Fatal stagger poisoning by consumption of *Festuca argentina* (Speg.) Parodi in goats from Argentine Patagonia

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Abstract

The present study describes the spontaneous and experimental poisoning of goats by *Festuca argentina* in Argentine Patagonia. In April 2017, eight seven-month-old Creole male goats were accidentally introduced into a paddock that contained *F. argentina*. After four days, two of the goats were found dead and four out of the six remaining goats were clinically affected. Two of the latter had to be later euthanized *in extremis.* The main clinical signs were progressive nervous signs, starting with moderate muscle tremors, wide-based stance and ataxia. Postmortem examination was performed on the two euthanized goats. Epidermal fragments of *F. argentina* were found in the rumen samples from the necropsied goats and the fecal samples from the four affected goats. For the experimental poisoning, fresh sheaths of *F. argentina* collected from the paddock were offered to two goats at 10 g/kg body weight for 3 days. After 24 – 36 h, both animals exhibited severe muscle tremors, reluctance to move, tetanic convulsions, and opisthotonus. In both the spontaneously and experimentally poisoned goats, gross lesions were similar and consisted of dehydration, petechial hemorrhages in the epicardium and congestion. The main microscopic findings consisted of degeneration and loss of Purkinje cells and torpedoes in the granular layer of the cerebellum. The *F. argentina* sheaths collected from the pasture were found to contain tremorgenic indole-diterpene alkaloids. Taken together, the results of the present study suggest that the tremorgenic syndrome observed in the spontaneously poisoned goats was due to poisoning by *F. argentina*.

Keywords. Cool-season grass; Indole-diterpenes; Tremorgenic syndrome; Goats; Patagonia

1. Introduction

The genus *Festuca* L., which belongs to the tribe Poeae, subtribe Loliinae (family Poaceae, subfamily Pooideae), comprises about 500 species distributed in temperate regions around the world, and can also, be found in the tropics. Although it is diverse in the Northern Hemisphere, about 220 taxa grow in South America, concentrated in the Andean and Patagonian regions (Ospina-Gonzalez, 2016). *Festuca argentina* (Speg.) Parodi is a perennial cool-season grass widely distributed in the steppes of Argentine Patagonia (Parodi, 1950). Anecdotal comments by farmers from different areas of Patagonia, including Andean and coastal steppes, suggest that *F. argentina* is toxic to livestock, mainly affecting young, naive animals foraging on overgrazed grasslands (Robles *et al.*, 2007).

Consumption of *F. argentina* and / or *Poa huecu*, another Patagonian cool-season grass, has been reported to produce clinical signs in sheep, goats and cattle, characterized by muscle tremors and ataxia, resulting in the disease commonly referred to as "Mal del huecú" (drunken disease), "tembleque" (staggers), or "chucho" (shaking) (Acosta, 1914; Gallo, 1979). According to Gallo (1979), the development of this toxicosis may be either acute, in which animals quickly develop clinical signs that last between 48 and 72 h and then result in death, or chronic, in which animals survive, maintaining subtle clinical signs for up to 20 days, as long as the diet is changed (Gallo, 1979).

Grasses such as *Festuca* spp. and *Lolium* spp. often form symbioses with endophytic fungi of the genus *Epichlöe* (Clavicipitaceae). These symbioses have been extensively studied because they lead to the production of four classes of alkaloids: ergot alkaloids, loline alkaloids, indole diterpene alkaloids, and peramine, which may affect both mammalian and / or insect herbivores. For example, *Lolium perenne*, a perennial ryegrass that grows in New Zealand and Australia, has been reported to cause a tremorgenic syndrome often referred to as

ryegrass staggers. *Lolium perenne* is associated with *Epichlöe festucae* var. *lolii*, which produces lolitrem B and other indole diterpene alkaloids that are responsible for the tremorgenic syndrome in livestock (Panaccione *et al.*, 2014; Reddy *et al*., 2019). *Festuca argentina* has been reported to be associated with the endophytic fungus *Epichlöe tembladerae* (Cabral *et al*., 1999). Molecular data suggest that *E. tembladerae* may produce indole diterpene alkaloids but not lolitrem B and that the endophyte would not produce ergot or loline alkaloids (Iannone *et al*., 2011). However, Casabuono and Pomilio (1997) reported the presence of the loline alkaloids loline, *N*-formylloline, *N*-methylloline, and 5,6-dehydro-*N*-acetylloline in *F. argentina*.

Herein, we report a field case of goats spontaneously poisoned by grazing on *F. argentina* in Argentine Patagonia. This is the first formal report of livestock poisoned after consumption of *F. argentina.* We also reproduced *F. argentina* poisoning in goats experimentally in a controlled feeding trial. We describe the epidemiological and clinical-pathological findings in both the spontaneously and experimentally poisoned goats and report the results of the chemical analysis of the *F. argentina* sheaths collected from the paddock where the goats had been grazing.

2. Material and Methods

2.1 *Spontaneous poisoning*

In April 2017, a farm near Paso de los Molles, Río Negro, Argentina (40.96471 S, 70.72341 W), was visited to determine the cause of an outbreak in a Creole goat flock. The farm is located in an ecological area characterized by hills and basaltic plateaus, with a cold semi-arid climate and an annual average rainfall of 200 mm. The landscape is composed of large steppes covered by shrubs and perennial grasses and small meadows covered by annual

and perennial grasses (Figure 1 B) (Bran *et al*., 2000). Eight seven-month-old Creole male goats had been accidentally introduced into a paddock that contained *F. argentina*. Two goats were found dead and two that were severely affected were sedated with 2% xylazine hydrochloride, euthanized *in extremis* with an overdose of 5% sodium pentobarbital, and necropsied. The remaining four goats were removed from the pasture.

2.2 *Diet analysis*

To identify the plant species and relative percentage consumed by the goats in the field, feces and rumen were microhistologically analyzed. Fecal and rumen samples were processed according to the microhistological technique described by Sparks and Malechek (1968) and modified by Williams (1969) and Latour and Pelliza Sbriller (1981). Briefly, the samples were oven-dried at 60 °C to constant weight, milled to <1 mm in a Wiley-type mill, depigmented with 70 % ethanol, rinsed with bleach, stained with alcoholic safranin and finally mounted on glycerin-gelatin. The rumen samples were previously washed with hot running water on a sieve to remove soluble substances that can coagulate and make further processing difficult. Five slides per sample were prepared, and 20 microscopic fields per slide were observed at a magnification of 100x, totalizing 100 microscopic fields observed for each sample (Holechek and Vavra, 1981). This allowed obtaining the frequencies of each plant item identified, following Holechek and Gross (1982). Epidermal and non-epidermal fragments of consumed plants were identified (Sparks and Malechek, 1968; Sepúlveda *et al*., 2004). The epidermal tissue of *F. argentina* that is generally detected in samples from the digestive tract of herbivores corresponds to the abaxial side of the leaves. It is characterized by rectangular-shaped cells of variable-length, with a thickened, wavy cell wall. Between those long cells, short cells are found, which can be better observed over the veins, while in

120 the rest of the blade they are seen as septum-like cells. On the abaxial side of the leaf, stomata
121 and macro-hairs are absent. For the identification of epidermal tissue, comparisons were made
122 with the Reference Collection of plant species patterns from the Patagonian region belonging
123 to the Microhistology Laboratory of the Experimental Station of the Instituto Nacional de
124 Tecnología Agropecuaria (INTA) at Bariloche, Río Negro, Argentina.
125
126 **2.3 *Experimental poisoning***
127 For the experimental poisoning, samples of *F. argentina* were collected from the
128 paddock in which the outbreak had occurred (Figure 1A). Plant samples (n=50) were taken
129 using two 250m-transect distanced between them by 25m, sampling a *F. argentina* specimen
130 each 10m. A whole specimen was sent to MSc. Donaldo E. Bran, CRP Herbarium, INTA
131 Bariloche (CRP #5991) for identification and registration. Leaf sheaths from individual plants
132 were evaluated microscopically by staining for fungal hyphae with Rose Bengal to confirm
133 the endophyte infection and rate of plant infection (Saha, 1988). Infected *F. argentina* sheaths
134 were stripped of dried leaves, and immediately stored at 4 °C for use in an experimental
135 feeding trial.
136 The feeding trial was performed following the ethical standards for animal
137 experiments in toxicological research recommended by the International Society of
138 Toxicology (Meier *et al*., 1993). Three seven-month-old Angora goats were used for the
139 experimental trial, housed in two indoor $8m^2$ pens. Two goats were offered a daily ration of
140 10 g/kg *F. argentina* sheaths for 3 days, while a third goat was not offered *F. argentina*. All
141 goats were also offered a concentrate (12% Crude Protein - 2.7 Kcal) and alfalfa (*Medicago*
142 *sativa*) hay, equivalent to 2% body weight, and water *ad libitum*. The amount of *F. argentina*
143 sheaths consumed daily by each experimental goat was recorded and the goats were clinically

examined twice a day at 9:00 am and 4:00 pm. This clinical examination was carried out by two veterinarian evaluators (AM and LB), and included neurological observations consisting in identifying signs of nervousness or agitation by respiratory and heart rate, changes in the normal body movements like limbs and head position, tremors of the body or head, and eye position. At the end of the trial, the treated goats were euthanized and necropsied.

2.4 *Histopathological and immunohistochemical analysis*

After performing a complete necropsy examination, samples for histopathological and immunohistochemical analysis were taken from both the spontaneously and experimentally poisoned goats. The central nervous system (CNS) was sectioned and samples of cerebrum, brainstem, cerebellum, spinal cord, liver, kidney, heart, lung, and spleen were collected and fixed in 10% buffered formalin. Samples were processed routinely for the production of 5-µm-thick hematoxylin and eosin (HE) sections. Representative sections of the cerebellum were immunostained for Iba-1, a microglia marker, and GFAP, an astrocyte marker. Iba-1 and GFAP staining were performed as previously described (Fenn *et al*., 2014). Briefly, tissue sections were subjected to antigen retrieval, peroxidase quenching and blocking of non-specific antigens prior to incubation with the primary rabbit anti-mouse Iba-1 antibody (Wako) or rabbit anti-mouse GFAP antibody (Dako). The subsequent steps of the immunohistochemistry protocol were performed by a commercial immunoperoxidase technique (EnVision+, Dako) and the samples were finally counterstained with Mayer's hematoxylin.

168 **2.5 *Chemical analysis***

169 The *F. argentina* samples collected from the paddock were frozen at – 80°C,
170 lyophilized and subsequently ground for chemical analysis. Only infected sheaths were
171 selected to determine the presence of indole diterpenes and ergot alkaloids. Plant material was
172 extracted and analyzed for the presence of indole diterpene alkaloids based upon published
173 reports by using high performance liquid chromatography – high resolution mass
174 spectrometry (HPLC-HRMS) (Rasmussen *et al.*, 2012; Lee *et al.*, 2017). The plant material
175 was also analyzed for lolitrem B. The ergot alkaloids lysergic acid amide and ergonovine and
176 their isomers were analyzed using a previously published method (Faeth *et al*., 2006),
177 whereas the ergot alkaloids ergovaline and ergonovine were analyzed by means of the
178 following procedure: a weighed aliquot of the ground plant material (50 mg) was placed into a
179 1.5-mL Eppendorf plastic snap cap tube. Then, the sample was added with 1.0 mL of 50%
180 methanol (25mM tartaric acid) solution (0.5 ppm ergotamine internal standard), and the
181 samples extracted by sonication for 1 h and centrifuged for 10 min (13,000 rpm). A 0.200-mL
182 aliquot was transferred to an autosample vial for analysis by HPLC-MS/MS using a Agilent
183 1100 binary HPLC pump, a Hypersil Gold C18 (100 x 2.1 mm) column and a Velos Pro LTQ
184 mass spectrometer with a heated electrospray ion source. The column was eluted with a
185 gradient flow of 20 mM ammonium acetate (A) and methanol (B) at a flow rate of 0.300
186 mL/min. The gradient conditions were: 25% B – 50% (0 – 1 min), 50% - 95% B (1-10 min),
187 95% B (10-15 min), 95%-25% B (15-16 min) and 25% B (16-21 min). The mass spectrometer
188 was set to scan MS/MS ions resulting from the fragmentation of the parent ions 582.3@HCD
189 35 and 534.3@HCD 50 and with selective monitoring of *m/z* 208 and 223.

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3. Results and Discussion

Eight seven-month-old Creole male goats were spontaneously poisoned after having been accidentally introduced into a paddock that contained *F. argentina*. After 4 days, two goats were found dead (animals #1 and #2) but no subsequent post-mortem studies were performed (Table 1). Upon clinical examination of the six remaining goats, four of them exhibited clinical signs. Two of them (animals #3 and #4) were sternally and laterally recumbent, respectively, and could not stand. Both of these goats were thus euthanized *in extremis* and necropsied (Table 1). The other two goats showing clinical signs (animals #5 and #6) showed moderate muscle tremors, a staggering gait, a wide-based stance, and ataxia (Figure 3A). These goats were removed from the paddock to a small pen where they were fed alfalfa hay and oats. These goats recovered completely after 10 and 14 days, respectively (Table 1). Finally, the two remaining goats (animals #7 and #8) exhibited no clinical signs and were also removed from the paddock (Table 1). During the anamnesis, the owner of the farm reported that the paddock where the outbreak had occurred had been closed to grazing for 20 years, because, historically, sheep or cattle grazing the paddock developed "Mal del huecú" every year in both summer and autumn.

As *F. argentina* was suspected to be the cause of the outbreak, its presence in the diet of the four poisoned goats that exhibited clinical signs was investigated by the microhistological technique previously described (Sparks and Malechek, 1968). Epidermal fragments of *F. argentina* were identified in the rumen and fecal samples from animals #3 and #4 as well as in the fecal samples from animals #5 and #6 (Figure 2). The percentage estimates of *F. argentina* compared to those of the other forage components in the diet are presented in Table 2. The percentage of *F. argentina* ranged from 3-9 % in the fecal samples

214 to 7-14 % in the rumen samples, demonstrating that the microhistological technique is a tool
215 that may aid in the diagnosis of plant poisoning (Stegelmeier *et al*., 2009). However, this
216 technique has not been widely used because it requires knowing the specific epidermal
217 characteristics of the species of interest and recognizing them in the content of the digestive
218 tract or feces of affected animals. Such a study can only be carried out by specifically trained
219 microhistologists. On the other hand, these epidermal characteristics must be resistant to
220 digestion because their identification and quantification by microanalysis are influenced by
221 the effect of digestion (Yaguedú *et al*., 1998). Thus, the diagnostic characters must have been
222 previously validated so that they are still identifiable after the passage of the plants through
223 the digestive tract of the animals (Cid *et al*., 2011). This technique provides an accurate
224 identification of the ingested plants, but not exact data on the amount consumed. However, it
225 has been successfully used to confirm ingestion of toxic species in acute (Giannitti *et al*.,
226 2013) and chronic plant poisoning outbreaks in Patagonia (Robles *et al*., 2000; Martinez *et*
227 *al*., 2019).

228 In the controlled feeding trial, two goats (animals #9 and #10) were experimentally
229 offered *F. argentina* to determine whether this grass was the cause of the tremorgenic
230 syndrome observed in the goats poisoned in the field. The onset and duration of clinical signs
231 in these experimentally poisoned goats are shown in Table 3. Animal #9 started to exhibit
232 muscle tremors in the head and apathy 24 h after being offered *F. argentina* sheaths. Animal
233 #10 started to exhibit muscle tremors in the head and reluctance to move 36 h after being
234 offered *F. argentina* sheaths. Over the next 24 - 48 h, the clinical signs became more
235 pronounced, with increased tremors, incoordination and falling when disturbed. Ataxia,
236 hypermetria, wide-based stance, abnormal postural reactions and strabismus were also

observed. On day 3, both goats showed the most severe clinical signs. Animal #9 had severe whole-body muscle tremors and total reluctance to move when disturbed, but was able to maintain a wide-based stance (Figure 3B). Animal #10 was laterally recumbent, with opisthotonus, and with recurrent tetanic convulsions with terminal paddling movements, so it was euthanized *in extremis* on day 4. On day 5, after *F. argentina* was discontinued, animal #9 slowly recovered without specific treatment apart from a concentrate, alfalfa hay and water *ad libitum*. On day 10, animal #9 still exhibited subtle tremors, and was subsequently euthanized and necropsied. The clinical outcome of animal #10 was classified as acute, as described by Gallo (1979), whereas that of animal #9 was classified as chronic.

Although unspecific, gross findings in the goats spontaneously poisoned in the field were similar to animal #10 experimentally poisoned, with dehydration corroborated by sunken eyes, petechial hemorrhages in the epicardium and congestion in lungs. The unique microscopic findings, summarized in Table 4, consisted of loss of Purkinje cells from the cerebellum, resulting in empty baskets. Torpedoes were also observed in the granular cell layer of the cerebellum. Another relevant finding was moderate microgliosis and astrocytosis with a pattern based on the clinical outcome. For example, animals #3, #4, and #10, which showed acute clinical outcomes, showed immunostaining by Iba-1 in the Purkinje layer, while animal #9, which was considered to have a chronic clinical outcome, showed immunostaining by Iba-1 in the molecular layer (Figure 4 and Table 4). We suspected that the immunostaining by Iba-1 in the Purkinje layer was in response to the death of Purkinje cells reported herein in animal #10, while immunostaining in the molecular layer was due to chronic or excessive activation in animal #9. The cerebellum`s microglia plays an important role in the pathogenesis of the ataxias (Ferro *et al.*, 2019). Activation of microglia has been correlated with disease severity and preceded Purkinje cell pathology, showing that the increase in

microglial number and TNF-α expression, could be a possible mechanism for microglia-driven neuroinflammation that contributes in pathogenesis in ataxia (Quek *et al.*, 2016). Likewise, we suspected that the pattern of GFAP staining observed in animal #9 may have been due to the involvement of astrocytes in the neurodegeneration that contributes to disease progression, similar to what occurs in diverse forms of human ataxias (Cerrato, 2020). No gross pathological or histopathological lesions were observed in other organs from either the spontaneously poisoned or experimentally poisoned goats. Histopathological findings are difficult to interpret in isolation because Purkinje cell degeneration or loss and torpedoes alone can be observed in animals affected by other neurological diseases (Mason, 1968), and thus do not constitute pathognomonic lesions of "Mal del huecú". However, the immunohistochemistry results could provide insight into the aetiopathogenesis of cerebellar disease cause by indole-diterpene alkaloids.

The epidemiological and clinical signs described in this study were similar to those of the perennial ryegrass stagger toxicosis (PRGT), commonly reported in New Zealand (Fletcher *et al.*, 1981) and Australia (Reed *et al*., 2000; Combs *et al.*, 2014). PRGT occurs in late summer and autumn and is clinically characterized by head tremors, muscle fasciculation of the neck and legs, hypersensitivity to external stimuli, and recumbency with a variable death rate (Combs *et al*., 2014). This poisoning occurs frequently in sheep and cattle grazing on *L. perenne* infected with *Epichloë festucae* var. *lolii*. These plants contain indole diterpene alkaloids, including lolitrem B, considered the most potent tremorgenic alkaloid (Panaccione *et al.*, 2014; Reddy *et al*., 2019). In Argentina, tremorgenic syndromes have been reported in livestock grazing on varied pastures as *Paspalum spp*. contaminated by *Claviceps paspali* (Lopez *et al*., 1985), *L. perenne* infected by *Epichloë festucae* var. *lolii* (Odriozola *et al*., 1993), *Cynodon dactylon* infected by *Claviceps cynodontis* (Odriozola *et al*., 1998), and *Poa*

285 *huecu* (Corbellini *et al.*, 1981), another grass that is mentioned as the cause of "Mal del
286 huecú". However, none of these plants were present on the farm where the spontaneous
287 poisoning occurred.

288 Grasses are reported to contain endophyte-produced ergot alkaloids and indole
289 diterpene alkaloids, both of which are toxic to mammalian herbivores (Panaccione *et al.*,
290 2014). *F. argentina* has been reported to be infected by the endophyte *Epichlöe tembladerae*
291 (Gentile *et al*., 2005; Iannone *et al*., 2011). In this study, the infection rate was estimated to be
292 66%, as 33 out of 50 plants contained a typical unbranched *Epichloë spp*. found in the
293 parenchymal tissues of *F. argentina* sheaths (Figure 1C). As mentioned previously, molecular
294 data reported by Iannone *et al*. (2011) suggested that *F. argentina* would likely contain indole
295 diterpenes but not the ergot alkaloids. Consistent with the previously reported molecular data,
296 we detected no ergot alkaloids in the *F. argentina* plants collected from the paddock where
297 the goats were poisoned. However, we did detect several indole diterpene alkaloids, including
298 terpendole C, paspaline, and an isomer of paxilline, which are known tremorgenic compounds
299 (Figure 5). Neither lolitrem B nor other several indole diterpene alkaloids, including
300 Terpendole K and 6,7-Dehydroterpendole A, were detected (Gardner *et al*., 2018). A
301 summary of the indole diterpene alkaloids detected in *F. argentina* is shown in Table 5.

302 The presumptive diagnosis of poisoning by *F. argentina* was based on
303 epidemiological, clinical and microhistological findings. The diagnosis was confirmed by the
304 experimental reproduction of the disease by feeding goats with *F. argentina* from the same
305 paddock where the spontaneous outbreak had occurred. Furthermore, the detection of indole
306 diterpene alkaloids in *F. argentina* is consistent with the clinical signs reported in the field
307 and experimental reproduction. Taken together, our results suggest that these compounds are
308 likely to be the toxic principle in *F. argentina*. This is the first formal report of the detection

of indole diterpene alkaloids in *F. argentina* and poisoning of livestock associated with the ingestion of *F. argentina*.

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435 **Table 1**. Clinical signs, care, outcome and samples taken from the goats affected by
436 spontaneous consumption of *Festuca argentina*.

Animal #	Clinical Signs	Care	Outcome	Samples
1	Not observed	Dead in paddock	Death	None
2	Not observed	Dead in paddock	Death	None
3	Severe muscle tremors Depression Sternal recumbency	Euthanized *in extremis*	Death	Fecal matter Rumen content CNS
4	Depression Lateral recumbency	Euthanized *in extremis*	Death	Fecal matter Rumen content CNS
5	Moderate muscle tremors Staggering gait Severe ataxia Incoordination Depression	Intensive care Change of diet	Recovered in 14 days	Fecal matter
6	Moderate muscle tremors Staggering gait Severe ataxia Incoordination Depression	Intensive care Change of diet	Recovered in 10 days	Fecal matter
7	None	Removed from the paddock	Survived	None
8	None	Removed from the paddock	Survived	None

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Table 2. Microhistological analysis of the rumen and fecal contents from spontaneously poisoned goats, expressed as percentages.

	Animal #3		Animal #4		Animal #5	Animal #6
% of Diet	Rumen	Fecal	Rumen	Fecal	Fecal	Fecal
F. argentina	13.4	6.6	7.3	3.7	9.0	6.9
Other grasses	20.2	21.7	17.8	11.8	14.4	19.3
Shrubs	43.5	70.7	73.4	82.7	64.4	70.3
Other	22.9	1.0	1.5	1.8	12.2	3.5

444 **Table 3.** Body weight, *F. argentina* intake, onset and duration of clinical signs, and outcome
445 of goats experimentally poisoned by *Festuca argentina*.

Animal #	Group	Body Weight (Kg)	Daily Intake[a] (g/Kg)	Days	Total Intake[a] (g)	Clinical Signs		Outcome
						Onset (h)	Duration (h)	
9	Dosed	12.4	5.9	3	223	24	240	Euthanized
10	Dosed	11.4	6.4	3	219	36	60	Euthanized *in extremis*
11	Control	13.1	0	3	0	---	---	Survived

446 [a] Fresh *Festuca argentina* plant material.

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Table 4. Histopathological and immunohistochemical findings in goats spontaneously and experimentally poisoned by *Festuca argentina.*

Animal #	Group[#]	Survive Days	Course	Histopathological finding^		IHC Stain[*]	
				Purkinje Loss	Torpedoes	Iba-1	GFAP
3	Spont	≤ 4	Acute	++	-	PL	-
4	Spont	≤ 5	Acute	+	+	PL	GL
9	Exp	10	Chronic	-	+	ML	ML
10	Exp	3	Acute	++	-	PL	-

[#]Spont: Spontaneous; Exp: Experimental.

^ -, no lesion; +, mild; ++, moderate, +++, severe.

*PL: Purkinje layer; ML: Molecular layer; GL: Granular layer

Table 5. Detection of indole diterpenes in *F. argentina* plants collected from the paddock.

Indole diterpene alkaloids[a]	MH+ (m/z)	Published RT[b]	*Festuca argentina*
Emindole SB	406.31044	35.1	x
13-Desoxypaxilline Isomer	420.25332	22.2	x
13-Desoxypaxilline Isomer	420.25332	26.8	x
13-Desoxypaxilline Isomer	420.25332	33.6	x
Terpendole B	422.26897	26.4	x
Paspaline	422.30535	33.7	x
Paxilline Isomer	436.24823	30.2	x
Paxitriol Isomer	438.26388	22.2	x
Paxitriol Isomer	438.26388	33.6	x
Terpendole E	438.30027	23.5	x
Terpendole H Isomer	452.24314	30.3	x
Terpendole I	454.25879	18.9	x
Terpendole I Isomer	454.25879	29.7	x
Terpendole I Isomer	454.25879	30.2	x
Terpendole D	506.32648	33.6	x
Terpendole C	520.30574	29.7	x
Terpendole J	522.32139	30.2	x
Terpendole A/M Isomer	536.30066	27.2	x

[a] indole diterpene alkaloids identified, according to Lee *et al.* (2017)

[b] Published retention time (RT) according to Lee *et al*. (2017)

Figure Legends

Figure 1. *Festuca argentina* (Speg.) Parodi from the paddock where an outbreak of "Mal del huecú" in goats had occurred. A) Specimen with many seeds, 80 cm tall; B) Environment of the paddock invaded mainly by the cool-season grass; and C) Typical hyphae (*Epichloë spp*-like) in intercellular space from *F. argentina* sheaths.

Figure 2. (A) Long and short cells of the epidermal tissue typical of the abaxial face of the leaf blade of the species are the identification`s keys as such observed in pattern of *Festuca argentina*. (B) Epidermal fragment from rumen samples collected from one of the goats spontaneously poisoned by *F. argentina* (animal #3).

Figure 3. (A) Goat spontaneously poisoned by *Festuca argentina* (animal #6) and (B) goat experimentally poisoned by *F. argentina* (animal #9). Both animals showed wide-based stance and depression. Paresis of thoracic legs was also evident in the goat from the field case.

Figure 4. Poisoning by *Festuca argentina* in goats. Cerebellum. Acute course (A, C, and E) and chronic course (B, D, and F). Staining: H&E (A and B), Iba-1 immunostaining (C and D), and GFAP immunostaining (E and F). Loss of Purkinje cells (A) and intact Purkinje cells (B), microgliosis around empty baskets of the Purkinje cell layer (C), no microgliosis around the Purkinje layer, but with a cicatricial point in the molecular layer (arrows) (D), no evidence of astrocytosis (E), and presence of astrocytosis by hypertrophy of cell bodies and processes on molecular layer (F)**.**

Figure 5. HPLC-HRMS ion chromatograms reconstructed from an isopropyl alcohol extract of (A) *Festuca argentina*, *m/z* 520.30574, terpendole C; (B) *Festuca argentina*, *m/z* 422.30535, paspaline; and (C) *Festuca argentina*, *m/z* 436.24823, paxilline isomer. Indole diterpenes identified according to Lee *et al*. (2017).

Figure 1.

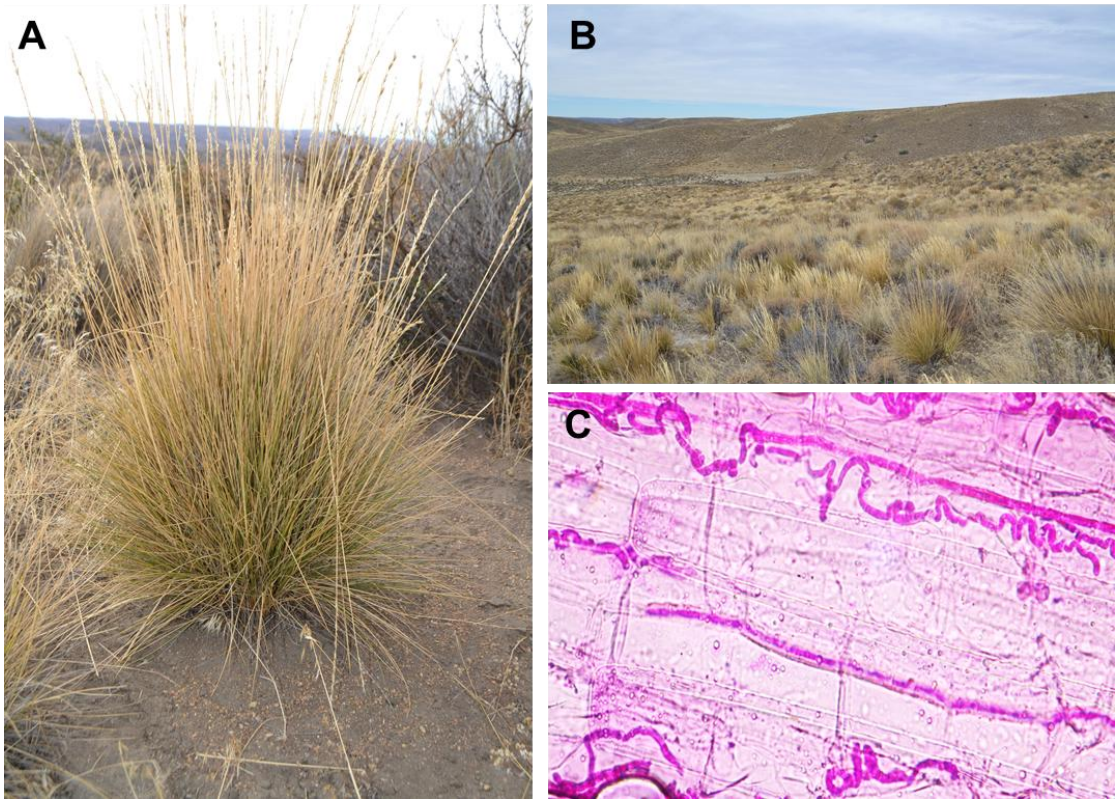


Figure 2.

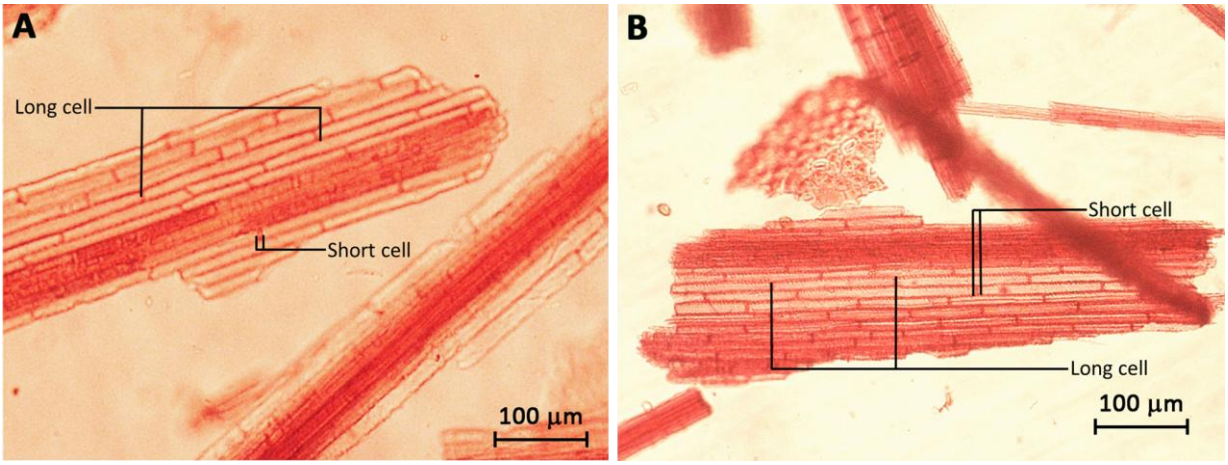


Figure 3.

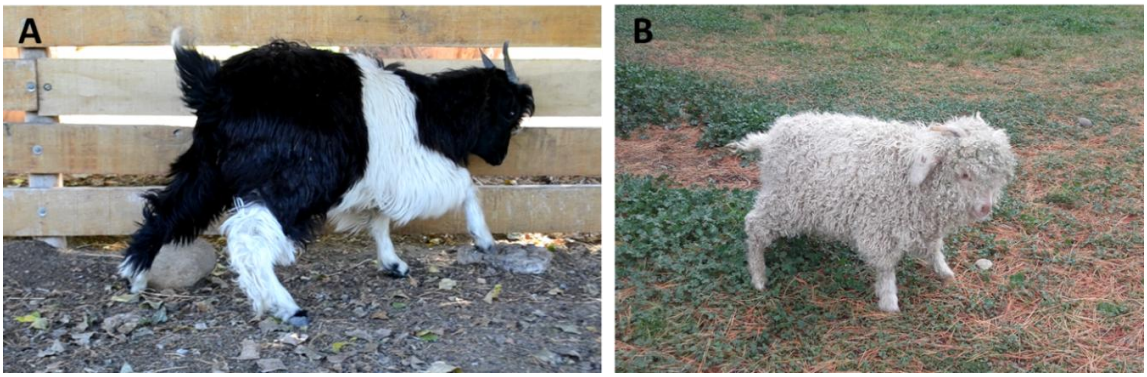
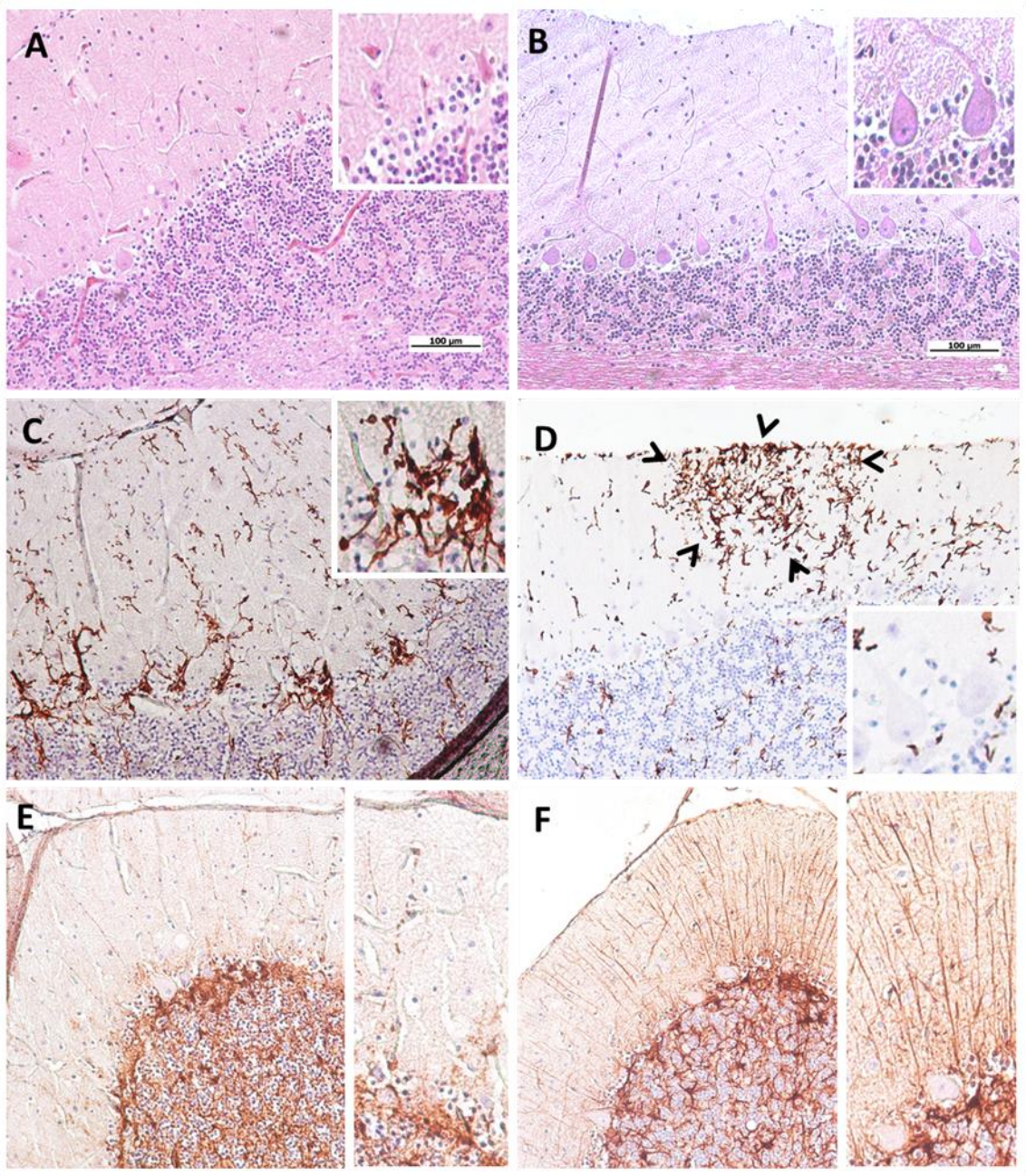
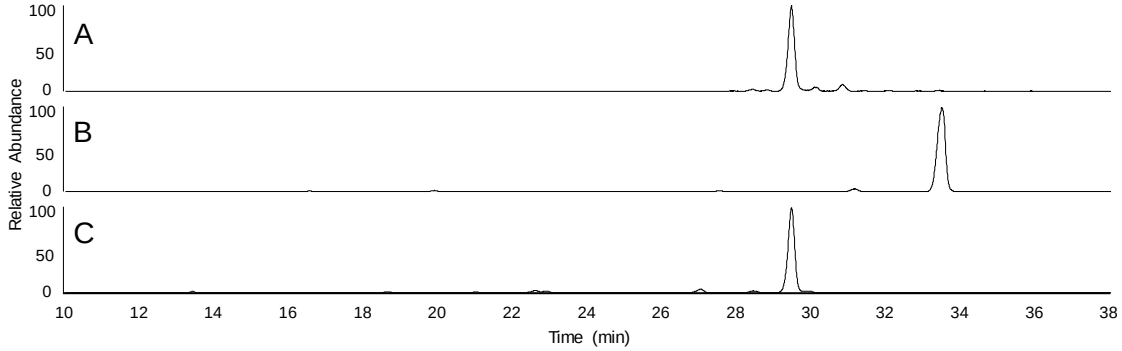


Figure 4.



524 **Figure 5**.



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Credit author statement

Agustín Martinez, Luciana Bain and Carlos Robles collected the data from the spontaneous and experimental studies. Daniel Cook, Stephen T, Lee and Dale R. Gardner did the chemical analysis. Agustin Martinez, Diego Sola and Cristina Acín did the immunohistochemical analysis. Laura Borrelli did the microhistological analysis. All authors wrote and reviewed the manuscript.