), insectivorous (e.g. bats, reptiles, birds, hedgehogs) and omnivorous (e.g. badger) as definitive hosts; coleopterans (beetles, crickets, cockroaches) as intermediate hosts and amphibians and some lizards were considered as paratenic hosts (2).

There are scant reports of physalopteriasis in *Myrmecophaga tridactyla*. Ortlepp (1922) described in his catalog the morphological characteristics of the *Physaloptera papillotruncata* collected from stomach of a *Myrmecophaga tridactyla* and the morphology of *Physaloptera magnipapilla* collected from stomach of a *Myrmecophaga tetradactyla* originating from Brazil (12). Silva et al. (2014) found *Physaloptera magnipapilla* and *Physaloptera semilanceolata* in stomach of giant anteaters from Brazilian Pantanal wetlands, but they did not describe gastric lesions (17). In Argentina, Santa Cruz found in 1997 *Physaloptera magnipapilla* in stomach of a *Myrmecophaga tridactyla* (personal communication). However, there are no reports describing lesions associated with *Physaloptera* parasitism in a free-living *M. tridactyla.*

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Reporting the occurrence of parasitic diseases of *Myrmecophaga tridactyla* can be beneficial to veterinarians and biologists dedicated to the conservation of this species. Therefore, the present report documents a case of anemia and hyperplastic gastritis due to *Physaloptera magnipapilla* parasitism in a free-living *Myrmecophaga tridactyla*.

Case report

A reintroduction program of giant anteaters is being carried out in Iberá Park in Corrientes Province of northeastern Argentina. In this context, on October 5, 2008 a free-living adult female of *M. tridactyla* was captured in an area of deforestation in Salta Province, northwest Argentina, for subsequent relocation in Iberá Park. On October 7, this animal came into quarantine and the clinical examination revealed a bodyweight of 30 kg, anorexia and ascites. The blood test revealed hypochromic microcytic anemia (Table 1), hypoproteinemia (Table 2) and increase of CPK, AST and LDH (Table 3). No clinical-improvement was noticed after treatment with antiparasitic drugs (ivermectin), antibiotics (penicillin and streptomycin) and fluid therapy. The giant anteater died on October 10, 2008.

Necropsy findings were ascites, hydrothorax, pulmonary congestion and severe catarrhal hyperplastic gastritis. The gastric wall was thickened with gastric mucosa diffusely hyperemic. The fundic mucosa was irregular with convoluted surface and thickened gastric rugae, with abundant brown-yellow catarrhal exudates and two clusters of numerous adult nematodes attached to the mucosa (Figs. 1 and 2).

Samples of worms were fixed in 2% formalin and sent to the Department of Parasitology of the Faculty of Veterinary Science of the University National of La Plata (UNLP), Buenos Aires, Argentina, and to the Department of Zoology and Ecology of the Faculty of Veterinary Science of the UNNE. Six males and 10 females were cleared in lactophenol for examination. Specimens were placed on glass slides and examined using a light microscope. The diagnostic characters evaluated were the morphology of the cephalic papillae, pattern and number of male caudal papillae, shape and size of spicules, uterus type, and vulvar position. The specimens were identified as *Physaloptera magnipapilla* based on Anderson et al. (2009) (1).

Samples of muscle, stomach, intestines, myocardium, liver, lung, lymph nodes, spleen, kidney and whole brain were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) for histopathological analysis.

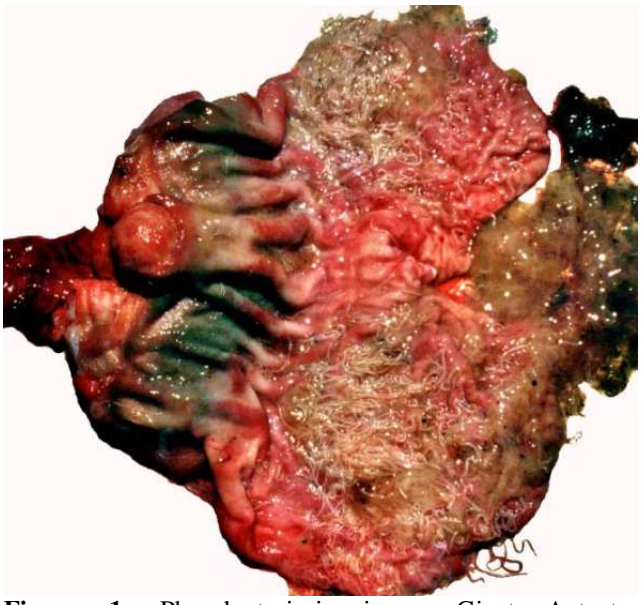


Figure 1. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Hyperplastic catarrhal gastritis. Two clusters of numerous *Physaloptera magnipapilla* attached to fundic mucosa.

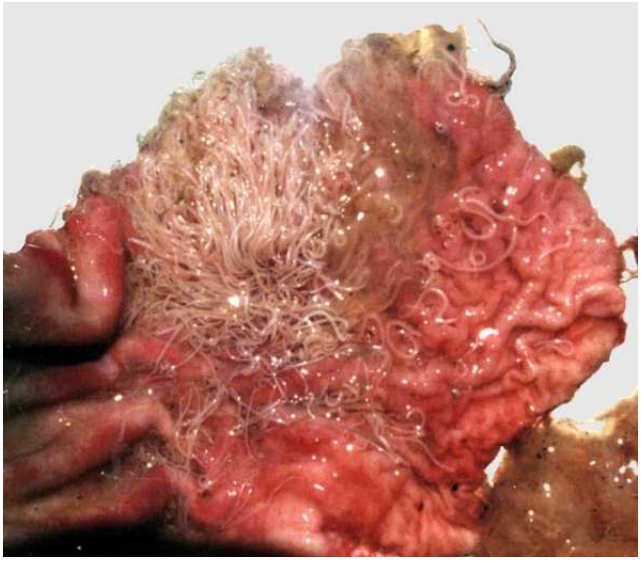


Figure 2. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Hyperplastic catarrhal gastritis. Fundic mucosa with gastric rugae thickened, covered with brown-yellow catarrhal exudate and numerous *Physaloptera magnipapilla* attached.

The microscopic findings were acute purulent bronchopneumonia, multifocal monophasic segmental coagulation necrosis of the muscle myofibers and hyperplastic gastritis. Gastric lesions were located at points of *Physaloptera magnipapilla* attachment, where there were well described punctate areas (pit-like structures) lined by ectatic and hyperplastic glands. The hyperplasia was composed of columnar mucous cells proliferation forming papilliform projections and irregular glandular

Table 1. Hematologic values in a giant anteater (*Myrmecophaga tridactyla*) parasitized by *Physaloptera magnipapilla* versus bibliographical reference values.

Variable	This case	Reference					
		(17)ℓ	(12)ℓ	(8)*	(16)*	(7)*	(4)*
Hematocrit (%)	25	33.93±0.75	35.54±7.98	36.1±6.6	37.7±1.06	35.6	34.9±5.45
RBCs (T/l)	2.10	2.19±0.07	4.5±0.95	2.65±0.5	2.36±0.14	2.36	2.38±0.38
Hb (g/dl)	8.79	10.91±0.47	-	14.2±2.7	11.8±0.52	13.1	13.8±1.69
MCV (fl)	119	153.86±20.32	78.73±3.2	136±3	165.12±8.71	150.3	147.9±7.22
MCHb (pg)	42	51.48±5.96	-	53±5	51.07±2.27	54.5	56.6±3.2
MCHbC (%)	35	32.79±2.45	-	39±3	31.26±0.96	36.1	38.4±1.25
Leukocytes (G/l)	8.25	9.73±0.76	10.44±3.39	10.82±2.81	11.87±2.88	8.42	8.55±2.69
Segs (%)	72	82.59	39.91±6.17	57±11	72.62±3.67	65.87	59.6±9.9
Bands (%)	10	0.45	-	-	-	0.48	0
Lymphocytes (%)	8	9	53.18±5.49	25±8	18.77±3.17	20.76	35±9.12
Monocytes (%)	2	4.46	2±1.55	1.4±0.7	1.69±0.04	3.45	3.2±1.74
Eosinophils (%)	8	3.32	4.90±2.2	15.9±9	6.92±1.67	8.09	2.5±2.3
Basophils (%)	0	0.16	0	0 to 1	0	1.35	0

RBCs: erythrocytes, Hb: hemoglobin, MCV: mean corpuscular volume, MCHb: mean corpuscular hemoglobin, MCHbC: mean corpuscular hemoglobin concentration. Segs: segmented neutrophils. Bands: band neutrophils. *: Reference values of captive giant anteater. ℓ: Reference values of free-living giant anteater.

Table 2. Serum proteinogram values in a giant anteater (*Myrmecophaga tridactyla*) parasitized by *Physaloptera magnipapilla* versus bibliographical reference values.

Variable	This case	References			
		(4)*	(8)*	(9)*	(17)ℓ
Total proteins (g/dl)	4.22	7.66±1.085	6.94±0.8	5.25±0.04	6.87±0.14
Albumins (g/dl)	1.41	3.62±0.508	3.36±0.21	2.03±0.06	1.53±0.03
α 1 globulins (g/dl)	0.40	-	-	0.66±0.10	-
α 2 globulins (g/dl)	0.88	-	-	0.64±0.22	-
β globulins (g/dl)	0.72	-	-	1.39±0.17	-
ɣ globulins (g/dl)	0.80	-	-	0.54±0.16	-
albumins/globulins ratio	0.50	0.95±0.210	0.94±0.1	0.63±0.04	-

*: Reference values of captive giant anteater. ℓ: Reference values of free-living giant anteater.

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Table 3. Serum enzymes values in a giant anteater (*Myrmecophaga tridactyla*) parasitized by *Physaloptera magnipapilla* versus bibliographical reference value.

Variable	This case	Reference: (9)*
CPK (Ui/l)	165	40±13.44
AST (Ui/l)	23	7±0.71
ALT (Ui/l)	10	4±2.83
LDH (Ui/l)	135	138±11.31

CPK: creatinephosphokinase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase. *: Reference values of captive giant anteater.

lumen (Fig. 3). The surrounding fundic glands presented with mucous metaplasia and loss of parietal and zymogen cells (Figs. 4 and 5). There was focal fibrosis and mild infiltration of plasma cells, lymphocytes, multinucleated giant cells and eosinophils in the lamina propria beneath the point of parasite attachment.

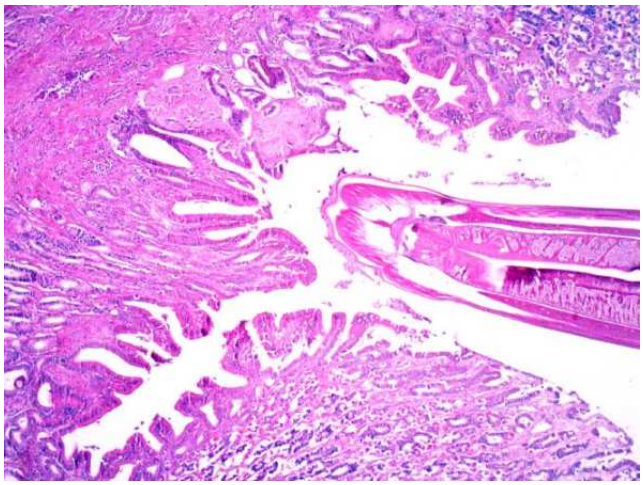


Figure 3. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Hyperplastic catarrhal gastritis. *Physaloptera magnipapilla* caused thinning of the mucosa on site of attachment with adjacent mucous cells hyperplasia. Focal fibrosis and focal mononuclear cells and eosinophils infiltration was present in the underlying lamina propria (HE, obj. 5x).

In cross section, the nematodes vary from 317 to 483 µm in diameter. They had a 5 to 20 µm thick cuticle, which varies from smooth to striate. Beneath the cuticle lies a thin layer (2 to 10 µm thick) of hypodermis which was projected in four symmetrically arranged hypodermal chords. The dorsal and ventral cords were very small. The lateral hypodermal chords were very large, narrow at the base and project into body cavity. A layer of polymyarian coelomyarian musculature was beneath the hypodermis. In the body cavity were observed the esophagus with triradiated lumen and the intestinal tract composed of a simple layer of columnar cells with a single basal nucleus and with a microvillar layer bordering the intestinal lumen (Fig. 6). The female's nematodes had a distended uterus containing numerous ovals, 45 by 20 µm, thick-shell embryonated eggs (Fig. 7). The eggs in the gastric catarrhal exudate were oval, 35 to 43 µm length by 21 to 26 µm width, with smooth thick shell (2,5 to 4 µm) containing larvae (Fig. 8).

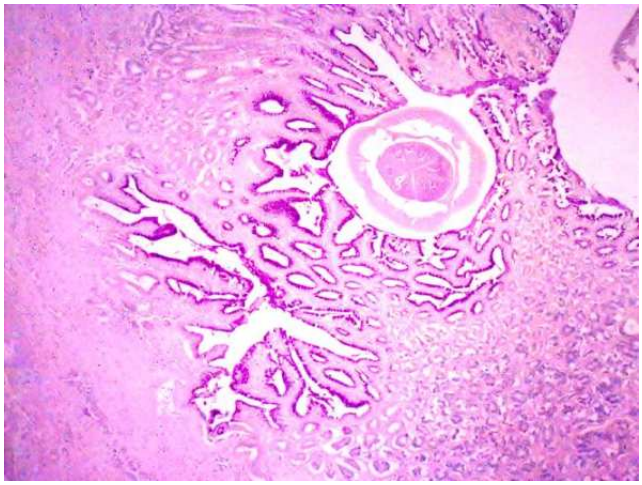


Figure 4. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Hyperplastic catarrhal gastritis. Mucous cells proliferation in the mucosa surrounding to *Physaloptera magnipapilla* (PAS, obj. 5x).

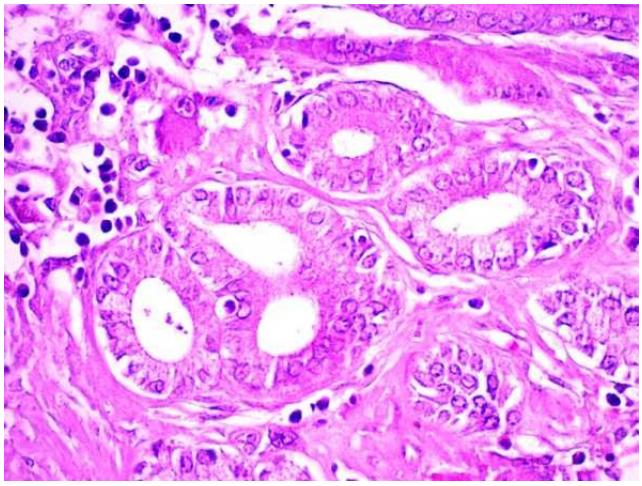


Figure 5. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Hyperplastic catarrhal gastritis. Fundic glands with loss of parietal and zymogen cells and displaced by hyperplastic mucous cells. Fibrosis and mild infiltration of plasma cells and lymphocytes were present in the lamina propria (HE, obj. 40x).

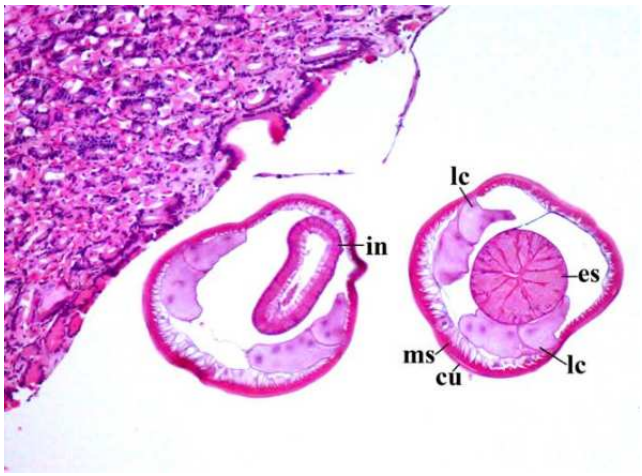


Figure 6. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Cross section through a *Physaloptera magnipapilla* showing the cuticle (cu), stalked lateral chords (lc), polymyarian coelomyarian muscle layer (ms), esophagus (es) and intestine (in) (HE, obj. 10x).

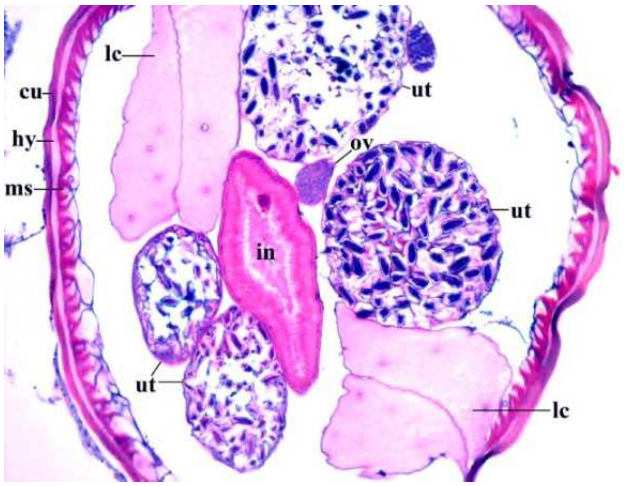


Figure 7. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Cross section through a female of *Physaloptera magnipapilla* showing cuticle (cu), hypodermis (hy), lateral chords (lc), somatic muscle layer (ms), intestine (in), uterus (ut) and ovary (ov) (HE, obj. 10x).

Discussion

Physaloptera magnipapilla as well as *Physaloptera semilanceolata* (17) and *Physaloptera papillotruncata* (12) has been previously diagnosed in stomachs of giant anteaters, but gastric injuries or clinicopathological findings were not reported. Other parasites found in stomach of the *Myrmecophaga tridactyla* were *Macielia chagasi*, *Macielia falsa*, *Macielia macieli*, *Graphidiops costalimai* (17), *Graphidiops assimilis*, *Graphidiops dissimilis* (17, 18) and *Paragraphidium pseudosexradiatum* (17).

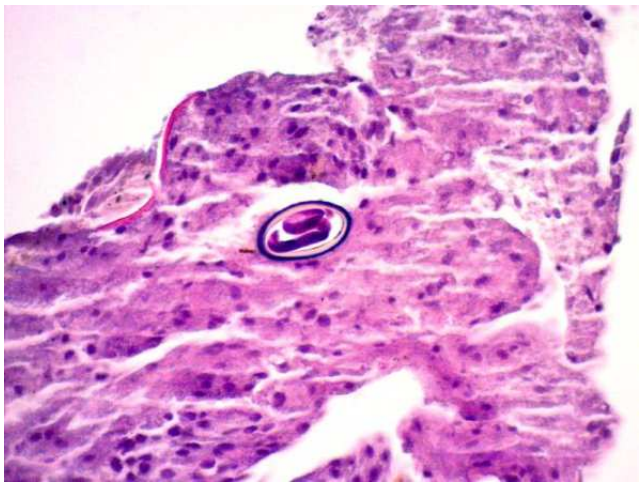


Figure 8. Physalopteriasis in a Giant Anteater (*Myrmecophaga tridactyla*). Cross section through an egg of *Physaloptera magnipapilla* (HE, obj. 40x).

The stomach lesion caused by *Physaloptera magnipapilla* observed in this case was hyperplastic (chronic) catarrhal gastritis characterized by pit-like structures and proliferation of mucous cells. These lesions were similar to those reported in the stomach of *Puma yagouaroundi* (13) and cats (10) with *Physaloptera praeputialis* and in stomach of hedgehogs with *Physaloptera clausa* (6). The gross changes reported in the gastric mucosa of cats with *Physaloptera praeputialis* were thickening, congestion, edema, tiny erosions and 0.8 to 5.5 cm long worms attached to the mucosa. Microscopic findings were congestion, edema, focal necrosis, and inflammatory infiltration of lymphocytes, macrophages and eosinophils. Proliferation of fibrous connective tissue among glands, hyperplasia of the mucosal glands and hyperplasia of the muscular layer were also described (10). The macroscopic lesions in hedgehogs infected by *Physaloptera clausa* included thickening, congestion and edema of the stomach wall with worms attached to the mucosa. The histopathological findings were gastritis with hyperemia, focal epithelial erosion, mild hemorrhage, diffuse infiltration of eosinophils in the lamina propria, and epithelial hyperplasia of the mucosal glands (6). Metaplasia and hyperplasia were not reported in the gastric mucosa of other physalopteriasis cases. *Physaloptera brevivaginata* caused ulcerative gastritis in insectivorous bats. In some bats complete perforations of the gastric wall were observed (2). Multifocal U-shaped erosions with mixed inflammatory response were seen at points where *Physaloptera retusa* were attached to the gastric mucosa of the sagebrush lizards (5).

The blood test from current case showed severe hypochromic microcytic anemia. The hematocrit, RBCs, Hb, MCV, MCHb and MCHbC were low when compared to reference values of captive (4, 7, 8, 15) and free-living giant anteaters (11, 16). *Physaloptera* spp. is a well-known agent of gastritis and anemia in dog (3). In this case the hypochromic microcytic anemia was possibly due to the

severe gastritis by *Physaloptera magnipapilla,* although deficit of copper or iron cannot be excluded. The current case also presented severe hypoproteinemia with significant decreases in all fractions of the serum proteinogram and decrease in albumins/globulins ratio, according to the ranges reported previously (4, 8, 9, 16). The possible cause of the hypoproteinemia was due to severe parasitism, although nutritional factors (starvation or deficiencies) also could be influenced. The activity of CPK and AST was increased compared to our baseline values (9). This could be attributed to muscle necrosis found in this case. The causes of bronchopneumonia and muscle necrosis were not determined. Despite having high parasitic load in stomach, the percentage of eosinophils was similar to reference values of clinically normal captive giant anteaters (7, 15). However, compared to others reference values of captive (4) or free-living giant anteaters (11, 16) our result indicate eosinophilia.

Since the life cycle of the *Physaloptera* involves insects as intermediate hosts (2). In this case the infection by *Physaloptera magnipapilla* could have occurred due to the myrmecophagous alimentary habit of *Myrmecophaga tridactyla*, based on ants and termites (14).

In conclusion, *Physaloptera magnipapilla* can cause anemia and hyperplasic catarrhal gastritis in free-living *Myrmecophaga tridactyla*.

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