






Outbreak of neonatal mortality in beef cattle associated with *Ateleia glazioveana* (Leg. Papilionoideae) poisoning in southern Brazil¹

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ABSTRACT.- Wiggers FHRSS, Menegatt JCO, Silveira BO, Melo SMP, Perosa FF, Keil E, Moraes JTR, Sanches MVG, Guagnini FS, Acatrolli G, Driemeier D, Panziera W. **Outbreak of neonatal mortality in beef cattle associated with *Ateleia glazioveana* (Leg. Papilionoideae) poisoning in southern Brazil.** *Pesquisa Veterinária Brasileira* 46:e07802, 2026. Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: weldenpanziera@yahoo.com.br

Ateleia glazioveana (Leg. Papilionoideae) is a cardiotoxic plant widely distributed in southern Brazil, where sporadic outbreaks of poisoning in cattle have been reported. Although neonatal mortality is a described clinical outcome in cattle, detailed epidemiological data and lesions associated with this form of intoxication remain scarce. This study reports an outbreak of neonatal mortality in a beef cattle herd in Rio Grande do Sul, Brazil, and characterizes the epidemiological, pathological, and immunohistochemical findings. The herd experienced one abortion and the loss of ten calves, all of which were born weak and died within three days. Two neonates were necropsied, revealing pale myocardial areas extending from the visceral pericardium to the endocardium. Histological analysis revealed multifocal myocardial fibrosis and cardiomyocyte necrosis, as well as vacuolization of the cerebral white matter. Masson's trichrome staining highlighted myocardial interstitial fibrosis, and anti-troponin C immunohistochemistry showed decreased or absent labeling in necrotic cardiac fibers. Polymerase chain reaction (PCR) testing for bovine viral diarrhea virus and *Neospora caninum* was negative. The presence of numerous sprouting *A. glazioveana* specimens in the pasture, with evidence of consumption and compatible lesions, supported the diagnosis. This report emphasizes *A. glazioveana* as a cause of reproductive losses and neonatal mortality in endemic regions, particularly during the plant sprouting phase.

INDEX TERMS: Abortion, cardiotoxicity, immunohistochemistry, toxic plants, timbó, cattle.

RESUMO.- [Surto de mortalidade neonatal em bovinos de corte associado à intoxicação por *Ateleia glazioveana* (Leg. Papilionoideae) no Sul do Brasil.] *Ateleia glazioveana* (Leg. Papilionoideae) é uma planta cardiotóxica amplamente

distribuída no Sul do Brasil, onde surtos esporádicos de intoxicação têm sido relatados em bovinos. Embora a mortalidade neonatal seja um desfecho clínico descrito na espécie, dados epidemiológicos detalhados e lesões associadas a essa forma de intoxicação ainda são escassos. Este estudo descreve um surto de mortalidade neonatal em um rebanho bovino de corte no Rio Grande do Sul, Brasil, e caracteriza os achados epidemiológicos, patológicos e imuno-histoquímicos. O rebanho apresentou um aborto e dez casos de bezerros nascidos fracos e que morreram dentro de três dias após o nascimento. Dois desses bezerros neonatos foram necropsiados e observou-se áreas pálidas no miocárdio, estendendo-se do pericárdio visceral ao endocárdio. Na análise histológica, observaram-se fibrose

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miocárdica multifocal e necrose de cardiomiócitos, além de vacuolização da substância branca no encéfalo. A coloração de tricrômico de Masson evidenciou fibrose intersticial cardíaca, e a análise imuno-histoquímica anti-troponina C demonstrou redução ou ausência de marcação em fibras cardíacas necróticas. Os testes de reação em cadeia da polimerase (PCR) para vírus da diarreia viral bovina e *Neospora caninum* foram negativos. A presença de numerosos exemplares de *A. glazioveana* em fase de brotação na pastagem, com evidência de consumo, associada às lesões compatíveis, suportou o diagnóstico. Este relato enfatiza *A. glazioveana* como causa de perdas reprodutivas e mortalidade neonatal em regiões endêmicas, especialmente durante períodos de brotação da planta.

TERMOS DE INDEXAÇÃO: Aborto, cardiotoxicidade, imuno-histoquímica, plantas tóxicas, timbó, bovinos.

INTRODUCTION

Ateleia glazioveana (Leg. Papilionoideae), popularly known as “Timbó”, is a deciduous tree with an average height of 5 to 15 meters, characteristic of the secondary vegetation of the seasonal deciduous forest, mainly in the basins of the Uruguay and Paraguay rivers. It has little commercial value and is recommended for the recovery of degraded ecosystems and for shading pastures (Carvalho et al. 2003). This plant is widely found in the western region of Santa Catarina and the northwestern region of Rio Grande do Sul, where it is classified as invasive, due to its vigorous reproductive and vegetative growth (Carvalho et al. 2003, Tokarnia et al. 2012). Traditionally, poisoning by this plant shows three clinical forms: abortion, neurological signs, and heart failure, the latter being the most common and widely studied (Stolf et al. 1994, Gava et al. 2001, Tokarnia et al. 2012).

Experimentally, the clinical forms depend on the amount ingested and the growth stage of the plant (Stolf et al. 1994, Gava et al. 2001). In the cardiac form, the heart lesions are chronic, characterized mainly by areas of myocardial fibrosis, in which sudden death may occur following movement or physical exertion (Gava et al. 2001, Tokarnia et al. 2012). The disease developed experimentally when the ingested doses were divided into daily doses of 2.5–7.5 g/kg, until a total of 100–150 g/kg was reached (Gava et al. 2001). In the abortion form, pregnant cows aborted between six and sixteen days after ingesting single or divided doses of 35 g/kg, and no other clinical signs were observed in the cows (Stolf et al. 1994). However, when receiving divided doses of 10 g/kg/day, the cows developed the neurological form, with encephalic lesions of *status spongiosus*, showing an overlap between the clinical presentations (Gava et al. 2001). The exact toxic compound in *A. glazioveana* is unknown; however, studies using gas chromatography have suggested the presence of flavones, indicating that its toxicity may be linked to this chemical class (Yokosuka et al. 2007, Fronza et al. 2025).

Different conditions predisposed to spontaneous poisoning include, primarily, starvation and the transfer of animals to pastures containing numerous *A. glazioveana* plants (Tokarnia et al. 2012). In addition, outbreaks are sporadic and reported at many-year intervals (around five-year intervals) due to the tree’s seeding, which produces many sprouts that are readily ingested because they are within the animals’ reach (Stolf et al. 1994, Tokarnia et al. 2012). Although it is one of the most common toxic plants in southern Brazil, reports of

neonatal mortality caused by *A. glazioveana* are scarce and, as previously mentioned, may occur only at long intervals. Adult animals often show no clinical signs but may have abortion at any stage of gestation or lead to the birth of weak calves that die a few days after birth (García y Santos et al. 2004).

The diagnosis of *A. glazioveana* poisoning in neonatal calves can be challenging because it requires a combination of epidemiological, clinical, and pathological aspects. Furthermore, this toxicosis is relevant, as it may represent a differential diagnosis for infectious diseases in cases of abortion and stillbirth in cattle. Therefore, the aim of this study is to describe the epidemiological, histopathological, and immunohistochemical features of a neonatal mortality outbreak caused by *A. glazioveana* in cattle in Rio Grande do Sul, characterized by concurrent cardiac and neurological lesions.

MATERIALS AND METHODS

Ethical approval. The procedures described here were conducted in accordance with the Research Committee of the Faculty of Veterinary of the “Universidade Federal do Rio Grande do Sul” (UFRGS), Brazil (no. 37662). Samples were submitted as part of routine clinical diagnostic testing following institutional and national guidelines.

The epidemiological data, including management practices, feeding, number of affected animals, and clinical signs of the outbreak, were obtained through interviews with the veterinarian and the farm owner. Two neonatal calves that died spontaneously were submitted for necropsy to the “Setor de Patologia Veterinária” (SPV) of the UFRGS. Tissue samples (skeletal muscles, heart, lymph nodes, spleen, liver, pancreas, rumen, reticulum, omasum, abomasum, small and large intestines, kidneys, adrenals, urinary bladder, lungs, and encephalon) were collected, fixed in 10% buffered formalin, routinely processed for histopathology, and stained with hematoxylin and eosin (HE). Fragments of heart were subjected to Masson’s trichrome (MT) staining according to a standard deparaffinization protocol, followed by the application of Weigert’s iron hematoxylin, fuchsin, phosphomolybdic acid, and aniline blue. Additionally, cardiac tissue sections were submitted to anti-troponin C immunohistochemistry (IHC) as described by Zorzan et al. (2024).

Fresh samples of spleen, heart, and brain from both calves were collected during necropsy and submitted to polymerase chain reaction (PCR) analysis for the detection of bovine viral diarrhoea virus (BVDV), as described by Bianchi et al. (2016), and *Neospora caninum*, as described by Macedo et al. (2017). Brain sections were stained with the Luxol fast blue (LFB) special stain. This stain visualizes myelin loss in the central nervous system, evidenced by non-colored areas in the white matter.

Samples of *Ateleia glazioveana* were collected from the same farm where the outbreak occurred and were subsequently identified (ICN 215630) at the Botanic Laboratory of the UFRGS, Brazil.

RESULTS

The cases occurred in the municipality of Palmeira das Missões, Rio Grande do Sul (30° 4’ 51” S, 51° 1’ 22” W), in May 2022. The herd consisted of 652 Angus beef cattle, of which 10 cows (between three and eight years of age) had a history of giving birth to weak, below-average-weight calves that failed to suckle and died between two and three days after birth. There was also one case of abortion, totaling 11 affected animals. No previous outbreaks of abortion and neonatal death were related to the property. Two neonatal Angus calves that died spontaneously were submitted for

necropsy at SPV-UFRGS (Calves 1 and 2, both three days old, one male and one female). During March (autumn), the cows were introduced into an area of native pasture containing numerous specimens of *Ateleia glazioveana* (Fig. 1), randomly distributed throughout the pasture, ranging in height from sprouts of only a few centimeters to adult trees up to 10 meters. The *A. glazioveana* trees were in the seeding and sprouting phases and showed moderate to severe evidence of consumption by the animals. In this area, there was food scarcity, and the cows were supplemented only with free-choice sodium and received no other source of feed. The dams received reproductive vaccination prior to the breeding season against infectious bovine rhinotracheitis, bovine viral diarrhea, *Campylobacter* spp., *Leptospira interrogans*, and *Histophilus somni*.

At necropsy of both calves, lesions were predominantly observed in the heart. There were mild to moderate pale areas on the visceral pericardial surface, more evident in the right ventricle (Fig. 2). These areas extended deeper into the myocardium on cut section, reaching the endocardial surface, and had a moderate firm consistency. In addition, Calf 1 showed mild deposition of gelatinous and translucent material (edema) in the subcutaneous tissue of the submandibular region. No other gross lesions were noted in the remaining organs. Histopathological examination of the heart revealed mild to moderate multifocal areas of necrotic cardiomyocytes characterized by hypereosinophilic cytoplasm and absent, pyknotic, or karyorrhectic nuclei. Furthermore, it was observed multifocal areas of proliferation of fibrous connective tissue dissecting cardiomyocytes, which were moderate in Calf 1 and mild in Calf 2 (Fig. 3). Predominantly in the cerebellum and telencephalic cortex (frontal, occipital and parietal) of both animals, multifocal randomly distributed areas of moderate vacuolization of the white matter were evident, varying approximately from 2 to 60 micrometers in diameter (Fig. 4). Despite the encephalic lesions, no related neurological signs were observed in the two neonatal Angus calves. No microscopic lesions were detected in the skeletal muscle or any other tissues from both calves.

Masson's trichrome staining of heart sections revealed areas of fibrous connective tissue proliferation among the myocardial fibers (Fig. 5). Immunohistochemical analysis of anti-troponin C showed moderate to marked multifocal areas of reduction or absence of troponin C immunolabeling in the cytoplasm of necrotic cardiomyocytes (Fig. 6). Complete absence of immunolabeling was observed in the most severely affected cardiac lesions identified histologically. Brain sections showed normal histochemical staining with Luxol fast blue staining in the white matter of both calves, indicating no loss of myelin. Furthermore, all tissue fragments tested by PCR were negative for both BVDV and *Neospora caninum*.

DISCUSSION

Diagnosis of abortion due to *Ateleia glazioveana* poisoning was made based on the macroscopic and microscopic findings in two necropsied animals, and epidemiological evidence. Epidemiological data had a crucial role in guiding the diagnosis, as multiple sprouting *A. glazioveana* specimens were found in the pasture, within the reach of the dams and showing evidence of consumption. Moreover, these findings occurred along with seasonal drought and food scarcity, which increase

the likelihood of plant consumption (Stolf et al. 1994, Tokarnia et al. 2012). Studies have shown that the highest incidence of abortion due to *A. glazioveana* poisoning occurs between November and May, a period when pregnant cows ingest the plant during the sprouting stage (Gava et al. 2001, Tokarnia et al. 2012). This aligns with the cases reported here, which occurred in May. Besides, it occurs at irregular intervals of several years, during which the plant produces a large number of seeds that sprout and are consumed by animals when within reach (Tokarnia et al. 2012). However, the reason for this biological behavior remains unclear.

The clinical manifestations associated with *A. glazioveana* poisoning are variable. They may include abortion and the birth of weak calves when pregnant cows ingest doses exceeding 22 g/kg of the plant (Stolf et al. 1994). In a study conducted by Gava et al. (2001), an outbreak of *A. glazioveana* poisoning in pregnant cows resulted in 30% mortality among adult animals and a 100% abortion rate. However, in some cases, adult animals may only exhibit reproductive effects (Tokarnia et al. 2012). In the present study, no clinical signs consistent with *A. glazioveana* poisoning were observed in cows, and the abortion rate was low compared to previous reports (Stolf et al. 1994, Gava et al. 2001).

Gross lesions in *A. glazioveana* poisoning in neonate calves are usually mild or absent (García y Santos et al. 2004). In our study, they were observed primarily in the heart and consisted of mild-to-moderate, multifocal white areas, interspersed within the myocardium, similar to the findings described by García y Santos et al. (2004). Histopathological examination revealed that those areas were characterized by fibrous connective tissue proliferation surrounded by degenerated and necrotic cardiomyocytes. Cardiac fibrotic and necrotic areas were further highlighted by MT staining and anti-troponin C IHC, respectively. Another important pathological feature, previously reported only once in a case of neonatal mortality in a calf, is the concurrent development of cardiac and encephalic lesions, the latter consisting of vacuolization within the white matter of the cerebellum and cerebral cortex (García y Santos et al. 2004). In our study, both calves exhibited simultaneous cardiac and neurological lesions, strongly supporting the diagnosis of *A. glazioveana* poisoning.

Masson's trichrome (MT) histochemical staining and IHC are not commonly used to identify lesions in cases of *A. glazioveana* poisoning, despite their usefulness as tools to improve diagnostic accuracy. In the present study, positive MT staining aided in the identification of interstitial fibrosis in the heart and highlighted chronic findings, an important feature for ruling out differential diagnoses. IHC for troponin C facilitates the detection of cardiomyocyte injury, as necrotic cells decrease the immunolabeling (Pavarini et al. 2012, Bandinelli et al. 2014, Zorzan et al. 2024). In this study, troponin IHC revealed reduced or absent labeling in areas of cardiac fiber degeneration and necrosis, as well as in regions where myocardium was replaced by fibrous connective tissue. These variations in the intensity of troponin C IHC labeling may be associated with troponin release peaks, which are greater between 48 and 72 hours after cardiac injury (Costa et al. 2016).

Ateleia glazioveana is classified as a cardiotoxic plant that causes subacute to chronic lesions, with *Niederzuehlla* (*Tetrapteryx*) spp. being its main differential diagnosis (Tokarnia

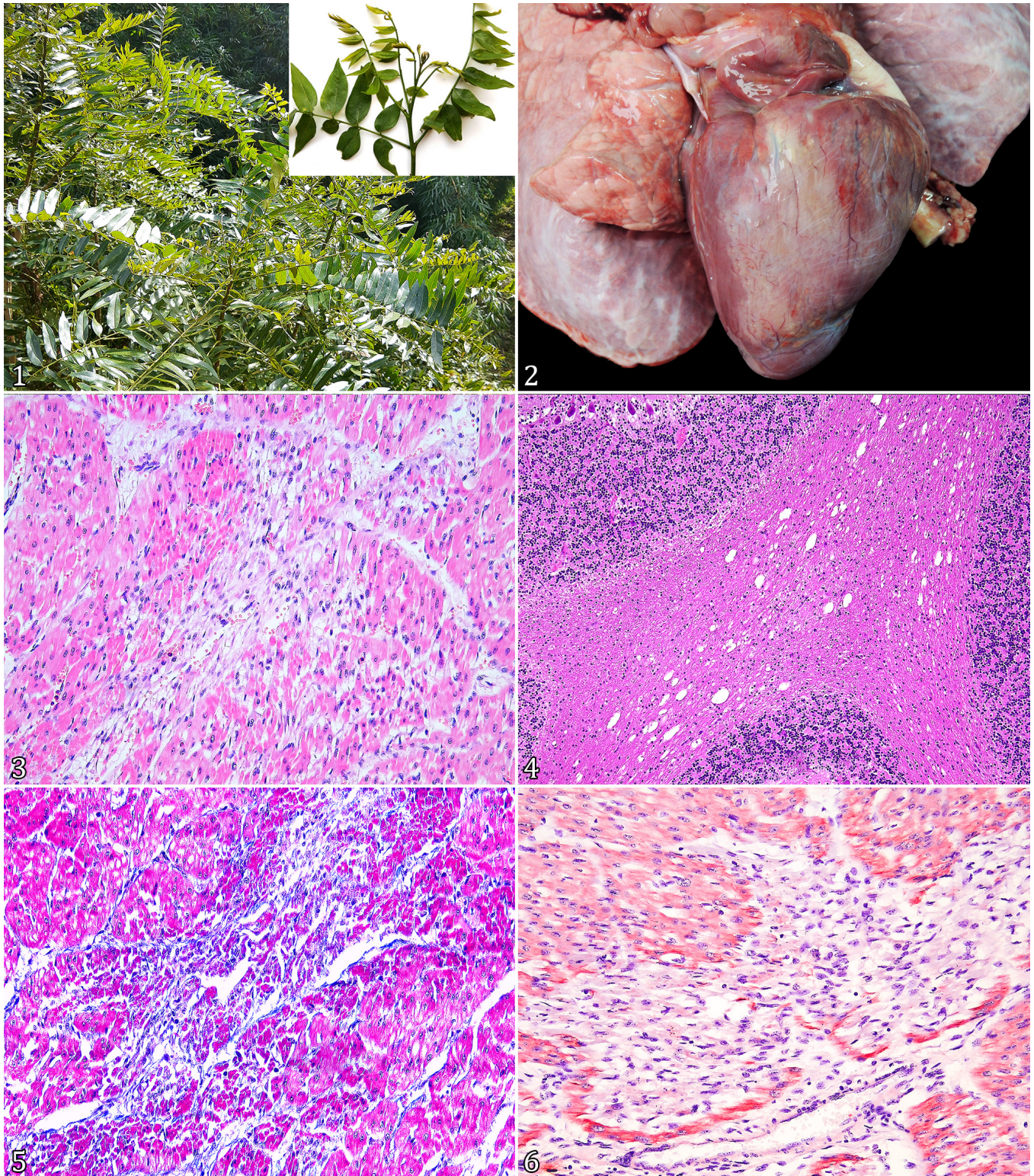


Fig. 1-6. Epidemiological and pathological findings in an outbreak of neonatal mortality in beef cattle associated with poisoning by *Ateleia glazioveana*. (1) *Ateleia glazioveana* specimens that were found in the paddock where cows were kept. Inset: closer look at the leaves. (2) Heart. A focally extensive pale area is visible on the visceral pericardial surface of Calf 1, affecting the right ventricle. (3) Heart. The focal area of fibrous connective tissue proliferation, dissecting cardiomyocytes, is evident. The affected region is surrounded by necrotic cardiomyocytes, characterized by hypereosinophilic cytoplasm and absent, pyknotic, or karyorrhectic nuclei. Calf 1. HE, obj. 200x. (4) Cerebellum. Multifocal and moderate white matter vacuolization. Calf 2. HE, obj. 100x. (5) Heart. Proliferation of fibrous connective tissue among myocardial fibers is highlighted by Masson's trichrome (MT) stain. Calf 1. MT, obj. 200x. (6) Heart. Anti-troponin C IHC demonstrating a marked absence of cytoplasmic immunolabeling in necrotic cardiomyocytes. Calf 1. Anti-troponin C IHC, DAB chromogen (brown color), obj. 200x.

et al. 2012, Riet-Correa et al. 2024). Both plants can affect adult animals and fetuses, inducing areas of cardiac fiber degeneration and necrosis, as well as myocardial fibrosis. Additionally, both intoxications may cause vacuolization of the brain's white matter, resulting in neurological clinical signs (Gava et al. 2001, Peixoto et al. 2011). The primary method to differentiate between the two plants is epidemiology: *A. glazioveana* is abundant in the western region of Santa Catarina and the northwestern region of Rio Grande do Sul, whereas *Tetrapteryx* spp. is restricted to the southeastern region of Brazil (Tokarnia et al. 2012). Other plants reported as causes of abortion in cattle include *Enterolobium contortisiliquum*, *Enterolobium gummiferum*, *Stryphnodendron coriaceum*, *Stryphnodendron obovatum*, and *Stryphnodendron fissuratum*. However, none of these induce lesions similar to those caused by *A. glazioveana*, and they are distributed in different regions of the country (Riet-Correa et al. 2012, Tokarnia et al. 2012). Poisoning by *Sida carpinifolia* primarily affects the fetal central nervous system, with microscopic lesions including vacuolization of neuronal cytoplasm, especially cerebellar Purkinje cells, as well as tubular epithelial cells of the kidney and thyroid follicular cells (lysosomal storage disease) (Pedroso et al. 2012, Reis et al. 2019). The pattern of lesion caused by *A. glazioveana* in the central nervous system, characterized mainly by white matter vacuolization, combined with the absence of *S. carpinifolia* on the farm where the outbreak occurred, allows differentiation between these two etiologies (Miller & Porter 2022).

Other differential diagnoses for abortion and neonatal mortality in cattle include bacterial etiologies such as *Brucella* spp., *Campylobacter* spp., and *Leptospira* spp.; viral agents such as BVDV; and protozoa, primarily *Neospora caninum* in cattle (Schlafer & Foster 2016). Notably, *N. caninum* is the main agent associated with abortions in studies conducted in Brazil (Corbellini et al. 2006, Antoniassi et al. 2013). In addition to the negative PCR results in the present study, this pathogen typically causes non-suppurative myocarditis and encephalic necrosis (Dubey et al. 2006, Antoniassi et al. 2013), lesions that were not observed here. Copper deficiency might be another differential diagnosis based on histopathological lesions in the brain (Tokarnia et al. 2010). However, neurological lesions in such cases are described mainly in lambs with enzootic ataxia and rarely in calves (Miller & Porter 2022). Moreover, LFB staining in cases of copper deficiency typically reveals reduced myelin sheaths, a finding not observed in the present study. In addition, copper deficiency does not cause the pronounced heart lesions in neonates (Tokarnia et al. 2010).

Distinct etiologies of toxic myodegeneration include vitamin E and selenium deficiency, excessive ionophore ingestion and intoxication with *Senna* spp. or gossypol (a substance present in cotton seeds). Nevertheless, no specimens of *Senna* spp. were observed, and there was no history of exposure to ionophores or cottonseed (gossypol). Vitamin E and selenium deficiencies were considered unlikely given the animals' age. In addition, these etiologies are rarely reported as causes of congenital cardiac lesions in cattle. Therefore, they were excluded from the differential diagnosis (Yamini et al. 2005, Takeuti et al. 2011, Valentine 2022).

CONCLUSION

This study describes a sporadic outbreak of neonatal mortality in cattle associated with *Ateleia glazioveana* poisoning. The diagnosis was supported by epidemiological findings, reproductive clinical signs, necropsy, histopathology, and ancillary exams. Masson's trichrome histochemical staining and anti-troponin C immunohistochemistry were valuable diagnostic tools for confirming heart lesions and supporting the diagnosis. These findings underscore the importance of recognizing *A. glazioveana* as a potential cause of reproductive losses, particularly during its sprouting phase, in endemic regions.

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Data availability statement. - The data presented are available in the report archives of the "Setor de Patologia" of the "Universidade Federal do Rio Grande do Sul" (SPV-UFRGS) and will be made available upon request.

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