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Authors

Almeida, Valdir M
Silva-Filho, Givaldo B
Bom, Hisadora ASC
et al.

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Encephalopathy caused by *Talisia esculenta* intoxication in pregnant ewes and their newborn lambs

Valdir M. Almeida, Givaldo B. Silva-Filho, Hisadora A. S. C. Bom, Silvio M. C. Fonseca, Franklin Riet-Correa, Francisco A. Uzal, Kevin D. Woolard, Francisco A. L. Souza, Fábio S. Mendonça[1]

Abstract. An outbreak of acute encephalopathy occurred in pregnant ewes and their newborn lambs associated with consumption of *Talisia esculenta* fruits and bark. Clinical signs in 5 adult pregnant ewes included drooling, bloat, tachypnea, depression, ataxia, body shaking, difficulty in rising, and recumbency. Three neonatal lambs born to some of those ewes had similar clinical signs. No significant gross abnormalities were observed on autopsy. Histologically, neuronal necrosis, axonal and dendritic swelling, and loss of Purkinje neurons were observed in the cerebellum. The observation of similar neurologic clinical signs and lesions in pregnant ewes and their neonatal lambs suggests that the toxic principle of *T. esculenta* crosses the placenta and reaches the fetus.

Keywords: ataxia; Brazil; encephalopathy; intoxication; lambs; pregnant; sheep; *Talisia esculenta*; talisin.

Toxic plants are a significant cause of morbidity and mortality of livestock in Brazil and elsewhere in the world. Brazil is home to a large number of plants that are toxic for animals. Among these, *Talisia esculenta* (*Sapindaceae*) is a 5–15 m tall tree (Fig. 1A, 1B) native to the South American Amazon area, and found commonly in Brazil, Colombia, Peru, and Bolivia. In Brazil, the plant is also found in the Brazilian Atlantic Forest, and in the Cerrado and Caatinga Biomes.[2,7,18] Flowering occurs from August to October, and the fruits ripen from January to March, depending on the region.[11] The fruit is commonly named "pitomba"; it is round or ellipsoid and 1.5–4 cm in diameter. Beneath the outer peel is the white, translucent, sweet-sour pulp with 1 or 2 large, elongated seeds (Fig. 1A, 1B).[2,11,20]

The pitomba fruit is rich in vitamin C and is sold in the northern and northeastern regions of Brazil for human consumption.[6] The tree is widely grown in domestic orchards and in pasturelands where it is used to provide shade to livestock.[11] The leaves and seeds of *T. esculenta* are used to make tea, which is considered to have medicinal properties.[2] However, seeds and leaves of *T. esculenta* contain talisin, a lectin with several anti-nutritional biological activities.[8,9]

Outbreaks of encephalopathy affecting cattle and sheep consuming leaves and fruits of *T. esculenta* have been reported in northeastern Brazil.[16,18] The intoxication has been reproduced experimentally in sheep fed this plant.[16,18] However, we found no reports of spontaneous intoxication of pregnant sheep by *T. esculenta*. We report here an outbreak of acute encephalopathy in pregnant ewes and their newborn lambs associated with the consumption of *T. esculenta* seeds and bark.

An outbreak of neurologic disease was observed in a flock of 240 Dorper sheep in a 200-ha farm in northeastern Brazil. The weather in this region is semiarid, with high mean temperatures and scarce and irregularly distributed rain throughout the year. The characteristic biome of this region is the Brazilian Caatinga, which is composed predominantly of xerophytic, woody, thorny, and deciduous vegetation.[3]

The flock consisted of 8 rams, 72 ewes, 120 yearling ewes and rams, and 40 newborn lambs. At the time of the outbreak, the sheep were on a degraded pasture composed mostly of buffel grass (*Cenchrus ciliaris*) and many *T. esculenta* trees that were producing fruit. In the evening, the animals were housed in a dirt floor corral, where they received chopped grass (*Pennisetum purpureum*), commercial pelleted feed containing ground whole corn, soybean meal, cottonseed meal, wheat bran, and a vitamin A, vitamin D3, and

Veterinary Hospital, Federal University of Campina Grande (Almeida, Riet-Correa), and Laboratory of Animal Diagnosis (Silva-Filho, Bom, Fonseca, Souza, Mendonça), Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil; California Animal Health and Food Safety Laboratory, San Bernardino (Uzal), and Department of Pathology, Microbiology and Immunology (Woolard), School of Veterinary Medicine, University of California–Davis, Davis, CA, USA.

[1]Corresponding author: Fábio S. Mendonça, Laboratory of Animal Diagnosis, Federal Rural University of Pernambuco, Rua Dom Manuel Medeiros s/n, Dois Irmãos, Recife, Pernambuco 52171-900, Brazil.
fabio.mendonca@ufrpe.br

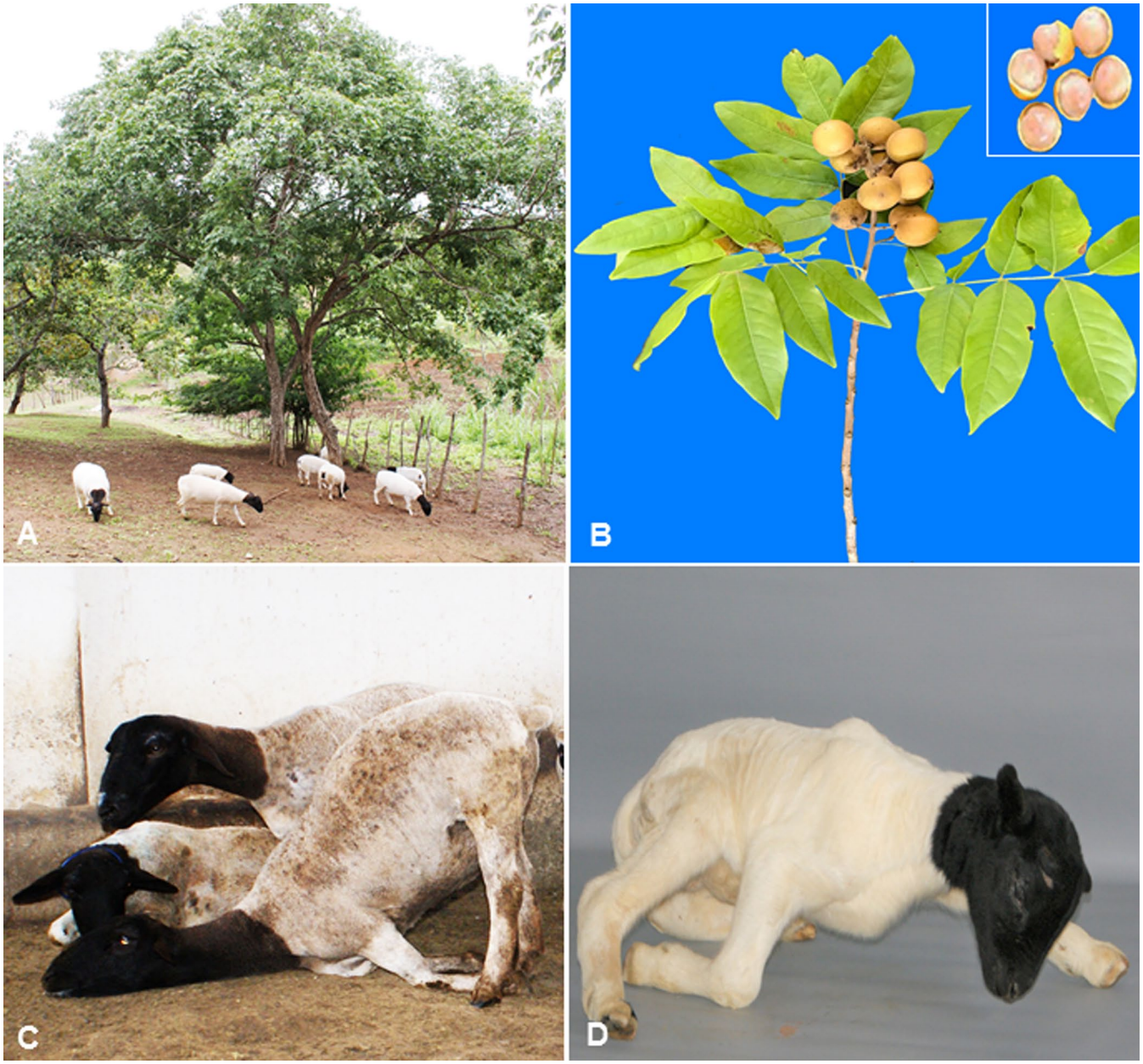

Figure 1. *Talisia esculenta* intoxication in sheep. **A.** *T. esculenta* ("pitombeira tree"), which can grow up to 5 m high. Sheep are consuming the fruit on the ground. **B.** Leaves and fruits of *T. esculenta*. **C.** Ewes with ataxia, sternal recumbency, and paresis. **D.** Neonatal lamb with difficulty standing.

vitamin E supplement. Additionally, the sheep received mineral salt containing bicalcium phosphate, sodium chloride, magnesium oxide, sodium selenite, calcium iodate, cobalt sulfate, iron sulfate, copper sulfate, and zinc sulfate. Water was available ad libitum 24 h/d.

At the end of May and beginning of June, the owner observed 18 ewes in late pregnancy gasping and with an uncoordinated gait. Clinical signs were observed 36–72 h after the sheep were moved to the pasture described above. Three ewes had severe clinical signs and died spontaneously. Six other ewes with moderate-to-severe clinical signs received supportive treatment and recovered fully 18–48 h after disease onset. Treatment consisted of a solution of activated charcoal (3.0 g/kg) given by orogastric tube, plus 2.5 L of a dextrose and electrolyte solution containing sodium, potassium, calcium, and magnesium chloride, given IV. Six other ewes with mild clinical signs recovered without supportive therapy. Immediately after birth, 4 lambs born to sheep severely affected by the intoxication had severe body trembling and inability to stand, followed soon by death.

Another 5 lambs, also born to affected sheep, had mild-to-moderate neurologic clinical signs and survived, but had permanent sequelae consisting of uncoordinated gait.

Five of the moderately affected pregnant ewes and 3 affected neonatal lambs were examined clinically, including neurologic examination. Blood samples were collected in EDTA tubes for complete blood counts. Additionally, rumen content was collected from the sheep using a gastric tube and used to evaluate pH, color, odor, appearance, flotation-sedimentation, methylene blue reduction, and protozoan activity.[5]

Two of the 5 pregnant ewes examined had moderate clinical signs that consisted of drooling, hyperemia and edema of the conjunctiva, ruminal hypomotility, mild bloat, tachypnea, and staggering gait. The other 3 pregnant ewes were more severely affected and died 3 d after the onset of clinical signs, which consisted of drooling, hyperemia and edema of the conjunctiva, dehydration, depression, ataxia, wide-based stance, staggering gait, spastic paresis, ruminal hypomotility, and bloat (Fig. 1C). When the ewes were forced to move, the clinical signs became more severe, the animals fell and remained recumbent, with tremors of the head, ears, neck, and limbs. The 3 severely affected ewes delivered 4 weak lambs that failed to nurse and had severe body tremors, followed by lateral recumbency, and death 3–4 d after birth (Fig. 1D). The average time between the observation of the first clinical signs of intoxication of the ewes and the onset of parturition was 24–36 h. The lambs that survived after developing neurologic signs, failed to develop normally, and had head tilt and a mild uncoordinated gait. Unfortunately, none of the surviving lambs was available for postmortem examination.

No hematologic alterations were observed, nor were changes in pH, color, or odor of the ruminal contents in the 5 pregnant ewes. However, there was a 60–80% reduction of the rumen protozoa and an increase in the methylene blue test.[5]

Autopsies were performed immediately after death on 2 of the severely affected ewes that had recently delivered lambs and had died spontaneously, and on 3 of the neonatal lambs severely affected that had also died spontaneously. On autopsy, a large amount of partially crushed bark and seeds compatible with *T. esculenta* were observed in the rumen of the 3 sheep (Fig. 2A). No other gross abnormalities were observed in any of the sheep or lambs.

The following tissues were collected and fixed in 10% neutral-buffered formalin (pH 7.2) for 24 h: brain, spinal cord, lung, heart, liver, pancreas, kidney, spleen, rumen, reticulum, omasum, and abomasum. All samples were processed routinely for the production of 4-µm thick hematoxylin and eosin (H&E) sections. Selected sections of cerebellum and brainstem were stained with Holmes silver nitrate and Luxol fast blue (LFB) and processed for glial fibrillary acidic protein (GFAP), *Listeria monocytogenes*, and border disease virus (BDV; *Flaviviridae*, *Pestivirus*, *Pestivirus D*) immunohistochemistry, following California Animal Health & Food Safety Laboratory system standard operating procedures.

Histologically, similar lesions were observed in the cerebellum of all ewes and neonatal lambs examined. Multifocally, the Purkinje cells were shrunken, and had hypereosinophilic cytoplasm, pyknotic or karyorrhectic nuclei (Fig. 2B, 2C), and often had swollen dendrites within the molecular layer (Fig. 2D) or swollen axons within the granular cell layer. Multifocally, there was also Purkinje cell loss ("empty baskets"), and occasionally these cells were disorganized and located in the internal granular layer. The LFB demonstrated swollen Purkinje cell processes and separation of the cellular processes (Fig. 2E). Immunohistochemistry for GFAP was strongly positive in the Purkinje cells and granular layers of the cerebellum, highlighting the foot processes of astrocytes (Fig. 2F). In the cerebrum, mild multifocal white matter rarefaction was noted. Occasionally, the Virchow–Robin spaces were mildly expanded by eosinophilic, homogeneous, proteinaceous fluid, or eosinophilic-to-amphophilic hyaline droplets, and capillary endothelial hypertrophy was occasionally observed. In the liver of the ewes, there was mild-to-moderate, multifocal, hepatocellular cytoplasmic vacuolation. No significant microscopic abnormalities were observed in any of the other tissues from the ewes or lambs examined. Immunohistochemistry for *L. monocytogenes* or BDV was negative.

The pasture of the affected animals was inspected by 2 of the authors (V. Almeida, F. Mendonça). This inspection revealed several *T. esculenta* trees, and the bark and fruits showed signs of consumption. The observation of crushed seeds and bark of *T. esculenta* in the rumen content of the ewes, and the evidence that these plants had been consumed in the pasture, are highly suggestive of the role of this plant in causing the clinical signs observed. The observation of identical microscopic lesions in the cerebellum of the pregnant ewes and their lambs suggests that the lambs were also intoxicated by this plant, and that the toxic principles of *T. esculenta* seeds cross the placenta and affect the fetus.

Spontaneous and experimental poisoning of cattle and sheep[16,18] and a dog[17] by the leaves and seeds of *T. esculenta* has been reported. The clinical picture observed in those cases was similar to our cases, although no gross or histologic lesions were observed.[16,18] In our cases, gross lesions were not found, but microscopic lesions were severe. It is possible that the difference between our results and those reported previously in experimental animals was associated with a higher dose of *T. esculenta* in our cases.

The microscopic lesions in our study included neuronal necrosis, dendritic and axonal swelling, and loss of Purkinje neurons. These changes may be observed in primary neurodegenerative diseases, such as neuroaxonal dystrophy, but may also occur secondary to neuronal injury.[10] Causes of selective neuronal injury include hypoxic–ischemic, hypoglycemic injuries, thiamine deficiency, and some chemical intoxications, such as those produced by organomercurials

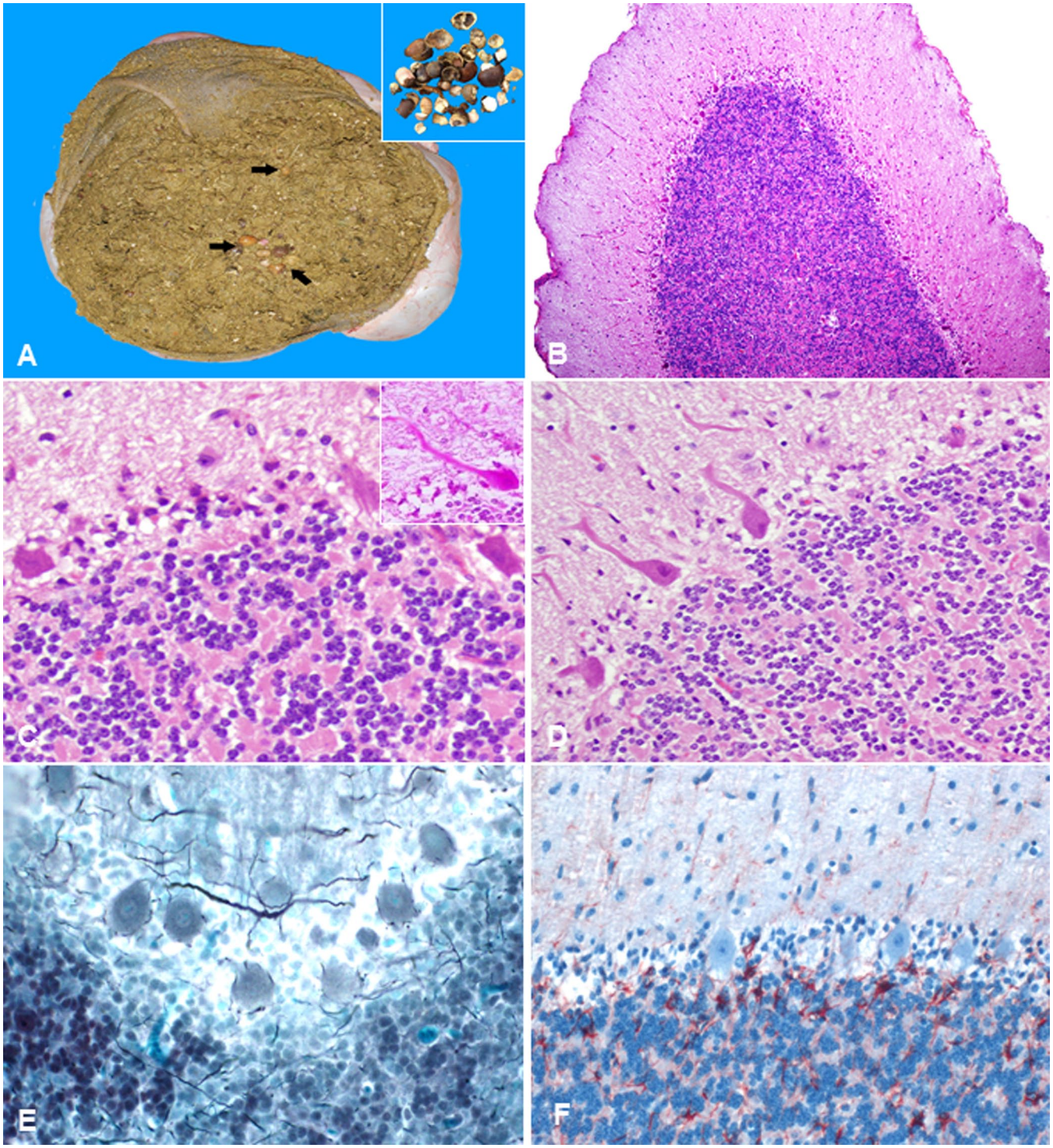

Figure 2. *Talisia esculenta* intoxication in sheep. **A.** Partially crushed seeds and bark of *T. esculenta* in rumen content of an affected sheep. Inset: seeds and bark removed from the rumen content of a ewe with severe clinical signs. **B.** Some Purkinje cells are necrotic or missing in the cerebellum of a pregnant ewe intoxicated by *T. esculenta*. H&E. **C.** Cerebellar Purkinje cells are shrunken and have hypereosinophilic cytoplasm, and pyknotic and/or karyolytic nuclei. Inset: higher magnification of Purkinje cell with a swollen dendrite within the molecular layer. H&E. **D.** Cerebellar Purkinje cells in a lamb have swollen dendrites within the molecular layer. H&E. **E.** Cerebellar Purkinje cell processes are swollen. Luxol fast blue. **F.** Astrocytosis in the cerebellum of a lamb. GFAP immunohistochemistry.

and sodium.[1] Toxic encephalopathy with similar clinical signs and high mortality has been reported in children consuming the fruits of *Litchi chinensis* (lychee tree), a plant from the same family as *T. esculenta*.[12,19] The acute encephalopathy was associated with 2 major toxins: hypoglycin A and methylenecyclopropylglycine,[19] substances found naturally in lychee seeds and fruits, known to cause hypoglycemia in animals by inhibiting β-oxidation of fatty acids and gluconeogenesis.[4,12,14,15]

The toxic principles of *T. esculenta* seeds are unknown. However, *T. esculenta* seeds contain talisin, a storage protein that has lectin-like activities characterized by inhibition of agglutination of human and animal erythrocytes in high concentrations (starting at 500 µg/mL).[7] In addition, talisin also has bovine trypsin inhibitory activity,[9] and induces a neutrophil and mononuclear cell inflammatory response by a mechanism related to a specific protein–carbohydrate interaction.[8,13] These interactions could serve as major signals in some cells, causing the release of pro-inflammatory mediators such as cytokines, nitric oxide, and leukotrienes. Additionally, talisin has an inhibitory effect on glucose and mannose in mouse neutrophils, and these activities may be related to different affinities of talisin for the glycosyl groups present on cell surfaces.[8] It is possible that at least some of these actions may have contributed to the neuronal necrosis observed in the sheep and lambs in our study.

Despite the severe nervous signs observed in the pregnant sheep poisoned by *T. esculenta*, most ewes recovered after supportive care, but this did not occur with the neonatal lambs. For adults, it is suggested that the poisoning may be reversible if the animals stop eating the seeds and supportive care is provided.[16,18] Newborn lambs appear to be more sensitive because lambs that did not die had sequelae. To prevent poisoning, farmers should avoid keeping ruminants in areas with *T. esculenta*.

Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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ORCID iDs

Silvio M. C. Fonseca https://orcid.org/0000-0002-8923-4473
Franklin Riet-Correa https://orcid.org/0000-0001-5738-7785
Francisco A. Uzal https://orcid.org/0000-0003-0681-1878
Fábio S. Mendonça https://orcid.org/0000-0001-5641-0883

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