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Health Assessment of Raptors in Triage in Belo Horizonte, Mg, Brazil

■Author(s)

Andery D de A[1],
Ferreira Junior FC[1],
Araújo AV de[1],
Vilela DA da R[2],
Marques MVR[1],
Marin SY[1],
Horta RS[1],
Ortiz MC1,
Resende JS de[1],
Martins NR da S[1,*]

[1] Avian Diseases Laboratory, Setor de Doenças das Aves, Departamento de Medicina Veterinária Preventiva, Escola de Veterinária da UFMG, Av. Antônio Carlos 6.627 – Caixa Postal 567, CEP 30123-970, Belo Horizonte, MG, Brazil
[2] Instituto Brasileiro de Meio Ambiente e Recursos Naturais Renováveis, IBAMA, Avenida do Contorno, 8121, CEP 30.110-120, Belo Horizonte, MG, Brazil

■Mail Adress

* Corresponding author e-mail address
Nelson Rodrigo da Silva Martins
E-mail: nrsmart@gmail.com

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ABSTRACT

Falconiformes (n=82), Strigiformes (n=84) and Cathartiformes (n=14) at a triage center (CETAS-Belo Horizonte, IBAMA, Brazil) were examined between 2008 and 2010 . No bird was reactive at hemagglutination-inhibition (HI) for antibodies against *Mycoplasma gallisepticum* (Mg). Two *Caracara plancus* (2/68) had HI titers (16-32) against Newcastle disease virus. No *Chlamydophila psittaci* DNA was detected in the liver (PCR; n=95). Blood smears (Giemsa; n=89) and spleen fragments (PCR; n=82) were 13.5% and 8.5% positive, respectively, for *Haemoproteus* only. Necropsy of Cathartiformes (n=10), Falconiformes (n=42) and Strigiformes (n=57) showed that trauma injuries were the main cause (63.3%) of admission and death, being fractures (38.5%) of the thoracic limbs (57.1%) the most frequent. Nematode (12.8%), cestode (1.8%), trematode (0.9%), and acanthocephalan (2.7%) parasite infections were relevant. Mites (Acari) were the most frequent (17.4%) external parasites, particularly *Ornithonyssus sylviarum* in *Asio clamator* and *Amblyomma cajennense* in *Tyto alba*. Chewing lice (10.1%) and *Pseudolynchia* spp. (9.2%) were also found. *Histomonas* spp. (6.4%) was found in the ceca of *Bubo virginianus*, *Athene cunicularia*, *Tyto alba*, and *Asio clamator*, but not in Falconiformes or Cathartiformes. *Trichomonas* spp. (oral cavity, pharynx and upper esophagus; 9.1%) was detected in Falconiformes and Strigiformes, but not in Cathartiformes. *Trichomonas* spp. were found in *A. cunicularia*, *Asio clamator*, *Glaucidium brasilianum* and *Tyto alba* (Strigiformes), and in *Rupornis magnirostris*, *Milvago chimachima*, *Falco femoralis*, *Falco sparverius* and *Caracara plancus* (Falconiformes). Coccidia (9.1%) (Sarcocystis spp., 6.4%) and mycosis were observed in most *Tyto alba* (70%). The evaluated Orders may not pose risks for commercial poultry production. Habitat loss and urban adaptation may be increasingly affecting raptors.

INTRODUCTION

Raptors are carnivorous birds of predatory habits, and are essential for population regulation because they are at the end of the food chain. They are also considered potential disease amplifiers and pathogen carriers. The Order Strigiformes is represented mostly by nocturnal owls. Falconiformes includes diurnal birds of prey, such as eagles, hawks, and falcons. The single family of Cathartiformes (Cathartidae) includes vultures, which are scavengers and which classification as bird of prey varies among authors. Despite not presenting determined adaptations for hunting, for practical reasons, especially due to their proximity to poultry farms, vultures were included in this study.

Brazil is known for its outstanding biodiversity; however, the intensification of human activities, such as the expansion of cities and the increasing demands for farming, generate strong pressure on the

various biomes, leading to habitat loss, fragmentation and degradation (ICMBio, 2008). The main threats to the survival of populations of birds of prey are related to these human activities, as well as hunting, trafficking, superstition, persecution, and conflict with humans (ICMBio, 2008).

Conservation threats make it an urgent priority the determination of the occurrence, incidence and distribution of diseases in raptors. Few diseases of birds of prey kept in captivity are well documented, but little is reported on free-living raptors (Joppert, 2007). Serological studies show that free-living birds are exposed to pathogens through contact with poultry waste and runoff from farms, or by ingestion of contaminated carcasses (Höfle *et al.*, 2002), being first victims, then potential sources.

Public and private institutions are responsible for receiving and maintaining rescued wild birds, including triage centers for wild animals (CETAS) rescued from trafficking or illegal captivity. Birds of prey account for 1-2% of all birds received by CETAS Belo Horizonte/MG (CETAS/BH). In the present study, we analyzed raptors received at CETAS/BH between December 2008 and August 2010, with the objective of describing the general health of these birds and the main diseases that may threat the commercial poultry production.

MATERIALS AND METHODS

Birds

Birds of orders Falconiformes, Strigiformes and Cathartiformes submitted to CETAS-BH (IBAMA) (S19°55' W43°57'), were analyzed in the period between December 2008 and August 2010. In total, 180 raptors, including 82 Falconiformes, 84 Strigiformes, and 14 Cathartiformes (Table 1), were evaluated.

Necropsy

Birds that died or were euthanized because they were suffering or it was clinically indicated due to the severity their injuries, were submitted to necropsy, according to a previously described protocol (Matushima, 2007). All necropsied birds were inspected to identify their species, origin, and injuries, such as fractures.

One hundred and nine (n=109) birds were necropsied. Seventeen sick birds eventually died and

Table 1 – Raptor avian species studied, tests performed and results.

Species	Total (N)	Necropsy	Blood smear[1]	PCR/ spleen[2]	SAT/ Mg[3]	APMV-1/HI[4]	PCR/ *Cp*[5]
Falconiformes	82						
Buteo albicaudatus	3	1	0/2	0/1	0/1	0/2	0/1
Buteo brachyurus	2	2	1/2	0/2	0/2	0/2	0/2
Caracara plancus	26	11	1/16	0/11	0/12	2/13	0/11
Falco femoralis	3	1	0/2	0/0	0/2	0/1	0/1
Falco rufigularis	1	0	0/1	0/0	0/1	0/1	0/1
Falco sparverius	8	5	0/1	0/3	0/2	0/1	0/4
Heterospizias meridionalis	1	0	0/2	0/0	0/1	0/1	0/1
Leptodon cayanensis	2	2	0/0	0/1	0/2	0/1	0/1
Milvago chimachima	10	3	1/8	0/2	0/5	0/6	0/3
Rupornis magnirostris	26	17	1/12	0/13	0/5	0/7	0/14
Strigiformes	84						
Athene cunicularia	11	11	0/1	5/6	0/1	0/1	0/9
Asio clamator	23	13	6/13	2/9	0/11	0/11	0/12
Asio stygius	7	4	2/4	0/4	0/3	0/4	0/4
Bubo virginianus	2	2	0/1	0/1	0/1	0/1	0/1
Glaucidium brasilianum	6	6	0/0	0/4	0/6	0/6	0/5
Megascops choliba	6	6	0/0	0/4	0/6	0/6	0/4
Strix huhula	1	0	0/1	0/1	0/1	0/1	0/1
Strix virgata	1	0	0/1	0/1	0/1	0/1	0/1
Tyto alba	27	15	0/15	0/13	0/27	0/11	0/14
Cathartiformes	14						
Coragyps atratus	14	10	0/9	0/8	1/9[6]	0/9	0/9

Notes. [1]Peripheral blood smear (Giemsa) results for hemoparasites / total; [2]Spleen PCR for *Haemoproteus* spp. - *Plasmodium* spp. /tested; [3]Serum agglutination for *Mycoplasma gallisepticum* results/ tested; [4]Haemagglutination-inhibition for antibodies to Newcastle disease virus; [5]Liver PCR for *Chlamydophila psittaci;* [6]*Coragyps atratus* retested by HI (negative).

were submitted both to clinical examination and necropsy.

Organ (liver and spleen) fragments were frozen for PCR investigation or fixed in 10% formalin for histological analysis. Intestinal content and mucosa scraping were examined under light microscopy (100 and 400x).

Parasites

Ectoparasites and feathers were collected in alcohol 70ºC for microscopy. Endoparasites were fixed in 10% formalin for subsequent identification. *Sarcocystis* sp. oocysts were cleared by centrifugation (10min/3000x*g*) in saturated NaCl solution, counted and 1,000/50µL resuspended in sterile PBS and intraperitoneally inoculated into Balb/c mice. Feces or intestinal contents containing oocysts were placed in potassium dichromate 2.5% for sporulation. Oocysts were characterized by their internal structures and morphometrics by microscopy (400x).

Blood samples

Birds were submitted to physical restriction in order to collect blood samples (0.5 to 1% of body weight) by ulnar or brachial venipuncture, with sterile disposable syringes and needles. Collected blood was used to prepare blood smears and for serum separation.

Serology

Fresh sera (not frozen) were individually tested by serum agglutination test (SAT) for antibodies against *Mycoplasma gallisepticum* (Mg) using a commercial kit (MYCO-TEST GALLI-Biovet®). Tests were performed according to PNSA (Brazil, 2001) and the manufacturer's recommendations, with equal parts of fresh serum and antigen (50µL). Known Mg positive and negative sera were used as controls.

Hemagglutination-inhibition (HI) test for Newcastle disease virus (APMV-1) antibodies was performed according to PNSA (Brazil, 2002), using La Sota strain of APMV-1 (New Vacin La Sota-Biovet®) as antigen (inactivated by β-propiolactone), fresh adult SPF chicken red blood cells (RBC) suspension (1%), and microtiter 96-well "u-bottomed" plates. Reference APMV-1 positive and negative sera were included in all tests, and retrotitration of the antigen to confirm 4 UHA. Two replicates were used for all tests, including controls. Serum titer was the reciprocal of the highest dilution showing complete RBC deposition, similar to normal RBC control wells.

DNA Extraction and Quantification

Silicon dioxide adsorption DNA extraction was previously described (Boom *et al.*, 1990) and modified by Caxito *et al.* (2006), by protein denaturation using sodium iodide. Briefly, about 200mg (or 200µL) of previously homogenized (liver orand spleen fragments) were added with three volumes (600µL) of sodium iodide (NaI, Labsynth, Brazil) and 50µl of a suspension of silica (Sigma-Aldrich Co., USA). The pellet was washed three times with a buffer (50% ethanol, 50mM Tris-HCl pH8.0, 10mM EDTA pH8.0) and once with acetone (-4°C). DNA adhered to the silica (pellet) was eluted by adding 60µl of TE buffer (10mM Tris-HCl pH8.0, 1mM EDTA pH8.0), and was then incubated (50°C, 10min) and centrifuged (2min, 14000rpm). The supernatant (template DNA) was removed and transferred into a fresh microtube and stored at -20°C until use. The extracted DNA samples were analyzed and quantified (ng/µL) by a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific Inc., USA).

Hemoparasites

Hemoparasites were investigated in peripheral blood smears of 89 birds (45 Falconiformes, 35 Strigiformes and nine Cathartiformes) and by PCR of 82 birds (33 Falconiformes, 41 Strigiformes and 8 Cathartiformes) . Two smears were prepared per bird with freshly collected blood, immediately dried in air, fixed in methanol, and stained with Giemsa (Valkiünas, 2005). Two hundred microscopic fields were analyzed by immersion light microscopy (1000 X) for the presence of hemoparasites.

PCR for *Haemoproteus* spp. and *Plasmodium* spp. was performed in spleen samples. DNA was extracted as described above, and PCR reactions were performed in a thermocycler (Maxygene, Axygen, USA) as previously described (Fallon *et al.*, 2003), using the primers 343F-5'GCTCACGCATCGCTTCT-3' and 496R-5'GACCGGTCATTTTCTTTG-3'. In the amplification reaction, 2µL of DNA template and 13µL of the reaction buffer (10mM Tris-HCl, pH8.5, 50mM KCl, Phoneutria®, Brazil), 2.0-2.5mM $MgCl_2$, 200mM dNTP, 0.5U *Taq* DNA polymerase (Phoneutria ®; Brazil) 0.4mM of each primer and sterile ultrapure water qsp) were used for 35 cycles of denaturation (94°C, 1min), annealing at 62°C for 1min and extension at 72°C for 70s. The initial denaturation was performed at 94°C for 2min and a final extension at 72°C for 3min. A final product with 192 base pairs was visualized by electrophoresis on non-denaturing 6% polyacrylamide

gel in 1X TBE buffer, fixed in ethyl alcohol solution at 10% and 0.5% acetic acid, stained with silver nitrate solution, and fragments of DNA as evidenced in a developing solution of sodium hydroxide and formaldehyde (Sanguinetti *et al.*, 1994). Genomic DNA of *Plasmodium gallinaceum* was used as positive control and SPF-chicken DNA sample extracted from the blood was used as negative control.

PCR for Chlamydophila psittaci

Chlamydophila psittaci (*Cp*) DNA was investigated in liver samples by PCR (Sachse *et al.*, 2009), using the primers *Cp*-F-5'-ACTACGGAGATTATGTTTTCGATCGTGT-3' and *Cp*-R-3'TCTTGGAGCGTYGGTGCACG-5'. In the amplification reaction, 200ng of template DNA, 2µL of 10X buffer (200mM Tris-HCl pH8.4, 500mM KCl), 1µL of 10mM dNTP, 1µL 50mM $MgCl_2$, 1µL of each primer, 0.1µL of Taq Polymerase 5U/1µL (Platinum Taq DNA Polymerase) and ultra pure water qsp were used to a final volume of 20µl. All reagents were supplied by Invitrogen® (USA). Reactions were performed in a thermocycler (Maxygene, Axygen, USA), with an initial denaturation cycle of 96°C for 60s, followed by 40 denaturation cycles at 94°C for 30s, annealing at 50°C for 60s, and extension at 72°C for 30s, plus a final extension at 72°C for 4min, yielding a final product of 418bp. DNA extracted from the liver of a bird diagnosed with *Cp*, as confirmed in other laboratories, was employed as positive control. The DNA extracted from liver of SPF chickens was used as negative control. PCR products were resolved by electrophoresis on 1% agarose gel, stained by ethidium bromide (10mg/mL) and visualized under UV transiluminator (Hoefer Scientific Instruments, USA).

RESULTS

Birds

The raptor species evaluated in the present study are shown in Table 1. Out of Cathartiformes, only Coragyps atratus was evaluated (n=14). The following were evaluated. Considering Falconiformes (n=82), *Caracara plancus* and *Rupornis magnirostris* (n=26), representing 63.4%, *Milvago chimachima* (12.1%, n=10) and *Falco sparverius* (n=8, 9.9%), both representing 85.4% of the total, were studied. Out of the Strigiformes (n=84), *Tyto alba* (32.1%, n=27), *Asio clamator* (27.3%, n=23), *Athene cunicularia* (13.0%, n=11), *Asio stygius* (8.3%, n=7), *Glaucidium brasilianum* (7.1%, n=6) and *Megascops choliba* (7.1%, n=6), representing 95.8% of owls, were examined.

Necropsy

Out of the 109 birds necropsied, 42 were Falconiformes, 57 Strigiformes and 10 Cathartiformes. Absolute number of deaths among Strigiformes was greater than the sum of the deaths in the other groups, which were 72%, 68%, 51% for Cathartiformes, Strigiformes and Falconiformes, respectively. Most birds died during triage. However, four owls and one *C. atratus* died immediately after being rescued. Eight Falconiformes, four Strigiformes and seven Cathartiformes were rescued and euthanized due to post-traumatic suffering. Ten percent of the dead birds were young and 90% were adults; 46.8% were males and 39.4% females; however, age and sex were not determined in 13.7% due to advanced autolysis of their bodies.

Trauma was the main cause of rescue of raptors at CETAS, affecting 63.3% (69/109) of birds necropsied, and according to taxonomic order, 66.7% (28/42) Falconiformes, 56.1% (32/57) Strigiformes, and 90% (9/10) Cathartiformes. Conditions included electrocution, fracture and traumatic amputation of extremities, traumatic brain injury (TBI), projectile injury, kite line or barbed wire cuts/amputation, fall from the nest and other undetermined causes. In 18.8% (13/69) of cases, it was not possible to determine the cause of the injury.

At necropsy, 38.5% (42/109) of the birds presented fractures, or 60.9% (42/69), of birds with trauma. According to taxonomic order, 42.9% (18/42) Falconiformes, 36.8% (21/57) Strigiformes, and 30% (3/10) of Cathartiformes presented fractures. Relative to fracture site, 57.1% (24/42) were located in the thoracic limbs, 14.3% (6/42) in the pelvic limbs, 11.9% (5/42) had multiple fractures, and 16.7% (7/42) in pelvic bones, beak, rib and skull.

Two birds suffered fatal electrocution (2/69): an *A. stygius* with foot burns, and an *C. atratus*, with about 50% of its body surface burnt. Traumatic amputation (3/69) of the extremities were observed at the metatarsus (*C. atratus*), and tarsus and 3rd finger (*R. magnirostris*). Cases of TBI were observed in four birds with traumatic conditions, including *R. magnirostris*, *A. cunicularia*, *Megascops choliba,* and *A. clamator*, with history of neurological signs. One case (*A. cunicularia*) of skull fracture was detected. Injuries caused by firearm (2/69) were observed in *R. magnirostris* and *Buteo brachyurus* by finding the projectile in the lesions, which included humerus fracture, skin and intercostal muscle perforation, rib fracture with ruptured lung,

and extensive pulmonary hemorrhage. Injuries caused by kite line (3/69) included two *C. plancus* and one *A. clamator* presenting skin, muscle and tendon laceration, and tissue loss in the forelimbs. One *A. clamator* had the right forelimb tangled in barbed wire. There were three cases of feathers covered with an oily chemical (3/109): one *Athene cunicularia*, one *T. alba,* and one *F. sparverius*. Cachexia was observed in 11 birds (11/109) with pectoral muscle atrophy, being nine Strigiformes and two Falconiformes, although not observed in Cathartiformes. The young birds that fell from the nest (3/69) included two *C. plancus*, one of which at hatch, and one *C. atratus*. Ophthalmic lesions (4/109) included corneal ulcer and opacity, exsudate, lens dislocation, hyphema, and exophthalmia. One bird had suppurative ocular injury associated with trichomoniasis at an advanced stage. Respiratory mycotic granulomas were observed in three Strigiformes, one Falconiformes and three Cathartiformes (7/109).

Parasites

Endoparasites (Table 2) were observed in 11% (12/109) of the necropsied birds, being 11.9% (5/42) Falconiformes and 12.3% (7/57) Strigiformes, but none in Cathartiformes. In some cases, the same bird showed parasitism by more than one species of helminths. Nematodes were found in 12.8% (14/109) of birds, cestodes in 1.8% (2/109), trematodes in 0.9% (1/109) and acanthocephalans in 2.7% (3/109). Endoparasitism by helminths in the air sacs were found in three *T. alba*, and were preliminarily identified as *Hamatospiculum* sp. A high parasite load of acanthocephalans fixed to the small intestine mucosa was found in one *Leptodon cayanensis*.

Coccidiosis, *Histomonas* spp. and *Trichomonas* spp. were the protozoan infections found. Coccidiosis was detected in 9.1% (10/109) of birds, *Histomonas* spp. in 6.4% (7/109) and *Trichomonas* spp. in 9.1% (10/109). Among *Trichomonas* spp. infections, 50% of cases were found in Falconiformes and 50% in Strigiformes, including *R. magnirostris*, *M. chimachima*, *F. femoralis*, *F. sparverius*, *C. plancus*, *A. cunicularia*, *A. clamator*, *G. brasilianum,* and *T. alba*. No case of trichomoniasis was observed in Cathartiformes. Diphteritic plaque lesions were observed in all *Trichomonas* infection cases in the oral cavity, and in some cases, involving the oropharynx, nasal cavity and infraorbital sinuses. All birds diagnosed with trichomoniasis died. The cases of *Histomonas* spp. were characterized by the observation of motile cells in cecal and cloacal lumen, in 12% Strigiformes of the species *B. virginianus*, *A. cunicularia T. alba* and *A. clamator*, but none in Falconiformes or Cathartiformes. *Heterakis gallinarum*, an intermediate host of the parasite in chicken, was not detected.

Table 2 – Endoparasites found in raptor birds rescued or at the triage center of CETAS-Belo Horizonte, Brazil between 2008-2010.

Parasite	Raptor bird host	Tissue location
Nematode		
Ascaridia sp.	*Rupornis magnirostris*	Small intestine
Filarid	*Asio stygius*	Cloaca
Hamatospiculum sp.	*Tyto alba*	Air sac
Hamatospiculum sp.	*Tyto alba*	Air sac
Hamatospiculum sp.	*Tyto alba*	Air sac
Physaloptera acuticauda	*Leptodon cayanensis*	Esophagus
Porrocaecum sp.	*Rupornis magnirostris*	Small intestine
Procyrnea mansioni	*Rupornis magnirostris*	Gizzard (ventriculus)
Spirurídeo (Subfamily Spirurinae)	*Asio stygius*	Esophagus
Streptocara pectinifera	*Tyto alba*	Gizzard (ventriculus)
Tetrameres sp.	*Athene cunicularia*	Proventriculus
Tetrameres sp.	*Asio stygius*	Proventriculus
Tetrameres sp.	*Asio stygius*	Proventriculus
Tetrameres sp.	*Leptodon cayanensis*	Proventriculus
Cestode		
Cestoda	*Asio clamator*	Small intestine
Cestoda	*Rupornis magnirostris*	Duodenum
Trematode		
Trematoda (Diplostomatidae)	*Rupornis magnirostris*	Small intestine
Acantocephala		
Acanthocephala	*Leptodon cayanensis*	Small intestine
Acanthocephala	*Asio clamator*	Small intestine
Centrorynchus sp.	*Leptodon cayanensis*	Jejunum

Three *T. alba* were parasitized by more than one protozoan, being two by *Sarcocystis* spp. and *Histomonas* spp., and one in addition to the previous two, also by *T. gallinae*. Despite the low occurrence, trichomoniasis was an apparently major cause of death in Falconiformes and Strigiformes. *Sarcocystis* spp. was observed in 77% (7/9) *T. alba* with coccidiosis, the only species affected.

Ninety percent of coccidiosis cases occurred in owls and 10% in *Coragyps atratus*, with no case in Falconiformes. Oocyst sporulation did not occur in two cases of *A. cunicularia*. *Sarcocystis* spp. were found sporulated in the feces of *T. alba*. Oocysts contained

two sporocysts surrounded by a thin membrane, giving an appearance of paired-oocysts. *Sarcocystis* oocysts (n=130) measured 19.41µm ± 1.14µm x 12.17µm ± 0.97µm and measurements of sporocysts were 12.17µm ± 0.97µm x 9.98µm ± 0.58µm. Diffuse residues were present within the sporocysts. Inoculated sporulated *Sarcocystis* sp. given intraperitoneally to Balb/c mice and incubated for 60 days, did not result in muscular lesions.

At necropsy, 9.2% (10/109) of birds (two Strigiformes, one Cathartiformes and seven Falconiformes) were parasitized by hippoboscid, 17.4% (19/109) by mites (sixteen Strigiformes, two Falconiformes and one Cathartiformes) and 10.1% (11/109) by chewing lice (one Strigiformes, six Cathartiformes and four Falconiformes).

Ornithonyssus sylviarum (Macronyssidae) caused clinical change in one *A. clamator* that died with a low hematocrit (17%), with intense pericloacal parasitism with skin crusting. Parasitism by *Amblyomma* spp. was observed attached close to the ear in one *T. alba*.

Serology

SAT for Mg

Sixty-five sera were analyzed (28 Falconiformes, 28 Strigiformes and nine Cathartiformes) for the presence of anti-Mg antibodies all proven negative. In one *C. atratus* a nonspecific SAT reactivity was detected, although shown negative by HI (Table 1).

HI for APMV-1 antibodies

Sera of 68 birds (30 Falconiformes, 29 Strigiformes and nine Cathartiformes) were tested for anti-APMV-1 antibodies. Only two *C. plancus* had titers (16 and 32), representing 15.4% (2/13) of the species and 6.7% (2/30) of the order (Table 1).

Hemoparasites

A general parasitism rate of 13.5% (12/89) was observed in the peripheral blood and 8.5% (7/82) in the spleen (Table 1). Hemoparasitism in Falconiformes was 8.9% (4/45), all determined in blood smears and none by PCR. In Strigiformes, blood smears were 22.8% (8/35) positive, but only 17.1% (7/41) were positive by PCR. No Cathartiformes showed hemoparasites. The morphology of the hemoparasites was consistent with genus *Haemoproteus*.

Fourteen birds were analyzed for hemoparasitism both by PCR and blood smear, and eight were negative in both tests, one was positive by both techniques, four were PCR negative but positive by blood smear (low parasitism), and one was positive only by PCR (Table 1).

Three birds (*B. brachiurus*, *A. stygius* and *R. magnirostris*) were positive (3/10) for *Haemoproteus* spp., and hippoboscid louse-flies (Hippoboscidae) were found. A sample of louse-fly was collected on *B. brachiurus* and submitted to PCR. DNA extraction (silica) was performed separately on the head and abdomen of the vector and *Haemoproteus* spp. specific DNA was found in both sections.

Chlamydophila psittaci

Ninety five liver samples (37 Falconiformes, 49 Strigiformes and 9 Cathartiformes) were tested negative for *C. psittaci* by PCR.

DISCUSSION

The number of individuals evaluated indicated that the populations of raptors suffer significant and varied health pressures when inhabiting the periurban environment. Joppert (2007) studied 114 raptors in São Paulo for 3.5 years, and most (65%) were Strigiformes, in agreement with our findings. Komnenou *et al.* (2005), in a 3-year retrospective study in Greece, registered only 18% owls among 402 raptors evaluated. Environmental, behavioral or ecological factors may explain these differences in incidence of taxonomic groups. The twenty studied species constitute approximately 21% of the total taxa recorded for the group in Brazil, being 15%, 39% and 17% Falconiformes, Strigiformes and Cathartiformes, respectively. However, the species *T. alba, A. clamator, R. magnirostris* and *C. plancus* represented more than half (57%) of birds studied, possibly because of their greater population in urban environments. Sick (1997) classified these taxa as generalists, capable of establishing populations in urban and peri-urban environments.

Trauma findings were in agreement with previous reports on raptors (Komnenou *et al.*, 2005), which described trauma as the most important cause of morbidity. Urban Falconiformes were mostly affected by vehicle collisions and electrocutions, while vehicular and window strikes are the most common causes of trauma in urban Strigiformes (Hager, 2009). Fractures were the single most important cause of morbidity, in agreement with previous reports (Naldo and Samour, 2004). According to Arnaut (2006), fractures of

thoracic or pelvic limbs are the most common, which is consistent with our findings. Cachexia was observed in Strigiformes and Falconiformes. Cathartiformes are mostly scavengers and do not need to hunt, searching for food in city waste.

The observed helminthic infection rates were lower than those previously reported by Joppert (2007), of 25.4%, and by Santoro *et al.* (2010), of 95%. The impact of those infections is generally considered low, except when associated with other diseases and stress. *Hamatospiculum* sp. was incidentally found in *Tyto alba* air sacs. On the other hand, an intense infection by an acanthocephalan was found in the small intestine mucosa of *Leptodon cayanensis*, and it is possibly associated with the bird's death. Acanthocephalans were previously described in Falconiformes and Strigiformes (Sanmartin *et al.*, 2004). The other endoparasites found (Table 2) are consistent with literature reports (Gomez *et al.*, 1993; Smith, 1996; Krone and Cooper, 2002; Fagerholm and Overstreet, 2008; Honisch and Krone, 2008; Kinsella and Forrester, 2008; Santoro *et al.*, 2011).

Hippoboscid flies, *Ornithonyssus sylviarum*, ticks and chewing lice were previously described in raptors (Philips, 2000) and were found in all Orders evaluated in the present study. These parasites may be associated with chickens, pigeons and sparrows (Arends, 2003). During rehabilitation, the proximity of raptors with free-living pigeons, sparrows, and other species, including reptiles and mammals, may allow their infection by ectoparasites. Mites had a negative effect on hematocrit values, which were below the 36% normally reported for *Asio clamator* (Sanchez *et al.*, 2005).

Trichomoniasis may have contributed for the morbidity and mortality of raptors (Forrester and Foster, 2008), and accounted in the present study for half of the protozoan occurrences in Falconiformes and Strigiformes. Pigeons have been diagnosed with trichomoniasis in the region (Resende *et al.*, 2001) and may have contributed as sources of infection. Higher morbidity and mortality associated with greater pigeon population was reported in urban raptors compared with rural ones (Boal *et al.*, 1998). Histomoniasis occurs in poultry of the region (unpublished data), and in the present study, *Histomonas meleagridis* (common in Phasianidae) affected only Strigiformes, and no *Heterakis gallinarum*, considered an intermediate host, was found in the infected owls, as previously described in *Buteo buteo* (Özmen *et al.*, 2009).

Coccidiosis occurred mostly in Strigiformes, and also in Cathartiformes, but not in Falconiformes. Sporulated oocysts of *Sarcocystis* spp. (Krone, 2007) were characterized by morphometry in *Tyto alba*. Measurements were similar to *Isospora buteonis* (Mathey, 1966), reclassified as *Sarcocystis* spp. (Lindsay and Blagburn, 1989; Modry *et al.*, 2004). However, *Sarcocystis dispersa* is considered specific of *T. alba* (Dolezel *et al.*, 1999). *Tyto alba* is also known for protozoan (*Sarcocystis* spp. and *Histomonas* spp.) co-infections. *Sarcocystis* sp. in *Buteo borealis* was not associated with clinical disease (Lindsay *et al.*, 1987), but in *Falco sparverius,* it was associated with mortality (Mathey, 1966). Sinantropic rodents may be source of infection for *T. alba* (Rommel and Krampitz, 1975). However, no lesions were detected in inoculated mice, suggesting important biological differences (Munday, 1977).

Serology for Mg was negative. Raptors in the triage center are kept for a short time in captivity , and those birds that able to fly are released after support therapy, while birds with irreversible trauma are euthanized. Transmission during triage may be low if compared with other bird classes, such as Psittaciformes or Passeriformes, which may remain longer in the center, thereby increasing the risk of cross-infections. In Brazil, PCR detection of Mg revealed that 51.9% of the dead Psittaciformes tested in a triage center (CETAS-BH) were positive (Gomes *et al.*, 2010), suggesting that large populations, common housing of birds of different origins and history of proximity with free-range poultry, could account for high levels of infection.

The studied raptors in Minas Gerais may not pose risks for commercial poultry, at least considering *M. gallisepticum*. However, several species of *Mycoplasma* were previously described in raptors (Panangala *et al.*, 1993; Poveda *et al.*, 1994; Oaks *et al.*, 2004; Lierz *et al.*, 2008a; Lierz *et al.*, 2008b; Loria *et al.*, 2008; Ruder *et al.*, 2009). Species considered pathogenic for commercial poultry were occasionally reported in raptors (Lierz *et al.*, 2008a). Distance and biosecurity are essential both for wild bird centers and poultry production sites, and there must be regular surveillance, as the infection may occur in both directions (Farmer *et al.*, 2005).

Our findings for antibodies against APMV-1 were consistent to those previously described (Schettler *et al.*, 2001). Low, but significant, titers were only found in *C. plancus*, which is commonly found close to human activity, including garbage dumps and poultry farms. Chickens usually receive live APMV-1 vaccines and

vaccine strain infection of free-living neighboring birds may be possible. Schettler *et al.* (2003) described a La Sota (vaccine-type) APMV-1 strain in *T. alba*. This strain is the most commonly used in Brazil for the protection of chicken flocks. The widespread use of live APMV-1 vaccines for poultry may render the correct evaluation of APMV-1 natural circulation difficult (Aldous and Alexander, 2001). Raptors fed with farm residues or living close to chickens presented a high level of infection (Chu *et al.*, 1976). Lentogenic APMV-1 was also detected in *Haliaetus leucocephalus* and *Bubo virginianus*, but not in *Accipiter cooperi, Carthartes aura* and *C. atratus* (Jindal *et al.*, 2010). Although most APMV-1 strains endemic in wild birds are lentogenic, deficient biosecurity in poultry facilities may enable challenge with virulent strains (Camenisch *et al.*, 2008). The raptors in this study may not pose risks to commercial poultry regarding APMV-1.

No hemoparasitism clinical signs were observed in the evaluated raptors and the incidence of *Haemoproteus* spp. was consistent with previous findings in raptors (Krone *et al.*, 2001; Andery *et al.*, 2010). The association of hippoboscid louse-flies and *Haemoproteus* (Remple, 2004) was here also observed. The triage center attracted free-living pigeons with hippoboscids, which were also found in positive raptors. *Haemoproteus* spp. DNA was found in both portions of the fly, possibly indicating vector infection. It was described that this agent replicates in the gut and migrate to the salivary glands of flies (Friend and Franson, 1999). Results of birds tested both by blood smears microscopy and spleen PCR lacked agreement. Conflicting results have also been described (Krone *et al.*, 2008), although PCR is considered more sensitive (Hellgren *et al.*, 2004). False negative results by PCR may result from genetic variation or low parasitemia (Fallon *et al.*, 2003).

Although *Cp* DNA was not detected in raptor livers, these results may not represent the actual status. False-negative results were described and were attributed to the intermittent nature of the infection (Andersen and Vanrompay, 2003). In São Paulo, *Cp*-specific antibodies were detected in 16% of the raptors in the Zoological Garden, but no *Cp* was detected by PCR of cloacal swabs (Raso *et al.*, 2005). However, birds may be negative for antibodies but present positive PCR in recent infections (Raso *et al.*, 2006). *Cp* detection was reported by Schettler *et al.* (2001), who studied lung and spleen by PCR and obtained 74% positivity, suggesting that raptors may play a role in the epidemiology of this disease.

Among the several anthropic-related affections, trauma represented the main cause for submission to triage and death. The urban adaptation of wild avian species, as a consequence of habitat loss and behavioral change, poses new challenges for the wild fauna. A new perception for the harmonious coexistence is needed for the preservation of these animals.

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