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Bovine diplodiosis in northwestern Argentina: description of three outbreaks

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Ruiz, A.F.[1,2]; Mercado-Cárdenas, G.[4]; Medina, D.M.[1,2,3]; Colque-Caro, L.A.[2,3]; Avellaneda-Cáceres, A.[2,3]; Aguirre, L.S.[1,2,3]; Sandoval, G.V.[1,2,3]; Singh-Ruiz, J.D.[1,2]; Micheloud, J.F.[1,2,3]

ABSTRACT

Diplodiosis, which is caused by *Stenocarpella maydis*, has emerged as a significant issue in Argentina, affecting cattle primarily in provinces like Buenos Aires, La Pampa, Santa Fe, and Córdoba. This work aims to document three outbreaks of cattle diplodiosis from 2015 to 2023 in the northwestern region of the country, highlighting clinical signs such as ataxia, muscle tremors, and paralysis, with affected animals often succumbing to the disease. Histopathological findings only revealed diffuse degeneration of cerebellar white matter, with no other significant lesions in other tissues. The disease is associated with toxic metabolites in contaminated maize, including diplodiatoxins and diplonine, although the exact pathogenic role of these toxins remains unclear. Epidemiological data showed varying incidence and mortality rates, and high lethality, with the appearance of the clinical signs after 10 to 15 days of exposure to the contaminated maize. These results highlight the need for further research into the toxicological mechanisms of *S. maydis* and its metabolites in cattle.

Keywords: Mycotoxins, neurological disease, cattle, *Stenocarpella maydis*.

RESUMEN

La diplodiosis, causada por Stenocarpella maydis, se ha convertido en un problema significativo en Argentina, afectando principalmente al ganado bovino en provincias como Buenos Aires, La Pampa, Santa Fe y Córdoba. Este estudio documenta tres brotes de diplodiosis bovina ocurridos entre 2015 y 2023 en el noreste del país, destacando signos clínicos como ataxia, temblores musculares y parálisis, con animales afectados que a menudo sucumben a la enfermedad. El análisis histopatológico reveló una degeneración difusa de la sustancia blanca del cerebelo, pero no se observaron lesiones macroscópicas significativas. La enfermedad está asociada con metabolitos tóxicos en maíces contaminados, incluyendo diplodiatoxinas y diplonina, aunque el rol exacto de estas toxinas en la patogénesis aún no está claro. Los datos epidemiológicos mostraron tasas variables de incidencia y mortalidad, y una elevada letalidad, con inicio de los signos clínicos luego de 10 a 15 días de exposición al maíz contaminado. Estos hallazgos subrayan la necesidad de más investigaciones para evaluar los mecanismos toxicológicos de S. maydis y sus metabolitos en el ganado.

Palabras clave: *micotoxinas, enfermedad nerviosa, ganado, Stenocarpella maydis.*

[1]Universidad Católica de Salta, Facultad de Ciencias Agrarias y Veterinarias, Campo Castañares (A4400EDD) Salta, Argentina. Correo electrónico: micheloud.juan@inta.gob.ar
[2]Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Investigación Animal del Chaco Semiárido, Área de Investigación en Salud Animal, (CIAP). Ruta Nac. 68 km 172,5 S/N, Cerrillos, Salta.
[3]Consejo Nacional de Investigaciones Científicas y técnicas (CONICET). CCT Salta-Jujuy. Rivadavia 941, 4400 Salta.
[4]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Salta, Área de Investigación en Salud Animal (CIAP), Laboratorio de fitopatología. Ruta Nac. 68 km 172,5 S/N, Cerrillos, Salta.

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INTRODUCTION

Diplodiosis, a mycotoxicosis that was initially identified in South Africa, Rhodesia, and Zambia, is caused by *Stenocarpella maydis*, formerly known as *Diplodia maydis* (Masango *et al*., 2015). Although it was first described in Africa, the disease has gained increasing relevance in some regions of South America (Odriozola *et al*., 2005, Odriozola *et al*., 2019). In Argentina, diplodiosis has become a recurring issue in cattle fed with contaminated corn or crop residues, especially in the central region of the country (Odriozola *et al*., 2005; Llada *et al*., 2016; Odriozola *et al*., 2019).

S. maydis-infected corn contains several toxic metabolites, such as diplodiatoxins, estenocarpine, carpelin, dipmatol, diplonine, and cetoglobosins K, L, M, and O (Masango *et al*., 2015). These toxins may contribute to clinical signs, either alone or in combination, potentially interacting in a synergistic or additive manner. Diplonine has been linked to nerve signal disruptions in guinea pigs, similar to those seen in ruminants (Snyman *et al*., 2011). However, while diplodiotoxin can be isolated, its role in poisoning is still unclear, as recent experiments with goats did not reveal significant clinical effects (Botha *et al*., 2020).

Diplodiosis is frequently diagnosed in Argentina, with cases being reported in central Argentina (provinces of Buenos Aires, La Pampa, Santa Fe, and Córdoba), where outbreaks are observed in cattle consuming contaminated corn (Odriozola *et al*., 2019; Llada *et al*., 2016; Odriozola *et al*., 2005). Outside this region, there is no literature about the disease. This work aims to describe three recent outbreaks of diplodiosis in cattle in northwestern Argentina (NOA), with a particular focus on clinical, pathological, and epidemiological findings associated with the disease.

MATERIALS AND METHODS

A retrospective study of cases of poisoning by *S. maydis* was conducted by the Animal Health Research Area "Dr. Bernardo Carrillo", IIACS, CIAP, INTA Salta. Samples obtained from field necropsies were fixed in 10% formalin. Subsequently, they were reduced and processed following classic histological paraffin inclusion techniques. Then, 3 µm cuts were made and stained with Hematoxylin-Eosin (H&E). The diagnosis was based on the presence of compatible neurological lesions, which were subsequently linked to the presence of corn infected with *S. maydis*. Data from records were analyzed by compiling information on the clinical, pathological, and epidemiological aspects of the outbreaks. The symptomatic description of the samples and morphological characterization of the fungal structures were carried out to identify the fungus. Each plant's basal part (first internode) was excised, surface-sterilized for 5 min in 10% sodium hypochlorite solution, rinsed in water, and air dried. Cross sections were incubated on potato dextrose agar (PDA) medium at 25°C in darkness.

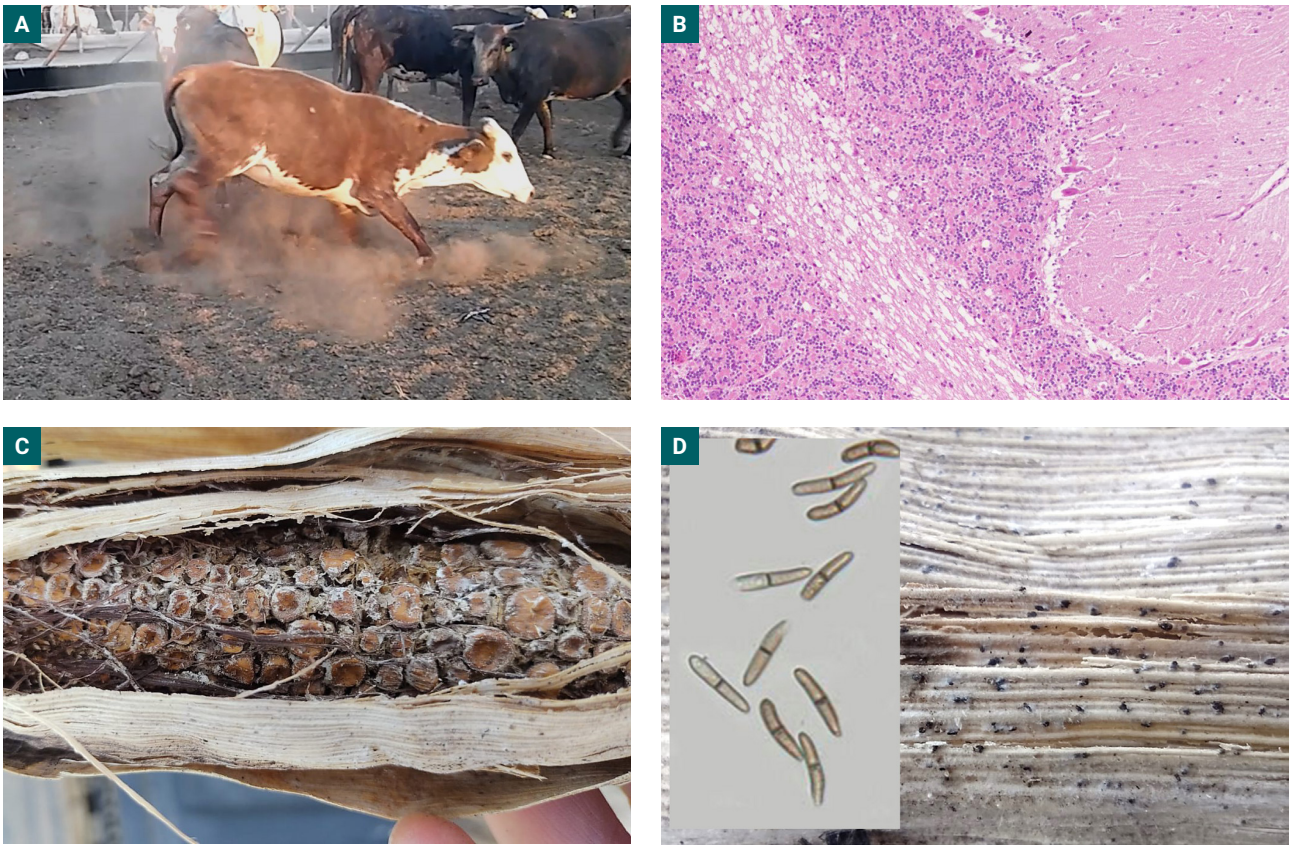

Figure 1. Diplodiosis in cattle. A) Clinical signs in cattle. B) Severe diffuse white matter status spongiosis, characterized by clear, round to oval vacuoles. C) Maize ear infected by *Stenocarpella maydis*, with pycnidia of fungus (D) on the husk of the ear. Insert: Detail of spore observation under the microscope.

RESULTS

From 2015 to 2023, 3 outbreaks of *Stenocarpella maydis* poisoning were confirmed out of the 490 bovine cases registered in that period (0.6% of bovine cases). The diagnosis of diplodiosis was based on the clinical findings, along with the presence of white matter degeneration in cerebellum tissues, which was associated with identifying the fungus on grazed corn.

The clinical findings included cerebellar ataxia, which is characterized by weakness, incoordination, myoclonus of the large muscle masses, and difficulty in moving. The animals then remained in sternal or lateral decubitus position until death. Some animals were in a state of alert, losing weight, and eventually progressing to sternal recumbency, unable to get up. Some of them recovered spontaneously within approximately a week, and others remained in lateral recumbency with opisthotonos until death occurred, leaving signs of pedaling at the site of death (fig. 1 A). In all the recorded clinical cases, the average duration of the clinical signs was 3-5 days, with a maximum of 7 days.

A total of four necropsies were performed. No significant gross lesions were observed in any of the cases. In some animals, muscular lesions and contusions due to prolonged recumbency were noted. Microscopically, a diffuse degeneration of the white matter, which was characterized by axonal loss and multiple vacuoles of variable size (spongiotic changes), was identified in the cerebellum of all the animals (fig. 1 B). The liver showed fatty degeneration. No other tissues presented relevant lesions.

Epidemiological data are shown in table 1. The outbreaks occurred in different locations within the NOA region and had different incidence rates, ranging from 1.83% to 6%, depending on the location and the category of the affected animals. Mortality rates also varied, being higher in the 2017 and 2023 outbreaks compared to the 2024 event. The duration of exposure to the feed after the initial clinical signs was a consistent factor in all the outbreaks, ranging from 10 to 15 days.

Some ears were entirely covered by the characteristic white mold with the stigmas adhered to the grains. On the surface of the mycelium, small dark-colored spots corresponding to the sporulation bodies of the fungus called pycnidia were also found (fig. 1 D). In all the specimens, the presence of *S. maydis* was identified through direct observation and isolation in potato dextrose agar culture medium (PDA 20%). The colonies grown on PDA were flat, with a felty to powdery appearance and a color varying from white-light brown to brown-dark orange with time. Globose pycnidia containing brown elliptical two-celled conidia, 5 to 7 × 28 to 30 µm in size, were observed. Based on its morphology, the fungus was tentatively identified as *S. maydis* (Berk.) Sutton (White, 1999).

DISCUSSION

Stenocarpella maydis is a corn pathogen that infects grains, leaves, and stems. The infection begins at the base of the cob, producing a dense mass of white-gray or light-brown mycelium. After the ripening of the corn, the fungus forms pycnidia that persist in the soil on grain debris. After winter and spring, conidia released from these pycnidia are dispersed by air or insects to infect corn during the summer (Riet-Correa *et al*., 2013; Riet-Correa, 1993). Intoxication typically occurs in winter (from July to September) during periods of higher precipitation in deferred corn or stubble where grain cobs contaminated by *S. maydis* remain as herein. The disease primarily affects cattle, with occasional cases in sheep (Riet-Correa *et al*., 2013; Riet-Correa, 1993). Morbidity rates range from 5% to 75%, and mortality rates from 2% to 20%, with animals of different ages being affected (Riet-Correa *et al*., 2013; Odriozola *et al*., 2005; Riet-Correa, 1993). The results described in this study are consistent with those reported in the literature.

In South America, the disease has been reported in Brazil and Argentina (Riet-Correa *et al*., 2024). In Brazil, it was diagnosed only once in cattle in Rio Grande do Sul (Riet-Correa, 1993). In Argentina, the disease is commonly observed in the central region of the country, primarily in the provinces of Buenos Aires, Santa Fe, La Pampa, and southern Córdoba (Odriozola *et al*., 2005, 2019; Cantón, personal communication). The outbreaks observed in this study indicate that the disease can occur outside of the regions where it is commonly seen and thus represent an emerging problem in the NOA region. Recent studies also describe that pregnant females can experience abortions and cases of congenital diplodiosis may be observed, characterized by the birth of dead or weak calves (Odriozola *et al*., 2019). The aforementioned findings were not found in the outbreak described here.

The clinical signs of the disease are characterized by tearing, salivation, muscle tremors, ataxia, and dysmetria, with exaggerated flexion of the limbs during movement. Some animals exhibit difficulty in walking, maintaining a flexed back, a lowered head, and spread limbs. Eventually, paralysis occurs, leading to permanent lateral or sternal recumbency and death. While recumbent, animals may display opisthotonos and exten-

Date	Location	Forage Type	Affected Category	Incidence	Mortality	Exposure Time
set/17	Lumbreras, Salta	Corn stubble	Cows	6% (9/150)	2.67% (4/150)	13 days
jun/23	Pozo Hondo, Sgo. del Estero	Deferred corn	Bulls	4.11% (6/146)	2.74% (4/146)	15 days
Aug-2024	Antillas, Salta	Deferred corn	Cows, heifers, and calves	1.83% (22/1200)	0.75% (9/1200)	10 days

Table 1. Incidence and mortality rates in cattle classified by location and forage type.
Samples of corn cobs with fungal growth were collected in all the cases. When separating the corn husk from the spike, the presence of mycelium was observed, with a whitish color that extended from the base to the apex and was mostly located between the rows of grains (fig. 1 C).

ded limbs. Animals removed from pastures may recover within 7 to 10 days (Riet-Correa *et al*., 2013; Kellerman *et al*., 1991). Similar clinical signs have been described in the literature, and the observations in this study were consistent with these reports. Newborn calves may show ataxia, opisthotonos, and difficulty in standing; when standing, they often do so with flexed metatarsals (Odriozola *et al*., 2019).

In terms of pathological findings, no relevant gross lesions were observed in the animals analyzed in this study. This is consistent with other reports (Riet-Correa, 1993; Odriozola *et al*., 2005). Microscopically, the characteristic lesion found in the analyzed outbreaks was diffuse spongiosis of the white matter of the cerebellum. This coincides with the description of spongiosis of the cerebral and cerebellar white matter in long-term cases reported in the literature (Odriozola *et al*., 2019; Odriozola *et al*., 2005; Kellerman *et al*., 1991). Additionally, in cases where calves died shortly after birth, spongiosis of cerebral, cerebellar, and spinal cord white matter is also noted, along with intramyelinic and astrocytic edema, which was only observed ultrastructurally (Odriozola *et al*., 2019). In this work, fatty degeneration of hepatocytes in the liver was also noted, but these findings are not relevant and are most likely the result of a prolonged period of anorexia in the animals caused by the disease.

Finally, the toxicological mechanisms of this fungus are still largely unknown. Only the pycnidium of *S. maydis* is toxic, and to reproduce the disease experimentally, the fungus must be cultured for 6 to 8 weeks. In experimental poisoning, 10-30 g/kg of cultured fungi is required to induce clinical signs; while in sheep, the toxic dose ranges from 10 to 130 g/kg. Contaminated corn with S. maydis can produce metabolites such as diplodiatoxin and diplonine, which may contribute to the disease individually or in combination (Masango *et al*., 2015; Snyman *et al*., 2011). Another toxic metabolite, diplodiatoxin, can be isolated from *S. maydis* cultures. However, its role in the pathogenesis of poisoning remains uncertain (Riet-Correa, 1993). In an experiment where diplodiatoxin was administered to goats at doses of 2 and 4 mg/kg over 3 to 5 weeks, no clinical signs were observed (Botha *et al*., 2020). Unfortunately, the toxin concentrations were not measured in the description of these outbreaks.

CONCLUSIONS

The study confirms the occurrence of diplodiosis in cattle from NOA, with classical clinical signs including tearing, salivation, muscle tremors, ataxia, and dysmetria. Recent outbreaks in non-traditional areas and the seasonal occurrence of the disease highlight a potential for a broader geographic spread than that previously considered, especially during winter and periods of high precipitation. The histopathological findings, such as diffuse degeneration of the cerebellar white matter and spongiosis, align with previous reports, although the study also identified fatty degeneration in the liver, a characteristic/a finding not previously documented. While several toxins of *S. maydis* and various metabolites have been identified, their precise roles and effects remain uncertain, with some metabolites showing variable toxicity in experimental works.

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