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Pathological findings and their role as causes of disease and death in free-ranging *Didelphis albiventris* from an urban area[1]

Julieni Bianchi-Morais[2,6], Ana R.M. Araujo[3], Wuglenya D.M. da Silva[2], Richard C. Pacheco[4], Dirceu G.S. Ramos[5], Marcos A. Souza[2], Valéria Dutra[3], Geovanna T. Martins-Silva[6], Nadia A.B. Antoniassi[6], Edson M. Colodel[2]*

ABSTRACT.- Bianchi-Morais J, Araujo ARM, Da Silva WDM, Pacheco RC, Ramos DGS, Souza MA, Dutra V, Martins-Silva GT, Antoniassi NAB, Colodel EM. **Pathological findings and their role as causes of disease and death in free-ranging *Didelphis albiventris* from an urban area.** *Pesquisa Veterinária Brasileira 46:e07821, 2026.* Laboratório de Patologia Veterinária, Hospital Veterinário, Faculdade de Medicina Veterinária, Universidade Federal de Mato Grosso, Av. Fernando Corrêa da Costa 2367, Boa Esperança, Cuiabá, MT 78060-900, Brazil. E-mail: edson.colodel@ufmt.br

This retrospective study aimed to characterize the main pathological findings and causes of morbidity and mortality in free-ranging *Didelphis albiventris* from an urban area of Cuiabá, Mato Grosso, Brazil. Twenty-nine specimens necropsied between December 2022 and April 2025 were evaluated. The primary cause of death was established in 27/29 (93.1%) cases. Non-infectious causes predominated (20/27, 74,1%), whereas infectious causes accounted for 7/27 (25,9%) cases. Lesions caused by physical agents, mainly associated with acute high-energy trauma or predation, were identified in 21/27 (77,8%) individuals. These lesions were characterized by multiple fractures, extensive lacerations, visceral rupture, cavitary hemorrhages, and circulatory shock as the immediate mechanism of death. Bacterial infections were identified in 9/27 (33,3%) cases and were associated with traumatic injuries, with fatal outcomes due to acute sepsis in 4/27 (14,8%) individuals. Inflammatory processes with defined etiologies were observed in 18/29 (62.0%) opossums, including granulomatous parasitic pneumonia associated with *Heterostrongylus heterostrongylus* (15/27; 55,6%) and intestinal acanthocephaliasis associated with *Oligacanthorhynchus microcephalus* (syn. *O. tortuosus*) infection (13/27; 48,1%). Extracellular deposition compatible with amyloid was observed in 11/27 (40,7%) individuals. The findings demonstrate that mortality in *D. albiventris* from urban environments is primarily associated with traumatic events related to anthropogenic pressures and interactions with domestic animals, resulting either in immediate fatal outcomes or secondary complications caused by bacterial infections. Additionally, chronic parasitic inflammatory diseases represent an important contributor to morbidity in this species.

INDEX TERMS: Opossum, necropsy, trauma, sepsis, parasitism, urban wildlife.

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[2] Laboratório de Patologia Veterinária (LPV), Hospital Veterinário (HOVET), Faculdade de Medicina Veterinária (FAVET), Universidade Federal de Mato Grosso (UFMT), Campus Cuiabá, Av. Fernando Corrêa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT 78060-900, Brazil. *Corresponding author: edson.colodel@ufmt.br

[3] Laboratório de Microbiologia e Biologia Molecular (LMBM), Hospital Veterinário (HOVET), Faculdade de Medicina Veterinária (FAVET), Universidade Federal de Mato Grosso (UFMT), Campus Cuiabá, Av. Fernando Corrêa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT 78060-900, Brazil.

[4] Laboratório de Parasitologia e Doenças Parasitárias (LPDP), Hospital Veterinário (HOVET), Faculdade de Medicina Veterinária (FAVET), Universidade Federal de Mato Grosso (UFMT), Campus Cuiabá, Av. Fernando Corrêa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT 78060-900, Brazil.

[5] Laboratório de Parasitologia Veterinária (PARASITOVET), Universidade Federal de Jataí (UFJ), Campus Jatobá, 3800, Jataí, GO 75801-615, Brazil.

[6] Laboratório de Patologia Animal (LAPAN), Hospital Veterinário (HOVET), Universidade Federal de Mato Grosso (UFMT), Campus Sinop, Av. Alexandre Ferronato, 1200, Setor Industrial, Sinop, MT 78557-267, Brazil.

RESUMO.- [**Achados patológicos e importância na causa de doenças e morte de *Didelphis albiventris* de vida livre provenientes de área urbana de Cuiabá, Mato Grosso, Brasil.**] Este estudo retrospectivo teve como objetivo caracterizar os principais achados patológicos e causas de doenças e morte em *Didelphis albiventris* de vida livre provenientes de área urbana de Cuiabá, Mato Grosso. Foram avaliados 29 espécimes necropsiados entre dezembro de 2022 e abril de 2025. A causa primária de morte foi estabelecida em 27/29 (93,1%), sendo as mortes do tipo não infecciosas predominantes (20/27; 74,1%), e causas infecciosas corresponderam a 7/27 (25,9%). Lesões não infecciosas por agentes físicos relacionadas com trauma agudo por impacto de alta energia ou predação foram registradas em 21/27 (77,8%) casos, caracterizadas por fraturas múltiplas, lacerações extensas, ruptura de vísceras, hemorragias cavitárias e choque circulatório como causa do desfecho clínico imediato. Adicionalmente infecções bacterianas foram identificadas em 9/27 (33,3%) casos, secundária aos traumas, notando-se desfecho fatal relacionado ao quadro lesional compatíveis com sepse aguda em 4/27 (14,8%) casos. Processos inflamatórios com etiologia definida foram observados em 18/27 (66,7%) gambás, destacando-se pneumonia granulomatosa parasitária associada a *Heterostrongylus heterostrongylus* (15/27; 55,6%) e acantocefalose intestinal relacionado a infecção por *Oligacanthorhynchus microcephalus* (sin*. O. tortuosus*) (13/27; 48,1%). Deposição extracelular compatível com amiloide foi observada em 11/27 (40,7%) indivíduos. Os achados demonstram que a mortalidade de *D. albiventris* em ambiente urbano resulta predominantemente de eventos traumáticos associados a pressões antrópicas e ataques por animais domésticos, com desfecho fatal imediato, ou complicados por infecções concomitantes. Além disso, doenças inflamatórias parasitárias desempenham papel importante no adoecimento desta espécie na área de estudo.

TERMOS DE INDEXAÇÃO: Gambá, necropsia, trauma, sepse, parasitismo, fauna urbana.

INTRODUCTION

Among Neotropical marsupials, *Didelphis albiventris* (Lund, 1840) is widely distributed throughout Brazil and exhibits remarkable ecological and behavioral plasticity (Brandão & Carmignotto 2012, Castro et al. 2021). With predominantly nocturnal habits and an omnivorous diet, this species exploits a wide range of ecological niches and performs important ecological functions, including seed dispersal and regulating populations of small vertebrates and invertebrates, including venomous animals (Bezerra-Santos et al. 2021).

The occurrence of *D. albiventris* in human-modified environments is largely driven by habitat fragmentation and the availability of anthropogenic resources, which increase interactions with humans, domestic animals, and urban infrastructure (Wilkinson et al. 2018, Borremans et al. 2019). This close association exposes the species to a variety of health and traumatic hazards, making it one of the wildlife species most frequently affected by vehicle collisions, dog and cat attacks, toxic waste, and infectious pathogens (Navas-Suárez et al. 2022). In urban environments, these factors often occur simultaneously, with infectious and parasitic diseases coexisting with traumatic injuries, resulting in the multifactorial pathological profile commonly observed in synanthropic wildlife (Bezerra-Santos et al. 2021, Chagas et al. 2024).

This broad exposure, together with the ecological role of *D. albiventris*, allows the species to serve as a sentinel, reflecting the circulation of infectious agents and the sanitary conditions of urban environments, an important aspect of the human-animal-environment health interface (Destoumieux-Garzón et al. 2018, Cupertino et al. 2020). Most *D. albiventris* individuals admitted to wildlife rehabilitation or research centers present with severe clinical conditions and are either euthanized or found dead upon arrival.

In this context, the dynamics of urban–wildlife interactions, including zoonotic diseases, should be understood within the framework of complex ecological systems. Hassell et al. (2017) emphasized the need for integrative, multispecies approaches to investigate disease emergence in urban landscapes. Therefore, systematic necropsy combined with histopathological examination is an essential tool for identifying disease patterns, determining the pathological significance of morphological findings, and, whenever possible, establishing the cause of disease and death in this species (Koshurnikova & Domskiy 2022). In addition, *post mortem* examination enables the collection of biological samples for diagnostic and epidemiological investigations, supporting the detection and characterization of pathogens circulating in anthropized environments (Koshurnikova & Domskiy 2022). These procedures provide a valuable opportunity for health surveillance of synanthropic wildlife and further highlight their importance within the One Health framework (Destoumieux-Garzón et al. 2018). Accordingly, this retrospective study aimed to identify the causes of death and characterize the major gross and histopathological lesions in *D. albiventris* originating from urban and peri-urban areas of Cuiabá, Mato Grosso, Brazil.

MATERIALS AND METHODS

Ethical approval. The study protocol was submitted to the Animal Use Ethics Committee of the "Universidade Federal do Mato Grosso" (CEUA-UFMT, Cuiabá Campus; SEI no. 23108.071864/2025-70) and was exempted from ethical review because it did not involve the use of live vertebrate animals, in accordance with Brazilian Federal Law no. 11,794 of October 8, 2008. The free-ranging opossums included in this study were either found dead or died during clinical care. They were subsequently submitted to the "Laboratório de Patologia Veterinária" of the "Hospital Veterinário", "Universidade Federal do Mato Grosso" (LPV-HOVET-UFMT, Cuiabá/MT, Brazil) for routine diagnostic investigation.

Case selection and data collection. Twenty-nine white-eared opossums (*Didelphis albiventris*) were retrospectively selected from necropsy reports issued by LPV-HOVET-UFMT from December 2022 to April 2025. Whenever additional diagnostic investigation or improved histological documentation was required, formalin-fixed paraffin-embedded (FFPE) tissues and samples stored in the HOVET-UFMT Genetic Biobank, coordinated by Prof. Richard C. Pacheco, were retrieved for complementary analyses.

For population characterization, data regarding origin (urban, peri-urban, or unknown), month and year of collection, sex, age class, body weight (g), body condition score (when available), and carcass preservation status were compiled. Age was primarily estimated based on tooth eruption and wear patterns and complemented by body measurements and evidence of sexual maturity. Individuals

were classified into four age categories: pouch young, juvenile, adult, and senescent (Astúa & Geise 2006).

Body condition was assessed semi-quantitatively using a five-point scoring system: 1 = cachectic, 2 = thin, 3 = normal, 4 = overweight, and 5 = obese. Scores were assigned according to body fat reserves, considering the amount of adipose tissue (absent, scarce, normal, abundant, or excessive), prominence of bony structures during external examination (marked, slight, or absent), and skeletal muscle mass (atrophied, preserved, or hypertrophied).

Pathological examination. Gross and histopathological findings were obtained from the diagnostic reports issued by LPV-HOVET-UFMT. Photographic archives were reviewed, and lesions involving the musculoskeletal system and the abdominal, thoracic, and cranial cavities were recorded.

Representative tissue samples of skin, trachea, lungs, heart, liver, spleen, kidneys, adrenal glands, urinary bladder, stomach, intestines, pancreas, lymph nodes, and brain preserved in 10% neutral buffered formalin were re-evaluated when available. Tissues were processed routinely, embedded in paraffin, sectioned at 3–5 µm, and stained with hematoxylin and eosin (HE). When indicated by the morphological findings, special histochemical stains, including Gram, Periodic acid–Schiff (PAS), and Masson's trichrome (AFIP protocol), were performed. Histological sections were examined by light microscopy, and lesions were characterized according to their morphological pattern, distribution, and severity (Barros & Rissi 2021).

Morphological findings consistent with bacterial sepsis were defined by combinations of lesions including: (a) multiple disseminated microabscesses; (b) septic emboli within small- and medium-caliber blood vessels; (c) multifocal random necrotic lesions associated with intralesional bacteria; (d) bridging hepatocellular coagulative necrosis; (e) acute pulmonary congestion and edema; (f) renal tubular congestion; and (g) multifocal multisystemic hemorrhages (Elbert & Rissi 2022, Cortellini et al. 2024).

Additional laboratory examinations. During necropsy, fresh samples of lungs, liver, spleen, kidneys, brain, heart, and segments of the gastrointestinal tract were collected for bacterial culture and identification at the "Laboratório de Microbiologia e Biologia Molecular" of the UFMT. Samples were individually inoculated onto 6% sheep blood agar (TM Media) and MacConkey agar (HIMedia) and incubated aerobically at 37 °C for up to 72 h. Isolated colonies were identified according to Markey et al. (2013) using Gram staining, catalase and oxidase testing, biochemical profiling, and carbohydrate utilization tests.

For parasitological evaluation, samples from the gastrointestinal tract, feces, and lungs were collected for the "Laboratório de Parasitologia Veterinária" (PARASITOVET) at the "Universidade Federal de Jataí" (UFJ). Nematodes were processed and mounted on microscope slides using either 50% glycerol or lactophenol (Hoffmann 1987) and identified according to standard taxonomic keys (Vicente et al. 1997, Anderson et al. 2009, Gibbons 2009, Costa-Neto et al. 2016). Acanthocephalans were stained with acid carmine, differentiated in 0.5% hydrochloric alcohol, dehydrated through graded ethanol solutions (70%, 80%, 90%, and absolute ethanol twice), clarified in eugenol (4-allyl-2-methoxyphenol), following a modified protocol of Amato (1985), and identified according to the taxonomic key of Schmidt (1972).

Diagnostic criteria. The primary cause of death was established by integrating available clinical history, gross and microscopic findings, and complementary laboratory results. The lesion or disease process considered most compatible with the fatal outcome was assigned as the cause of death following the criteria proposed by Correia et al. (2023). Causes of death were categorized as infectious or non-infectious. Animals euthanized for welfare reasons were classified according to the underlying condition that prompted euthanasia. Morphological alterations unrelated to the immediate cause of death were recorded as co-pathologies or associated findings. Cases involving severely mutilated or crushed carcasses, advanced autolysis or putrefaction, or the absence of diagnostic lesions that precluded determination of the primary fatal event were classified as inconclusive.

Lesion classification. For descriptive analysis, lesions were grouped into three major categories: (1) traumatic and hemodynamic lesions; (2) degenerative and necrotic diseases; and (3) inflammatory diseases of defined etiology (parasitic or bacterial) (Stromberg et al. 2019). Lesions that could not be assigned to these categories were grouped as "other disorders," including degenerative, necrotic, and inflammatory changes interpreted as secondary to other pathological processes.

Traumatic lesions were classified according to gross morphological characteristics and biomechanical principles of trauma pathogenesis. Blunt-force injuries were defined by tissue crushing, fractures, and hemorrhage consistent with the dissipation of kinetic energy over a broad contact area (Siqueira et al. 2016, Ressel et al. 2016, Navas-Suárez et al. 2022). Penetrating blunt injuries were characterized by the combination of skin perforation and adjacent blunt tissue damage, compatible with simultaneous penetration and tissue compression (Haddad Junior 2022, Díaz et al. 2023). Incised and puncture wounds were distinguished based on wound edge morphology and the relationship between wound depth and length (Díaz et al. 2023). Cases presenting multiple independent traumatic injuries affecting different body regions were classified as polytrauma, considering lesion distribution and the occurrence of multiple tissue injuries typical of high-energy trauma in free-ranging mammals (Navas-Suárez et al. 2022). The adopted classification was based exclusively on lesion morphology and did not imply identification of the traumatic agent or the specific circumstances in which the injuries occurred.

RESULTS

Population characteristics

Diagnostic records from 29 *Didelphis albiventris* specimens necropsied from December 2022 to April 2025 were evaluated. Most animals originated from urban areas of Cuiabá (25/29; 86.2%), followed by peri-urban areas (2/29, 6.9%), whereas the origin was unavailable for two cases (6.9%). Most individuals were adults (18/29, 62.1%), followed by juveniles (7/29, 24.1%) and pouch young (4/29, 13.8%). Eighteen animals were males (18/29, 62.1%), and 11 were females (11/29, 37.9%). Body weight ranged from 30 to 1,615 g. Body condition was classified as normal in 13/29 (44.8%) and thin in 13/29 (44.8%) individuals, whereas no body condition score was available for three animals (10.3%). Carcass preservation ranged from excellent to poor; however, in most cases, tissue preservation was considered suitable for histopathological evaluation (Table 1).

Causes of death

A primary cause of death was established in 27/29 (93.1%) opossums, whereas two cases (6.9%) were classified as inconclusive because of advanced autolysis and the absence of diagnostic lesions (Table 1). Among cases in which the cause of death was determined, non-infectious causes predominated,

Table 1. Numerical sequence of *Didelphis albiventris* specimens necropsied at LPV-HOVET-UFMT (Cuiabá/MT, 2022–2025), including information on sex, age group, month and year of death, principal pathological findings, and primary cause of death

Case	Sex	Age	Month -year of death	Primary cause of death	Major lesions (by category)
1	M	Adult	12-2022	Multisystemic parasitic inflammatory disorder	Inflammatory (defined): parasitic granulomatous pneumonia (+++); intestinal acanthocephaliasis (+++); parasitic necrotic enteritis (+). Others: gastric repletion by *Turgida turgida* (+++); amyloidosis (+++).
2	M	Adult	02-2023	Euthanasia (multisystemic parasitic inflammatory disorder)	Inflammatory (defined): parasitic granulomatous pneumonia (+++); intestinal acanthocephaliasis (++); esophagus with *Heterostrongylus heterostrongylus* (+). Others: gastric repletion by *T. turgida* (+++), lymphoplasmacytic myocarditis (+), amyloidosis (+++).
3	M	Juvenile	02-2023	High-energy impact	Physical agent: acute polytrauma with multiple fractures; extensive and deep lacerations; splenic rupture; hemoperitoneum. Inflammatory (defined): parasitic granulomatous pneumonia (+).
4	F	Adult	03-2023	Predation	Physical agent: penetrating-blunt trauma with deep muscle laceration and pelvic hematoma. Inflammatory (defined): parasitic granulomatous pneumonia; intestinal acanthocephaliasis (++); parasitic gastritis; purulent dermatitis (BCTS) (++). Others: gastric repletion by *T. turgida* (++); amyloidosis (++).
5	F	Pouch young	03-2023	Predation	Physical agent: penetrating-blunt trauma with deep lacerations (head and thoracic limb); cranial fractures; intracranial hemorrhage. Others: non-purulent meningoencephalitis (-/+), necrosuppurative dermatitis (BCTS) (+).
6	M	Adult	03-2023	Predation	Physical agent: penetrating-blunt trauma with perforating lacerations and purulent wounds (abdomen and limbs); hematomas (++). Inflammatory (defined): parasitic granulomatous pneumonia (+++); intestinal acanthocephaliasis (+); purulent bacterial pneumonia (++); purulent bacterial tracheitis (++); purulent bacterial nephritis (++). Others: gastric parasitism by *T. turgida* (++), amyloidosis (+).
7	M	Juvenile	04-2023	High-energy impact	Physical agent: blunt trauma with hypovolemic shock due to splenic rupture; hemoperitoneum and pulmonary hemorrhage. Inflammatory (defined): penetrating-blunt trauma and septic arthritis; systemic bacterial thromboembolism (+++); parasitic granulomatous pneumonia (+++); esophagus with H. heterostrongylus (+). (+). Others: gastric repletion by T. turgida (++).
8	F	Adult	05-2023	Bacterial sepsis (predation)	Physical agent: blunt trauma with hypovolemic shock due to splenic rupture; hemoperitoneum and pulmonary hemorrhage. Inflammatory (defined): penetrating-blunt trauma and septic arthritis; systemic bacterial thromboembolism (+++); parasitic granulomatous pneumonia (+++); esophagus with H. heterostrongylus (+). Others: gastric repletion by T. turgida (++).
9	M	Juvenile	07-2023	High-energy impact	Physical agent: blunt trauma with mandibular fracture and hematoma; hepatic fissure and hemoperitoneum.
10	M	Juvenile	08-2023	High-energy impact	Physical agent: blunt trauma with metacarpal fracture and hematoma. Inflammatory (defined): parasitic granulomatous pneumonia (++); intestinal acanthocephaliasis (+). Others: gastric repletion by *T. turgida* (+); amyloidosis (+).
11	F	Adult	09-2023	High-energy impact	Physical agent: craniofacial polytrauma with craniofacial fractures (mandible, maxilla, parietal, temporal, and supraorbital regions). Inflammatory (defined): intestinal acanthocephaliasis (++). Others: gastric repletion by *T. turgida*, periportal hepatitis (+)
12	F	Pouch young	10-2023	Predation	Physical agent: blunt trauma with hypovolemic shock due to thoracic perforation; hemothorax; deep lacerations
13	M	Adult	12-2023	Predation	Physical agent: penetrating-blunt traumas with laceration of skin and abdominal and left thoracic limb muscles, and necrosuppurative dermatitis (BCTS): + Inflammatory (defined): parasitic granulomatous pneumonia (++); intestinal acanthocephaliasis (+). Others: myocarditis; interstitial nephritis (+); gastric repletion by *T. turgida* (+); amyloidosis (++).
14	M	Adult	11-2023	High-energy impact	Physical agent: blunt trauma with multiple pelvic fractures. Inflammatory (defined): intestinal acanthocephaliasis (+); necrosuppurative dermatitis (BCTS) (++) Other: gastric repletion by *T. turgida* (-/+).
15	F	Pouch young	01-2024	Bacterial sepsis (predation)	Inflammatory (defined): fibrinosuppurative bacterial pericarditis (+); fibrinosuppurative bacterial pneumonia (++); purulent dermatitis (BCTS) (++).

Case	Sex	Age	Month -year of death	Primary cause of death	Major lesions (by category)
16	M	Juvenile	01-2024	Predation	Physical agent: acute cranial polytrauma with cranial bone fractures; encephalic hemorrhage; tongue rupture Inflammatory (defined): parasitic granulomatous pneumonia (++); pulmonary bacterial embolism (++). Other: gastric repletion by *T. turgida* (-/+).
17	M	Adult	02-2024	High-energy impact	Physical agent: blunt trauma with hypovolemic shock due to splenic rupture; hemoperitoneum. Inflammatory (defined): parasitic granulomatous pneumonia (++); intestinal acanthocephaliasis (++). Others: gastric repletion by *T. turgida* (+); amyloidosis (+).
18	M	Adult	05-2024	Bacterial sepsis (predation)	Physical agent: penetrating-blunt trauma with exposed fracture; extensive muscle laceration associated with necrosuppurative dermatitis (BCTS) (++); fibrinous bacterial peritonitis. Inflammatory (defined): parasitic granulomatous pneumonia (++); intestinal acanthocephaliasis (++). Others: interstitial nephritis (++); amyloidosis (+); gastric repletion by *T. turgida* (+).
19	M	Adult	02-2024	Bacterial sepsis (predation)	Physical agent: systemic thromboembolism and penetrating-blunt periocular trauma. Inflammatory (defined): purulent dermatitis (BCTS) (++); microabscesses in spleen, liver, and kidneys; fibrinous peritonitis and purulent pneumonia; multisystemic septic emboli (+++).
20	F	Adult	07-2024	Predation	Physical agent: penetrating-blunt trauma with thoracic perforation; hemothorax; pulmonary alveolar hemorrhage and edema (+++)
21	M	Adult	08-2024	Euthanasia (predation)	Physical agent: penetrating-blunt trauma with laceration of the right knee, bone exposure of the mandible, and perforations near the neck. Inflammatory (defined): parasitic granulomatous pneumonia (+++). Others: necrosuppurative dermatitis (BCTS) (++).
22	M	Juvenile	08-2024	Euthanasia (high-energy impact)	Physical agent: blunt trauma with tibia/fibula fracture; muscle laceration with edema and hemorrhage in tarsus and metatarsus. Others: purulent dermatitis (BCTS) (++)
23	M	Pouch young	09-2024	Starvation	Hepatocellular atrophy (+++). Acute multisystemic congestion (+)
24	M	Juvenile	09-2024	Predation	Physical agent: polytrauma with hypovolemic shock, deep lacerations with thoracic perforation; pulmonary and renal rupture; hemothorax, traumatic tail amputation.
25	M	Adult	10-2024	Multisystemic parasitic inflammatory disorder	Inflammatory (defined): parasitic granulomatous pneumonia (+++); intestinal acanthocephaliasis (++). Others: gastric repletion by *T. turgida* (++); amyloidosis (+++).
26	F	Adult	10-2024	High-energy impact	Physical agent: severe polytrauma with exposed fractures of lumbar vertebrae; abdominal laceration. Inflammatory (defined): parasitic granulomatous pneumonia; intestinal acanthocephaliasis; parasitic granuloma in omentum. Others: gastric repletion by *T. turgida* (+); amyloidosis (+); hepatic telangiectasia (+).
27	F	Adult	11-2024	Euthanasia (predation)	Physical agent: penetrating-blunt trauma with extensive abdominal lacerations and myiasis. Inflammatory (defined): parasitic granulomatous pneumonia (++); intestinal acanthocephaliasis (+++). Others: gastric repletion by *T. turgida* (+); amyloidosis (+++).
28	F	Adult	01-2025	Undetermined	Advanced *post mortem* autolysis
29	F	Adult	02-2025	Undetermined	Advanced *post mortem* autolysis.

F = female, M = male; Physical agent: traumatic events; Inflammatory (defined): inflammatory processes considered etiologically defined and directly associated with the identified causal agents; Others: includes findings not related to the immediate clinical outcome; Intensity of alteration: (-/+) minimal; (+) mild; (++) moderate; (+++) marked; BCTS = bacterial contamination at the trauma site.

accounting for 20/27; 74,1% of all animals. These were mainly associated with acute high-energy trauma, including vehicle collisions (Fig. 1–2) or predation (Fig. 3–4), accompanied by secondary hemodynamic disturbances. Infectious causes accounted for 7/27 (25,9%) cases and included multisystemic inflammatory parasitic disease and bacterial sepsis with multisystemic thromboembolism.

Cases 1, 2, and 25 presented with severe multisystemic parasitic inflammatory disease in the absence of evidence of high-energy trauma or predation. Opossum 1 was found in a comatose state, unresponsive to external stimuli, and died spontaneously. The principal lesions consisted of granulomatous pneumonia, granulomatous enteritis, and intestinal parasitic granulomas. Opossum 2 was euthanized because of severe respiratory distress accompanied by apathy, profound depression, absence of reflexes, and lateral recumbency. Gross and histopathological findings included granulomatous pneumonia, intestinal parasitic granulomas,

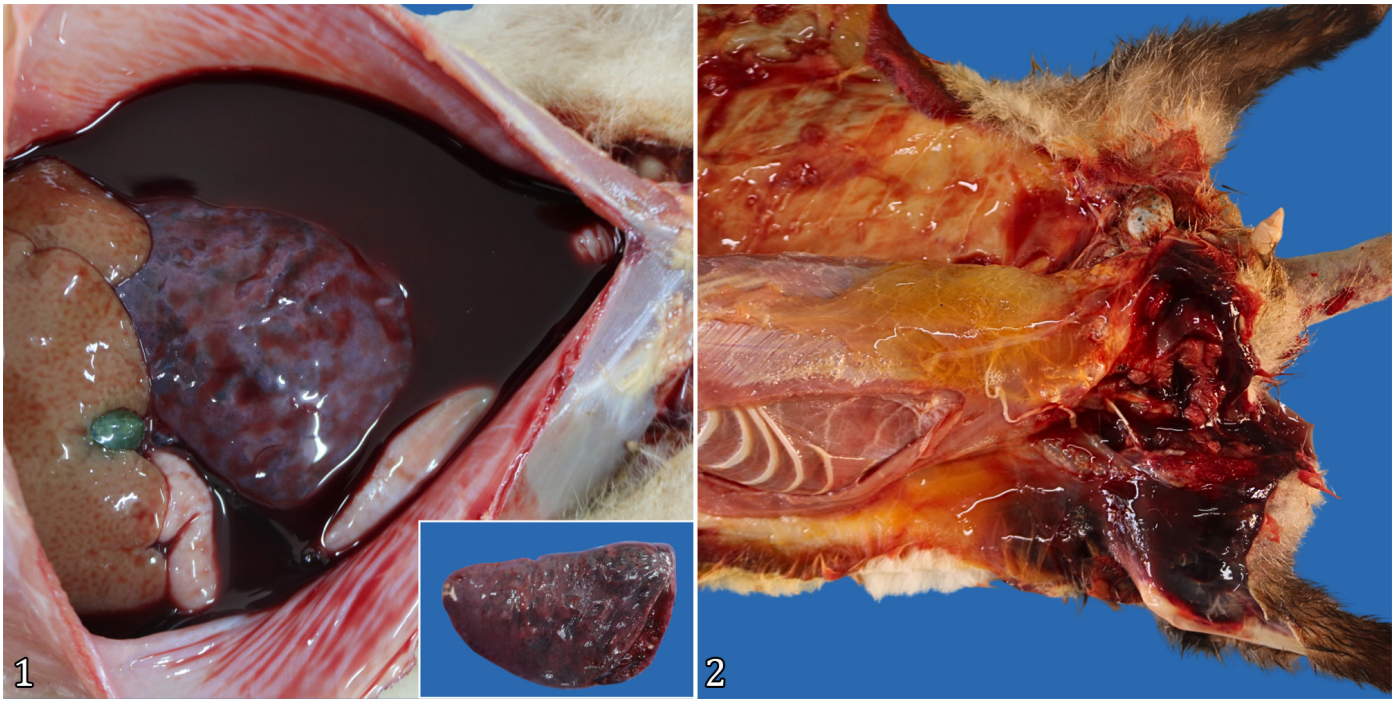


Fig. 1–2. Traumatic lesions in Case 3, resulting from high-energy impact in *Didelphis albiventris*. (**1**) Abdominal cavity showing pronounced hemoperitoneum and splenic rupture. Inset: the spleen exhibits an irregular surface with extensive parenchymal disruption. (**2**) Right pelvic limb, femoral region, presenting fracture accompanied by severe hemorrhage and marked edema of the surrounding tissues.



Fig. 3–4. Traumatic lesions resulting from predation in *Didelphis albiventris*, Case 24. (**3**) In the lateral thoracic region, deep lacerations and muscular perforation are evident (white arrow); the tail is absent, leaving an irregular stump. Inset: lung exhibiting hemorrhage associated with profound, extensive, and symmetrical perforations. (**4**) Cranial region demonstrating a severe laceration with bone exposure and multiple fractures attributable to compressive forces, consistent with penetrating–blunt trauma induced by the bite of a domestic animal.

and nonspecific lymphoplasmacytic myocarditis. Opossum 25 was admitted in a debilitated condition, showing dehydration and moderate icterus. Histopathological examination revealed granulomatous pneumonia and intestinal parasitic granulomas.

In all three cases, the pulmonary lesions were associated with *Heterostrongylus heterostrongylus* infection. Cross and longitudinal sections of adult and immature nematodes were observed predominantly within the alveolar lumen and bronchioles, accompanied by macrophage-rich inflammatory infiltrates and interstitial pneumonia characterized by thickening of the alveolar septa. Intestinal lesions attributed to *Oligacanthorhynchus microcephalus* (syn. *O. tortuosus*) consisted grossly of firm nodules within the small intestine associated with luminal dilation and thinning of the intestinal wall. Microscopically, mural or transmural nodules were associated with attachment of the parasite proboscis. Early lesions were characterized by necrosis, hemorrhage, and acute inflammation, whereas chronic lesions consisted of parasitic granulomas containing intact parasites surrounded by a fibrocollagenous capsule with marked reactive fibroplasia. In addition, hyaline deposits were observed in renal glomeruli and hepatic and splenic sinusoids. These deposits stained positively with Congo red and exhibited birefringence under polarized light, consistent with amyloid deposition.

Bacterial inflammatory lesions were observed in 9/27 (33,3%) opossums. In five cases, inflammation was primarily associated with secondary infection of the skin and subcutaneous tissues at sites of predation-related or high-energy traumatic injuries. In these cases, no samples were available for microbiological culture, and bacterial infection was diagnosed based on the presence of coccoid and/or bacillary bacterial structures in hematoxylin and eosin-stained sections and confirmed by Gram histochemical staining.

Deaths due to bacterial sepsis were diagnosed in Opossums 8, 15, 18, and 19. Opossum 8 (Fig. 5–6) had suppurative septic arthritis accompanied by bacterial thromboembolism involving the kidneys, esophagus, liver, heart, brain, and lymph node. *Pseudomonas* sp., *Streptococcus* sp., and *Klebsiella* sp. were isolated from affected tissues. Opossum 15 presented with fibrinosuppurative pericarditis and bacterial pneumonia, from which *Pseudomonas* sp., *Neisseria* sp., and *Micrococcus* sp. were isolated. Opossum 18 had suppurative dermatitis and severe fibrinous peritonitis, with isolation of *Staphylococcus* sp. Opossum 19 exhibited suppurative dermatitis secondary to trauma, microabscesses in the spleen, liver, and kidneys, fibrinous peritonitis, and suppurative pneumonia accompanied by septic emboli within small- and medium-caliber blood vessels. *Enterococcus* sp., *Staphylococcus* sp., and *Pasteurella* sp. were isolated in this case.

Gram-stained histological sections of inflammatory lesions revealed variable numbers of Gram-positive and Gram-negative bacteria, displaying both coccoid and bacillary morphologies, with bacterial abundance varying among cases.

Pathological findings and co-pathologies

The pathological findings associated with the clinical outcome demonstrated the frequent coexistence of traumatic injuries, hemodynamic disturbances, infectious inflammatory lesions, and gastrointestinal and respiratory parasitic infections

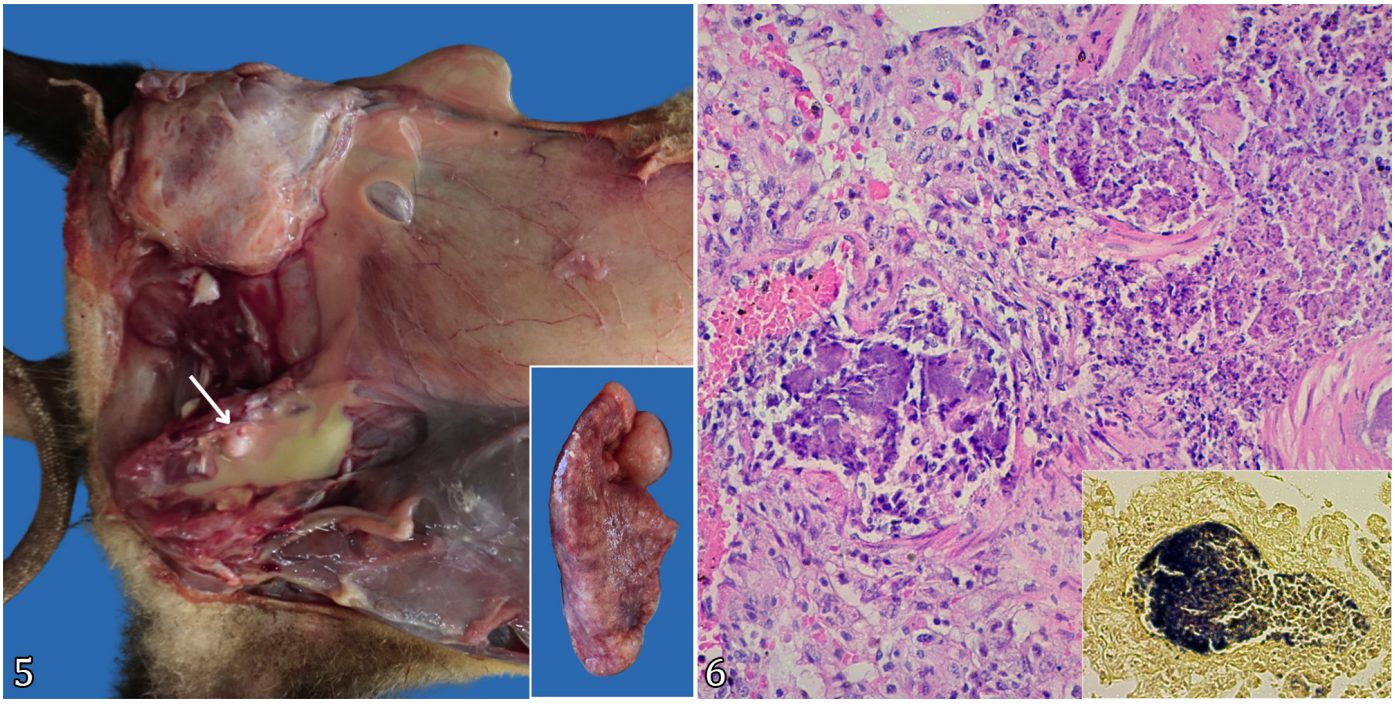


Fig. 5–6. Systemic bacterial thromboembolism associated with septic arthritis in *Didelphis albiventris*, Case 8. (**5**) Section of the right coxofemoral joint revealing the outflow of a yellow-brown, creamy liquid content (pus). Inset: right lung with irregular pleural surface, diffusely light-red coloration, and multifocal, irregular, raised whitish-gray areas consistent with embolic pneumonia. (**6**) Photomicrograph of the lung demonstrating an intravascular embolus composed of fibrinous material and bacterial aggregates, associated with marked neutrophilic inflammatory infiltrate and disruption of the adjacent pulmonary parenchyma. HE, obj. 20x. Inset: Gram-positive bacterial aggregates within the embolus highlighted by Gram staining, obj. 20x.

(Table 1). Lesions caused by physical trauma represented the predominant pathological pattern in the study population and were identified in 21/27 (77,8%) opossums. Within this group, regional hematomas and cavitary hemorrhages were observed in 18 cases. The anatomical regions most frequently affected were the head, abdomen, and thoracic and pelvic limbs, each involved in seven cases (33.3%), followed by the trunk (6/21, 28.6%) and tail (2/21, 9.5%) (Fig. 7).

Based on their morphological characteristics, traumatic injuries were classified as penetrating blunt trauma (9/21, 42.9%), blunt trauma (6/21, 28.6%), or polytrauma (6/21, 28.6%). Multiple fractures, extensive lacerations, visceral rupture, and severe cavitary hemorrhage characterized polytrauma cases. Blunt trauma was characterized predominantly by fractures associated with hematomas and hemoperitoneum. Penetrating blunt injuries consisted of deep lacerations with exposure of muscle and bone, frequently compatible with predation, and, in some cases, perforation of the thoracic cavity accompanied by hemothorax and compressive atelectasis.

Multisystemic parasitic inflammatory lesions were recorded in 18/27 (66.7%) opossums. Except in Cases 1, 2, and 25, these lesions occurred concurrently with traumatic injuries and were interpreted as co-pathologies. The respiratory system was the most frequently affected (15/18, 83.3%), with parasitic granulomatous pneumonia associated with *H. heterostrongylus* (Fig. 8–9). Grossly, the lungs exhibited an irregular pleural surface with multifocal to coalescing white-to-pale-yellow nodules and irregular areas of pulmonary consolidation. Histologically, lesions consisted of multifocal to diffuse granulomatous inflammation, thickening of the alveolar septa, bronchus-associated lymphoid tissue (BALT) hyperplasia, and cross and longitudinal sections of nematodes within the pulmonary parenchyma.

Among opossums with parasitic pneumonia, microscopic examination of the esophagus in 2/15 animals revealed adult *H. heterostrongylus* embedded within the esophageal mucosa without an associated inflammatory response.

Intestinal acanthocephaliasis, characterized by multiple intestinal parasitic granulomas caused by *O. microcephalus* (Fig. 10–11), was identified in 13/18 (72.2%) opossums. In one of these animals, an additional parasitic granuloma was present in the omentum, most likely resulting from parasite migration through the intestinal wall.

Turgida turgida was detected in 15/18 (83.3%) opossums. In 6/15 (40.0%) animals, only a few nematodes were present within the gastric lumen and were not associated with gastric distension or inflammation of the gastric mucosa. In 7/15 (46.7%) animals, larger numbers of nematodes caused gastric distension without significant inflammatory changes, whereas in 2/15 (13.3%) animals, mild eosinophilic gastritis was observed in addition to gastric distension.

Other disorders interpreted as co-pathologies were observed in 21/27 (77,8%) opossums. Inflammatory lesions of undetermined etiology included lymphocytic meningoencephalitis (1/27, 3,7%), lymphoplasmacytic myocarditis (4/27 , 14,8%), interstitial nephritis (2/27, 7,4%), and periportal hepatitis (1/27, 3,7%). These lesions were interpreted as nonspecific co-pathologies and were not considered primary causes of death.

Necrotic lesions secondary to trauma or bacterial sepsis were identified in 12/27 (44,4%) opossums, affecting the skin (7/27, 25,9%), liver (5/27, 18,5%), kidneys, and skeletal and cardiac muscles, with no evidence of isolated primary degenerative disorders.

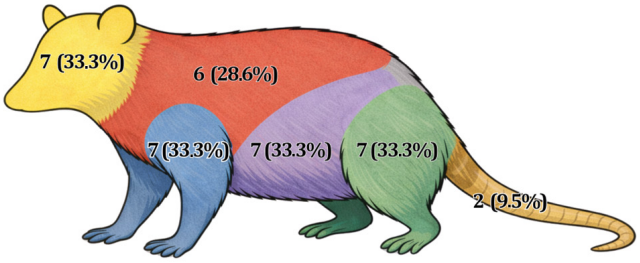
Degenerative hepatic changes included vacuolar hepatocellular degeneration in 8/27 (29.6%) opossums, attributed to glycogen accumulation and/or hepatic lipidosis and interpreted as secondary to metabolic disturbances or systemic stress.

Extracellular deposits consistent with amyloid were observed in 11/27 (40.7%) individuals, predominantly affecting the liver, kidneys, and spleen. Histologically, these deposits consisted of homogeneous eosinophilic hyaline extracellular material of variable abundance that stained positively with Congo red and exhibited birefringence under polarized light. In the kidneys, amyloid deposition involved both the glomeruli and renal interstitium. In the spleen, deposits were located predominantly within the splenic sinusoids. In the liver, amyloid initially accumulated within the space of Disse and, in more advanced cases, was associated with marked sinusoidal thickening.

Hepatic telangiectasia was observed in one opossum (1/27, 3.7%) and was interpreted as an incidental necropsy finding.

DISCUSSION

The findings of this study demonstrate that, in urban environments, the primary cause of death in *Didelphis albiventris* was associated with high-energy traumatic events and predation. Infectious diseases represented the second



	Predation	High-impact
Head	4	3
Thoracic limbs	4	3
Pelvic limbs	3	4
Trunk	4	2
Abdomen	3	4
Tail	1	1

Fig. 7. Schematic illustration of the regional frequency of traumatic lesions in *Didelphis albiventris*. The values indicated within the body of the diagram correspond to the number of records and the frequency of occurrence by anatomical region. These figures do not represent mutually exclusive categories, as some individuals exhibited lesions in multiple body sites. The coloured diagram highlights the topographic distribution of trauma, while the associated columns provide information regarding the causal mechanisms: predation (n = 11) and high-energy impact (n = 10).

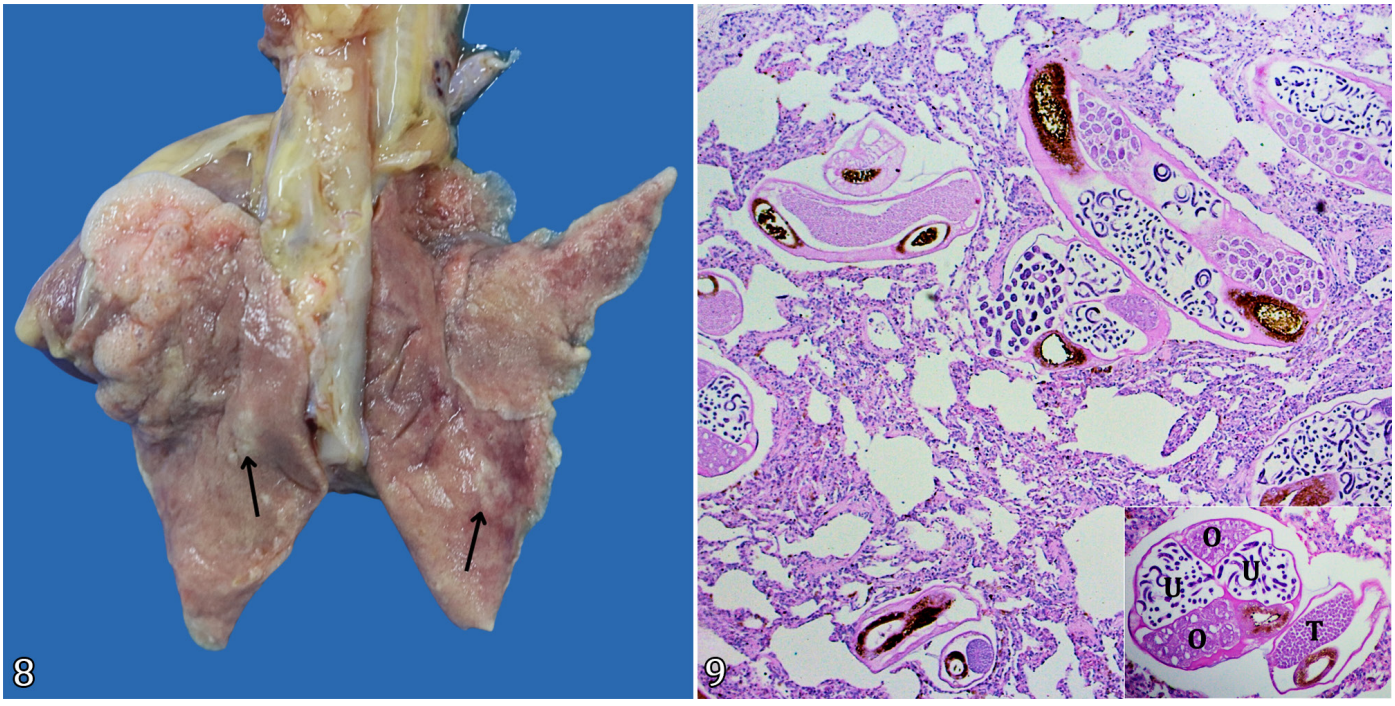


Fig. 8–9. Parasitic granulomatous pneumonia caused by *Heterostrongylus heterostrongylus* in *Didelphis albiventris*, Case 2. (**8**) The lung is consolidated, firm, with an irregular pleural surface containing multifocal to coalescent nodular areas varying from white to pale yellow (arrows). In the cranial lobes, the borders are intensely emphysematous. (**9**) Photomicrograph of the lung showing structural disarray and increased interstitial cellularity due to thickening of the alveolar septa associated with sections of female and male parasites. HE, obj. 5x. Inset: cross-section of parasites showing uteri (U) filled with larvae and ovaries (O) in a female, and testicle (T) in a male. HE, obj. 40x.

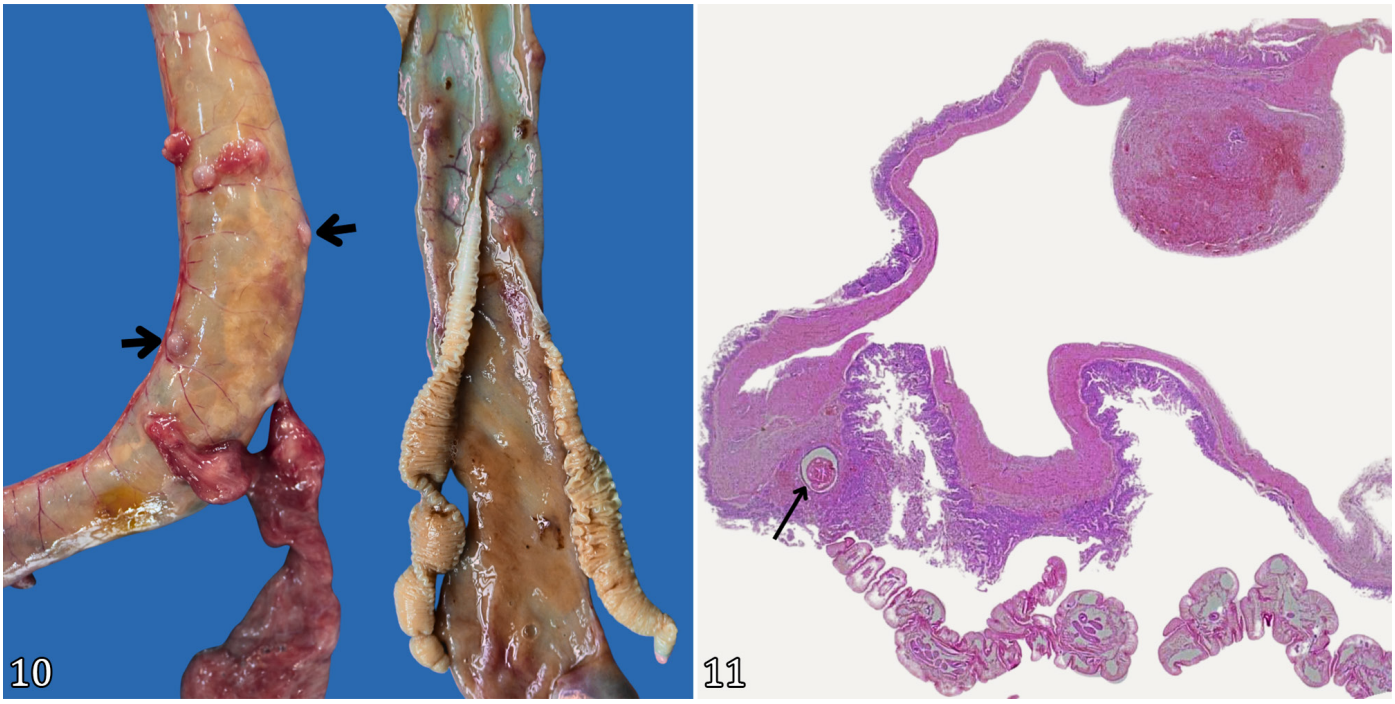


Fig. 10–11. Parasitic granulomas caused by *Oligacanthorhynchus microcephalus* (syn. *O. tortuosus*) in the small intestine of *Didelphis albiventris*, Case 1. (**10**) On the left, the small intestine exhibits multiple transmural, exophytic, reddish nodules (arrows), accompanied by luminal dilatation and thinning of the intestinal wall. On the right, the intestinal lumen contains elongated, multi-ringed acanthocephalans attached to the mucosa, with attachment sites corresponding to the nodules. (**11**) Photomicrograph of the small intestine. The mucosa appears irregular and discontinuous, with the parasite anchored to the mucosal surface (arrow), and a granuloma extending through the serosa. HE, submacroscopy.

most frequent cause of death, particularly bacterial infections with morphological features consistent with sepsis. However, in all affected opossums, these infections occurred secondary to previous traumatic injuries involving disruption of the skin barrier.

Multisystemic parasitic infections, together with associated inflammatory and degenerative lesions, were also identified and contributed to the overall burden of disease in this population. This mortality pattern is consistent with that reported for other synanthropic species exposed to multiple environmental stressors in urbanized landscapes (Hassell et al. 2017, Wilkinson et al. 2018, Bezerra-Santos et al. 2021). In the present study, no consistent seasonal pattern or statistically robust association between the month of occurrence and the primary cause of death could be identified. These findings should therefore be interpreted with caution, given the limited sample size, and longer-term surveillance studies are needed to confirm potential temporal trends.

Traumatic injuries represented the predominant pathological finding, affecting 72.4% of the examined opossums. In 68.9% of the cases, trauma was considered the direct cause of death. It included lesions consistent with high-energy blunt-force trauma, injuries resulting from hostile animal interactions — predominantly perforating-contusive wounds — and polytrauma. Polytrauma occurred with similar frequency in animals showing morphological patterns compatible with high-energy impact and in those with lesions consistent with predation, indicating that multiple traumatic mechanisms should be considered when investigating the cause of death in this species.

Cases classified as blunt-force trauma, particularly those consistent with high-energy impacts, exhibited multiple fractures, visceral rupture, and extensive internal hemorrhage. This lesion pattern is compatible with abrupt kinetic energy transfer, with acute hypovolemic shock considered the underlying mechanism leading to the clinical outcome (Ressel et al. 2016, Navas-Suárez et al. 2022). The severity of blunt-force injuries is determined by the amount of kinetic energy transferred, which depends on the mass and velocity of the impacting object, as well as the impact area and the structural and mechanical properties of the affected tissues (Ressel et al. 2016). According to Ressel et al. (2016), parenchymal organs such as the liver and spleen are more susceptible to rupture due to their limited tolerance to mechanical deformation, explaining severe hemorrhagic events resulting from visceral rupture, such as hemoperitoneum associated with splenic rupture. Although blunt-force trauma is frequently associated with vehicle collisions (Bauni et al. 2017, Navas-Suárez et al. 2022), the classification adopted in this study was based exclusively on morphological patterns; therefore, the specific etiological circumstances could not be conclusively determined in all cases.

Additionally, 33.3% of the opossums presented perforating-contusive and incised injuries compatible with predation by domestic animals, particularly dogs and cats. Attacks by domestic animals represent a significant threat to urban wildlife, with high morbidity and mortality rates documented across different geographic contexts (Chaves et al. 2022, Díaz et al. 2023). Data from wildlife rehabilitation centers indicate that a large proportion of wild vertebrates admitted following cat attacks do not survive (Loyd et al. 2017), and longitudinal studies have confirmed the cumulative impact of domestic dogs and cats on wildlife populations (Rufino et al. 2025). Morphologically, dog bites produce crushing injuries associated with irregular lacerations, necrosis, and damage to muscular and osseous tissues due to mandibular force (Haddad Junior 2022), a pattern consistent with the lesions observed in the present study.

Age distribution demonstrated that trauma affecting dependent young and juveniles represented an important cause of death, suggesting increased vulnerability of younger individuals to abrupt environmental events, possibly related to reduced locomotor experience and lower escape capacity associated with age, as described in other mammalian species (Young et al. 2022). Furthermore, trauma affecting adult females may indirectly impact offspring survival through starvation and hypoglycemia, as observed in one case in this study, highlighting the indirect consequences of maternal mortality on dependent young.

In human medicine, sepsis is broadly defined as life-threatening organ dysfunction resulting from a dysregulated host response to infection (Singer et al. 2016). In veterinary medicine, although universal diagnostic criteria have not yet been established, death is a common clinical outcome of septic conditions in both domestic and wild animals (Relja et al. 2020). Vascular bacterial invasion promotes endothelial injury, activation of inflammatory cascades, microthrombus formation (vascular bacterial dissemination), and impaired tissue perfusion, ultimately leading to multiple organ dysfunction (Shi & Pamer 2011, Chen et al. 2018, Maneta et al. 2023). In the four cases in which lesions consistent with sepsis were identified, microbiological examination of submitted samples revealed bacterial genera including *Pseudomonas*, *Klebsiella*, *Staphylococcus*, *Enterococcus*, *Pasteurella*, *Streptococcus*, *Neisseria*, and *Micrococcus*. These microorganisms are recognized as opportunistic agents capable of causing septic conditions in humans and animals (Chun et al. 2015, Sousa et al. 2022, Sousa et al. 2025, Maneta et al. 2023, Mu et al. 2023). The microbiological findings corroborated the morphological identification of Gram-positive and Gram-negative bacterial structures, including coccoid and bacillary forms, observed within tissue sections exhibiting septic lesions in the four *D. albiventris* evaluated in this study.

The observed microbial heterogeneity supports the interpretation that mixed bacterial infections represented secondary complications associated with traumatic injuries. In these cases, sepsis occurred in opossums with predation-related injuries, which likely served as portals of entry for bacteria, resulting in local infection and subsequent systemic dissemination (Haddad Junior 2022, Atan et al. 2025). In the present study, immediate death was a frequent consequence of trauma; however, individuals surviving the initial event may paradoxically be at increased risk of progressive infection leading to sepsis. Therefore, both direct inoculation of microorganisms from the predator's oral cavity and secondary exposure to environmental bacteria represent plausible mechanisms of infection, potentially progressing to bacteremia and sepsis. These findings indicate that synanthropic wildlife, living in close association with human waste and contaminated environments, may serve as indicators of the circulation of potentially pathogenic bacteria in urban areas. Further investigations, including antimicrobial susceptibility testing and

molecular characterization of resistance genes, are warranted to better assess associated risks. Additionally, these findings reinforce the importance of surveillance approaches within the One Health framework.

Parasitic infections were frequent and were detected even in individuals in whom the primary cause of death was attributed to trauma or bacterial sepsis. The high prevalence of parasites in opossums is consistent with continuous exposure to parasitic life cycles in anthropized environments (Bezerra-Santos et al. 2021, Chagas et al. 2024, Sogari et al. 2025), reinforcing the chronic nature of the health challenges faced by this population. Among helminth-associated diseases, granulomatous pneumonia caused by *Heterostrongylus heterostrongylus* was particularly relevant, as well as acanthocephaliasis caused by *Oligacanthorhynchus microcephalus,* which resulted in extensive mural, transmural, and omental parasitic granulomas. Additionally, *Turgida turgida* was frequently observed within the gastric lumen, generally associated with gastric distension and occasionally with eosinophilic gastritis. Although the inflammatory response was mild and inconsistent, the high parasite burden suggests a potential role as a cofactor in the deterioration of health status in this species, and clinical monitoring would be valuable to better understand its significance. These findings corroborate previous studies indicating that opossums of the genus *Didelphis* harbor high parasite diversity, with many helminths acting as co-pathogens or as common parasitic infections, frequently associated with chronic inflammatory processes or mild and subclinical alterations in free-ranging animals (Zabott et al. 2017, Bezerra-Santos et al. 2021, Chagas et al. 2024).

Among other disorders, inflammatory lesions of undetermined etiology suggest persistent immune challenges that may compromise physiological reserves and increase susceptibility to acute events (Relja et al. 2020). Degenerative hepatic changes, including glycogen accumulation and hepatic lipidosis, were interpreted as reactive alterations associated with metabolic stress or systemic responses to injury (Relja et al. 2020). Multifocal necrotic lesions affecting multiple organs were considered secondary changes associated with trauma, hypoxia, or sepsis, representing part of the expected spectrum of acute hemodynamic instability (Relja et al. 2020). Extracellular deposits consistent with amyloid, observed in 11/29 (37.9%) individuals, may be associated with persistent inflammatory stimulation (Penticoff et al. 2021). Hepatic telangiectasia was interpreted as an incidental finding without evident clinical relevance. Hemodynamic alterations, including congestion and multisystemic edema, were frequently secondary to terminal events and did not represent independent primary disease processes.

In this study, lymphoplasmacytic myocarditis was observed in four cases and was characterized by multifocal inflammatory infiltrates of variable intensity, composed predominantly of lymphocytes and plasma cells, with fewer macrophages. These infiltrates were associated with hyaline degeneration, necrosis, and loss of cardiomyocytes, as well as mild interstitial fibrosis. This inflammatory pattern is compatible with chronic processes described in marsupials of the genus *Didelphis*, including trypanosomiasis-associated lesions (Teixeira et al. 2011, Madureira et al. 2026, Pontarolo et al. 2025).

Although intracytoplasmic pseudocysts containing *Trypanosoma* spp. amastigotes were not identified, the absence of visible parasitic forms does not exclude the possibility of infection. Recent studies have reinforced the role of didelphid marsupials as potential reservoirs of *Trypanosoma cruzi* and other trypanosomatids, including in wild-urban interface areas. Madureira et al. (2026) documented the occurrence of *T. cruzi*, *Trypanosoma lainsoni*, and *Leishmania* spp. in *D. albiventris* and other small mammals in Minas Gerais, highlighting the potential risk of zoonotic transmission in fragmented landscapes. Similarly, Pontarolo et al. (2025) confirmed the presence of *T. cruzi* TcI in *D. albiventris* from southern Brazil, reinforcing the role of these animals as synanthropic hosts.

Therefore, molecular investigations are essential to clarify the etiology of these lesions (Macchiaverna et al. 2025). Furthermore, integrating parasitological, molecular, and epidemiological approaches is crucial for assessing the risk of transmission to humans and domestic animals in areas where interactions between wildlife and urban environments are increasing.

CONCLUSIONS

In *Didelphis albiventris* from urban and peri-urban areas of Cuiabá, the main causes of death were high-energy traumatic events, including vehicle collisions and attacks by domestic animals, followed by bacterial sepsis. Chronic conditions, such as parasitic pneumonia and intestinal acanthocephaliasis associated with systemic amyloid deposition, were also identified and may reflect persistent inflammatory stimulation resulting from continuous exposure to infectious agents, contributing to disease burden in the studied population.

Morphological evaluation through gross and histopathological examination represents an important tool for identifying both immediate causes of death and associated co-pathologies, supporting the health monitoring of a species that plays a relevant role as a sentinel at the wildlife-environment interface within the One Health framework.

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Credit author statement.- Julieni Bianchi-Morais, Edson Colodel, and Nadia Antoniassi – study design. Julieni Bianchi-Morais, Edson Colodel, Luis Quillas, Wuglenia Martins-Silva, and Marcos Souza – necropsies, laboratory processing, and pathological analyses. Julieni Bianchi-Morais and Geovanna Taináh Martins da Silva – database organization, tabulation of necropsy information, and evaluation of macroscopic photodocumentation. Ana Rebecca Maia Araujo and Valéria Dutra – microbiological analyses and bacterial isolation. Richard Pacheco and Dirceu Ramos – parasitological analyses and taxonomic identification of parasites. Edson Colodel – project administration.

Data availability statement.- The data from this research are stored in the archives of LPV-HOVET-UFMT, Cuiabá, and will be made available upon request to the corresponding author.

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