# Spontaneous poisoning in sheep caused by pseudofruits of *Hovenia dulcis* (japanese grape)

# Intoxicação espontânea em ovinos causada por pseudofrutos de *Hovenia dulcis* (uva japonesa)

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**ABSTRACT**
An outbreak of spontaneous intoxication by the pseudofruits of the japanese grape (*Hovenia dulcis*) was observed in a flock of 270 sheep in Rio Grande do Sul, Brazil, resulting in the death of nine animals. The clinical picture was characterized by digestive, nervous, and respiratory alterations with progression to death after 15 to 48 hours. The main microscopic findings were hepatic necrosis, necrosis of the rumen and abomasum mucosa, pulmonary edema and atelectasis, kidney degeneration, cerebral spongiosis, and vacuolization of Purkinje neurons. *Hovenia dulcis* is wide spread in Brazil, especially in the south of the country. However, this is the first report of spontaneous poisoning in sheep caused by its pseudofruits.

**Keywords**: sheep, toxic diseases, *Hovenia dulcis*, japanese grape, poisonous plants.

**RESUMO**
Um surto de intoxicação espontânea pelos pseudofrutos da uva japonesa (*Hovenia dulcis*) foi observado em um rebanho de 270 ovelhas no Rio Grande do Sul, Brasil, resultando na morte de nove animais. O quadro clínico foi caracterizado por alterações digestivas, nervosas e respiratórias com evolução para óbito após 15 a 48 horas. Os principais achados microscópicos foram necrose hepática, necrose da mucosa do rúmen e abomaso, edema pulmonar e atelectasia, degeneração renal, espongiose cerebral e vacuolização dos neurônios de Purkinje. A *Hovenia dulcis* está amplamente difundida no Brasil, principalmente no sul do país. No entanto, este é o primeiro relato de intoxicação espontânea em ovinos causada por seus pseudofrutos.

**Palavras-chaves**: ovinos, doenças tóxicas, *Hovenia dulcis*, uva japonesa, plantas tóxicas.

## 1 INTRODUCTION

*Hovenia dulcis* (Rhamnaceae) is native to China, Japan, Korea, and the Himalayas (CARVALHO, 1994; MAIEVES and RIBANI, 2013). This plant, introduced to Brazil in 1987, is currently widely distributed and adapted to the country's soil and climate, especially in its southern region (SELLE et al., 2009). *Hovenia dulcis* flowers from August to February, and the fruiting season lasts from March to October. The tree has a

globose capsular fruit attached to a thickened and fleshy fruit stalk, which is very palatable at the end of its maturation. The peduncle, botanically a pseudofruit, is commonly referred to in Brazil as japanese grape (CARVALHO, 1994; COZZO, 1960).

Pharmacological studies of the pseudofruits, seeds, leaves, and peels of *H. dulcis* have shown that they contain abundant quantities of simple phenols, saponins, and tannins, with smaller amounts of steroids, flavonoids, and xanthones; however, the active ingredient responsible for the pathogenesis of intoxication remains unknown (ALVARENGA, 2012; PINTO, 2013). In Brazil, spontaneous poisoning by *H. dulcis* pseudofruits was first described in 1998 in the state of Rio Grande do Sul after the deaths of six goats showing clinical signs and neurological and gastrointestinal anatomopathological findings (COLODEL et al., 2004). In the following years, experimental toxicity studies on *H. dulcis* pseudofruits in bovines reported clinical and pathological results similar to those produced by hepatotoxic plants (CARDOSO et al., 2015; GAVA et al., 2004). Spontaneous infections have also been reported in bovines, with clinical, histopathological, and necropsy findings that are mainly limited to the nervous, respiratory, and digestive systems and liver (BERNARDI et al., 2016), which is consistent with the results of experimental studies (CARDOSO et al., 2015).

Empirical information obtained from sheep farmers and veterinarians from southern Brazil suggests that *H. dulcis* is toxic to sheep, goats, and bovines. However, no studies have described the clinical and anatomopathological picture of spontaneous intoxication in these animals. Therefore, the present study aims to describe, for the first time, the epidemiological, clinical-toxicological, and anatomopathological aspects of an outbreak of spontaneous intoxication caused by *H. dulcis* pseudofruits in sheep.

The outbreak of intoxication examined in this study occurred in on a farm in northern Rio Grande do Sul, Brazil. During the outbreak, nine sheep became ill and died, even after receiving veterinary care. Epidemiological, clinical, and necropsy data were obtained from the farm's records and those of the veterinary doctor who treated the animals and performed necropsies on two of the deceased sheep. During the necropsy, macroscopic observations were performed, and fragments of all organs were collected and fixed in formalin. The samples were subsequently processed using conventional histochemical methods, sectioned into 5µm slices, stained with hematoxylin and eosin (H&E), and sent to the laboratory for histopathological examination.

## 2 CASE REPORT

The outbreak occurred in May a flock of 270 Ile de France sheep of both sexes and various age groups. The animals were raised in a semi-intensive production system in which they received balanced commercial feed and corn silage placed in a trough every morning. After feeding, the sheep were transferred to an area of native pasture divided into paddocks. One of these paddocks was shaded by "japanese grape" trees (Figure 1A), which were fruiting when the flock was transferred.

According to information obtained from the farm's records, the flock remained in the paddock with the fruiting trees for 15 days after being transferred, and some sheep began to eat fallen pseudofruits from day one. However, consumption of the fallen pseudofruits increased during the seven days preceding the recorded illnesses and deaths. The nine sheep that fell ill received veterinary care as soon as the first clinical signs were detected but showed no improvement and subsequently died.

The poisoning of sheep by plants can be facilitated by certain climatic factors. In the present case, native forage was negatively affected by drought and posteriorly by an early frost. In addition, the time during which the flock was placed in the paddock coincided with the period of fruiting and maturation of the highly palatable pseudofruits and their availability on the ground (Figure 1B).

The observed morbidity in the flock was 3.3%, with a mortality rate of 100% among affected individuals. The evolution from the first clinical signs to death ranged from 15 to 48 hours. Monitoring the cases made it possible to observe and describe the clinical presentation of the two sheep that were later necropsied. The initial clinical signs were depression and anorexia, progressing to other clinical nervous signs and death. Table 1 summarizes the clinical signs observed in the necropsied sheep. The other sheep showed the same clinical disorders, although with varying intensity.

Table 1. Clinical alterations, evolution, and outcomes after spontaneous poisoning in sheep caused by pseudofruits of *Hovenia dulcis*

| Clinical signs | Sheep 1 | Sheep 2 |
|---|---|---|
| Depression | + + +* | + + + |
| Anorexia | + + + | + + + |
| Episodes of watery diarrhea | + + | + + |
| Reduction of ruminal movements | + + | + + + |
| Tympanites | + + | + + |
| Polydipsia | + + | + + |
| Frequent urination in small volumes | + + | + + |
| Compulsive movements of the head | + + + | + + + |
| Ataxia | + + + | + + + |
| Spontaneous falls | + + + | + + + |

| | | |
|---|---|---|
| Lateral or sternal decubitus | + + + | + + + |
| Tremors | + | + + |
| Resting head on the ground | + + | + + + |
| Rhythmic retraction of nostrils | + + + | + + + |
| Shortness of breath | + + + | + + + |
| Bilateral mucus-sanguineous nasal discharge | + + | + + |
| Increased volume in the ventral cervical region | + + + | + + + |
| Sialorrhea | + + | + + + |
| Increased body temperature (40 - 41ºC) | + + + | + + + |
| Evolution of the clinical picture (hours) | 15 | 48 |
| Case outcome | Death | Death |

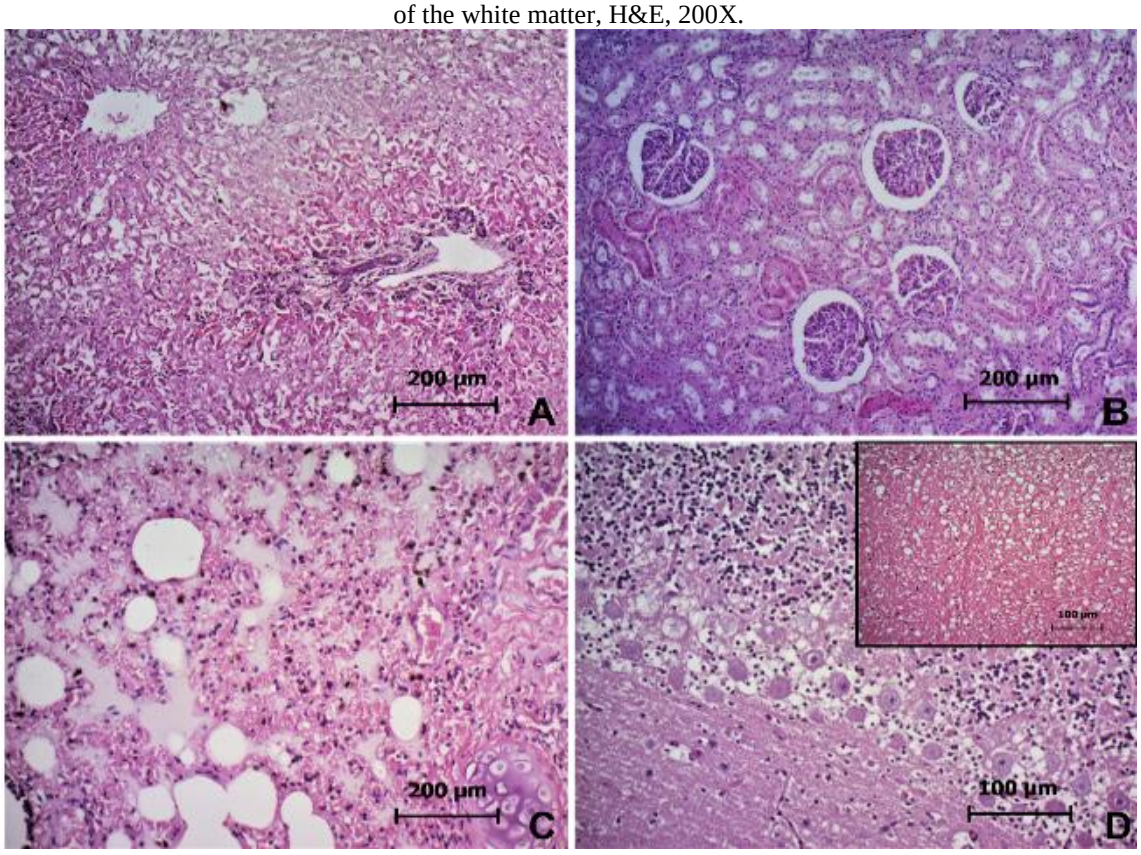
* Clinical signs: + mild, ++ moderate, +++ marked.

During the necropsies, the main macroscopic findings were an enlarged, congested liver showing a pronounced accentuation of the lobular pattern (Figure 1C). The digestive system (rumen, abomasum, and small intestine) presented serosa with petechiae and focal suffusions, as well as numerous fragments of *H. dulcis* pseudofruits and seeds (Figure 1D). The kidneys exhibited pale medullary and cortical regions, with pelvic dilatation also present. Edematous, congested lung parenchyma displaying areas of consolidation were also found, as well as mild cerebral hyperemia.

Figure 1. A) Paddock with fruiting trees of *H. dulcis*. B) Pseudofruits and fruits of *H. dulcis*. C) Sheep 1. Liver with a pronounced accentuation of the lobular pattern. D) Sheep 2. Ruminal content showing pseudofruits, fruits, and seeds of japanese grape.

Histological observations included hepatocellular degeneration and centrilobular necrosis (Figure 2A), congestion, and intrahepatocyte cholestasis in the liver, in addition to moderate multifocal necrosis of the rumen and abomasum mucosa. Moderate multifocal renal degeneration was also detected, characterized by vacuolization, eosinophilia, pyknosis, and cylinders (Figure 2B). Other histological observations were diffuse edema, severe pulmonary congestion (Figure 2C), moderate multifocal atelectasis, moderate interstitial and alveolar hemorrhage, and moderate mononuclear interstitial inflammatory infiltrate. In the brain, the necropsy showed moderate multifocal gray and white matter spongiosis, mild multifocal perivascular and perineural edema, mild eosinophilia, and vacuolization of Purkinje neurons (Figure 2D).

Figure 2. A) Sheep 2. Liver showing centrilobular degeneration and necrosis; H&E, 100X. B) Sheep 1. Renal degeneration characterized by vacuolization, eosinophilia, pyknosis, and cylinders; H&E, 100X. C) Sheep 2. Lung with edema and severe congestion; H&E, 200X. D) Sheep 1. Cerebellum with vacuolization and eosinophilia of Purkinje neurons, H&E, 200X. Sheep 2. Detail of brain with spongiosis of the white matter, H&E, 200X.




## 3 DISCUSSION

The diagnosis of intoxication by *H. dulcis* was based on epidemiological data, clinical signs, macroscopic and microscopic findings, and the presence of pseudofruits and seeds of *H. dulcis* in the gastrointestinal contents of necropsied sheep. Spontaneous

poisoning by *H. dulcis* has already been diagnosed and described in goats and cattle, with a clinicopathological picture involving the nervous and digestive systems (BERNARDI et al., 2016; COLODEL et al., 1998). In experimental and spontaneous poisoning, bovines have exhibited a clinical and pathological condition similar to that of poisoning by acutely hepatotoxic plants (BERNARDI et al., 2016; CARDOSO et al., 2015; GAVA et al., 2004). The evolution of the outbreak, together with reports of poisoning in goats and cattle, therefore point to a diagnosis of plant toxicity in sheep.

From an epidemiological point of view, three factors (together or separately) can predispose sheep to suffer poisoning from the ingestion of *H. dulcis* or other inedible plants. In decreasing order of importance, these factors are changes in how sheep are managed, scarcity of forage in some periods of the year, and the presence in or near the paddocks of toxic plants and/or trees with poisonous fruits (TOKARNIA et al., 2012; VIANA, 2008). The sheep on the farm on which the outbreak occurred were kept under a semi-intensive production regime and, at the time of the outbreak, were kept in a paddock containing fruiting *H. dulcis* trees. Combined with a period of low forage availability due to drought and an early frost, the animals increasingly consumed *H. dulcis* pseudofruits, leading to episodes of acute intoxication.

The monitoring and data collection provided important insights into the clinical picture of the intoxication. In contrast to previous studies of spontaneous intoxication events in goats and cattle, it was possible to follow the progression of the clinical picture among the sheep affected by this outbreak. The initial clinical signs were depression, anorexia, episodes of watery diarrhea, decreased ruminal movements, tympanitis, polydipsia, frequent urination in small amounts, compulsive head movements, and ataxia. Over the hours, the clinical picture evolved to spontaneous falls, lateral or sternal decubitus, tremors, head resting on the ground, rhythmic retraction of the nostrils, short breathing, bilateral mucus-bloody nasal secretion, edema in the ventral cervical region, drooling, and increased body temperature (40-41°C), progressing to death. Many of the symptoms observed in sheep are consistent with the manifestations in cattle and goats noted in previous studies (BERNARDI et al., 2016; CARDOSO et al., 2015; GAVA et al., 2004; COLODEL et al., 1998).

The clinical course of spontaneous poisoning in this outbreak lasted 15-48 hours. In goats, cases of spontaneous intoxication led to death within 2-24 hours after the onset of clinical signs, while the clinical course lasted 48 hours in bovines (BERNARDI et al., 2016; TOKARNIA et al., 2012). Experimentally intoxicated cattle, meanwhile, exhibited

the first clinical signs 8-17 hours after ingestion of the pseudofruits and/or seeds of *H. dulcis*, while the only death occurred 107 hours after ingestion (CARDOSO et al., 2015; GAVA et al., 2004). The clinical and pathological picture of experimentally and/or spontaneously intoxicated cattle was similar to that observed for sheep in the present study.

The low morbidity rate is likely due to the flock being removed from the paddock immediately after the necropsies were completed. According to the farmer, the clinical signs observed in this study were similar to those detected in sheep that had died on the farm in previous years in the same season and paddock. However, it is impossible to impute the same cause to the earlier deaths, since these animals were not necropsied and their clinical signs were not sufficiently observed.

Biondo et al. (2014), conducting an experimental study on sheep, reported that the ingestion of low doses of *H. dulcis* pseudofruits (6.4g/kg and 13.46g/kg) did not alter clinical parameters (heart rate, respiratory rate, and ruminal movements), behavior, and urinalysis findings, nor did it cause death. However, after evaluating the ruminal fluid, the authors found that even these low doses of pseudofruits reduced the pH of the rumen in 24-36 hours, which can trigger acidosis and lead to death. The administration of the pseudofruits was therefore suspended, following which the animals recovered (BIONDO et al., 2014). In this study, the nine sheep that became ill did not show evidence of recovery and died despite receiving veterinary care. This result may be related to the amount and/or toxic potential of the ingested pseudofruits and intrinsic factors of the individual animals' health.

The necropsies revealed very similar macro- and microscopic alterations in both animals, suggesting consistent outcomes due to the reported poisoning by *H. dulcis* pseudofruits. Moreover, the hepatic, gastric, respiratory, renal, and nervous alterations found in the necropsies are consistent with observations of japanese grape poisoning in goats and bovines (BERNARDI et al., 2016; CARDOSO et al., 2015; COLODEL et al., 1998; GAVA et al., 2004). Similarly to affected bovines, the necropsied sheep showed macro- and microscopic foci of mucosal necrosis in the rumen and abomasum, characterized by easy detachment. The cause of these injuries to the compartments of the pre-stomach may be related to the intense ruminal acidification due to a high concentration of sugars, excess saponins, and pseudofruit fermentation (ALVARENGA, 2012; TOKARNIA et al., 2012). Saponins of some plant species can cause symptoms that range from irritation to severe necrosis and inflammation in the rumen mucosa

(DAVIS et al., 2009; KINGSBURY, 1964). Molyneux et al. (1980) attributed these lesions to the lytic action of saponins on the cell membranes of the mucosa.

Intoxicated sheep showed an enlarged and congested liver with an accentuated lobular pattern, symptoms consistent with findings in intoxicated bovines (BERNARDI et al., 2016; TOKARNIA et al., 2012). These hepatic changes may indicate the acute hepatotoxic potential of the pseudofruits of *H. dulcis*. This finding is reinforced by the microscopic analysis, which detected intense centrilobular degeneration and necrosis, diffuse congestion, and cholestasis. These acute lesions have been reported in cases of poisoning (whether spontaneous or experimental) in cattle, which also report subacute to chronic changes such as biliary epithelial proliferation and fibrosis (BERNARDI et al., 2016; CARDOSO et al., 2015; GAVA et al., 2004). The two necropsied sheep exhibited mild bile duct hyperplasia and discrete foci of fibrosis. However, these lesions should be interpreted with caution since they may suggest chronic liver injury due to the consumption of pseudofruits in large amounts and/or regularly or even the possibility of chronic lesions produced by other agents prior to intoxication. The occurrence of chronic liver injury has also been reported in humans diagnosed with *H. dulcis* poisoning (KIM et al., 2012).

Analyses of the cranial cavity revealed hyperemia of the meninges, consistent with reports in goats (COLODEL et al., 1998). In addition, microscopic observations showed multifocal spongiosis in the gray and white matter, multifocal perivascular and perineuronal edema, eosinophilia, and mild vacuolation of Purkinje neurons. The nerve injuries detected in the sheep coincided with those described in goats (COLODEL et al., 1998) and bovines (BERNARDI et al., 2016) after spontaneous intoxication with *H. dulcis*.

The description of macro- and microscopic alterations in the kidneys and lungs of the necropsied sheep is the first record of such a potential effect due to spontaneous poisoning by pseudofruits of japanese grape on these organs. These findings are therefore a potentially novel symptom of *H. dulcis* poisoning in sheep. Interestingly, the histological alterations found in the lungs of sheep intoxicated by *H. dulcis* were similar to those reported by Wouters et al. (2013) in a study on sheep poisoned by *Trema micrantha*, which is also widely used as a shade tree in rural and urban settings. The authors found that *T. micrantha* triggered severe disorders and injuries in the respiratory and renal systems, with clinical signs similar to those reported in the present study. Colodel et al. (2004) found that the consumption of fruits from *Erythroxylum deciduum*

also caused respiratory and nervous disorders in sheep. These relevant alterations in the signs of sheep health are crucial for diagnosing flocks from areas where *H. dulcis* and other plants occur.

We highlight that this is the first report of spontaneous poisoning in sheep caused by the pseudofruits of *H. dulcis* in Brazil. The diagnosis was obtained by combining information on the presence of the fruiting trees in a grazing paddock and with clinical and pathological findings.

## 4 FINAL CONSIDERATIONS

This study provides essential information for the diagnosis of *Hovenia dulcis* intoxication cases and the implementation of control and prevention measures in the regions where the plant occurs. Through the study, it was also demonstrated the importance of performing necropsies, histopathological examinations and, mainly, of a careful epidemiological investigation for diagnostic elucidation of outbreaks of plant poisoning, regardless of the animal species.

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