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Mycoflora of the toxic feeds associated with equine leukoencephalomalacia (ELEM) outbreaks in Brazil

M.C.A. Meireles,[1] B. Corrêa,[2] O. Fischman,[3] W. Gambale,[2] C.R. Paula,[2] N.O. Chacon-Reche[2] & C.R. Pozzi[4]

[1]*Faculdade de Veterinária, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brasil;* [2]*Seção de Micologia, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo (ICB-USP), São Paulo, SP, Brasil;* [3]*Setor de Micologia, Disciplina de Biologia Celular, Escola Paulista de Medicina (EPM), São Paulo, SP, Brasil;* [4]*Instituto de Zootecnia (IZ), Secretaria de Agricultura do Estado de São Paulo, Nova Odessa, SP, Brasil*

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Abstract. The mycoflora of 39 feed samples associated with 29 Equine Leukoencephalomalacia (ELEM) outbreaks was studied from 1988 to 1990, in Brazil. Microbiological examination indicated *Fusarium* spp. as the most frequent mold which occurred in 97.4% of samples followed by *Penicillium* spp. in 61.5% and *Aspergillus* spp. in 35.9%. The moisture content of feed implicated in death of horses was above 15% which can favor the development of *Fusarium* spp. From the genus, *F. moniliforme* was the predominant species with an occurrence of 82.0%. Two additional species, not commonly associated with animal toxicosis, were isolated in low frequency, *F. proliferatum* (12.8%) and *F. subglutinans* (2.6%). It is important to emphasize that the isolation of *F. proliferatum* and *F. subglutinans* from feed obtained from the epizootic areas has not been documented previously in Brazil.

Key words: Equine, Feeds, *Fusarium proliferatum*, *Fusarium subglutinans*, Leukoencephalomalacia, Mycoflora

Introduction

Equine leukoencephalomalacia (ELEM), is a neurotoxic disease of equidae, characterized by multifocal liquefactive necrosis of the white matter in one or both cerebral hemispheres. It is a seasonal mycotoxicosis, most common when a hot and dry season is followed by a wet and cold period [1]. The syndrome has been associated with the consumption of feed contamined with fumonisins (FB_1 and FB_2), produced especially by *Fusarium moniliforme* and probably by other species of the genus [2–4]. *F. moniliforme* has been the predominant fungus isolated from moldy feed causing outbreaks of ELEM in the USA [5–7], Republic of South Africa [8–10], Egypt [11], New Caledonia [12], Argentina [13], China [14] and Brazil [15–19] and has also been noticed in Greece and probably in Germany [5].

In 1971, Wilson & Maronpot [20] experimentally reproduced the typical disease and established toxigenic *F. moniliforme* as the fungal agent responsable for the mycotoxicosis. ELEM was induced by the oral administration or intravenous injection of FB_1 produced by *F. moniliforme* to horses [21–23]. Other species of the genus, *F. proliferatum* and *F. subglutinans*, have been also associated with outbreaks of ELEM and Porcine Pulmonary Edema (PPE) [3, 4, 24].

This paper reports the mycoflora of 39 feed samples associated with 29 ELEM outbeaks from four Brazilian states.

Material and methods

Equine feed samples. Each sample associated with ELEM outbreaks was packaged in paper sacs, approximately 1 kg of each one, identified and transport-

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Table 1. Geographic location and type of 39 feed samples

Type of feed	Geographic location (states)				Total
	R.G. do Sul	São Paulo	S. Catarina	Minas Gerais	
Corn	15	2	2	1	20
Ground corn & straw	3	4	–	1	8
Ground corn	4	2	–	–	6
Commercial pellets	–[a]	2	–	–	2
Oats	1	–	–	–	1
Straw	–	1	–	–	1
Rice grass	1	–	–	–	1
Total	24	11	2	2	39

[a]Sample not taken.

ed to the laboratory. The 39 samples were obtained from farms in four Brazilian states (1988–1990): Rio Grande do Sul (24), São Paulo (11), Santa Catarina (2) and Minas Gerais (2) (Table 1).

Determination of mycoflora according to Busta et al. [25]. Fungi were determined by blending a 10 g portion of each sample in 90 ml of phosphate buffered saline (PBS). Serial dilutions, until a 10^{-5} concentration, was made from each material: 1 ml of each dilution was spread on each of two Sabouraud glucose agar (pH 5.6) plates, containing chloramphenicol (100 μg/ml) and sodium azide (300 μg/ml). The plates were incubated for 7 days at 25 °C and observed daily. Fungal colonies were selected for subculturing and identified according to the methods for each genus [26–28].

Results and discussion

From 1988 to 1990, 29 ELEM outbreaks were studied in four Brazilian states: Rio Grande do Sul (13), São Paulo (12), Santa Catarina (2) and Minas Gerais (2) (Table 2 and Fig. 1). Approximately a hundred purebred horses died, most within 12 hours after clinical signs appeared. The syndrome was characterized clinically by acute death with neurological signs. All of the necropsied horses showed focal to diffuse uni or bilateral areas of liquefative necrosis of cerebral white matter. The animals had been maintained in confinement.

Outbreaks of ELEM are usually seasonal. Those reported here occurred from late fall through early spring and were most common in winter (June, July, August and September months). (Table 2 and Fig. 1).

In Brazil, most authors [15, 17, 18] correlate ELEM with low temperatures without taking into consideration other climatic factors except Barros *et al.* [16] emphasized the role of pre-harvest rainfall. Moisture is important to the growth of *Fusarium* spp. and colonization is recognized by a pink to reddish brown color overlying the grain. In 75% of the cases, the moisture content of the corn kernels was above 15% which can favor the development of *Fusarium* spp. Corrêa *et al.* [29] reported moisture content higher than 16% in stored maize, contaminated with *Fusarium* spp. in Brazil.

Mycological examination of 39 feed samples indicated the presence of 3 genera of filamentous fungi shown in Table 3 and Fig. 2. *Fusarium* spp. was the most frequent mold which occurred in 97.4% of samples followed by *Penicillium* spp. in 61.5% and *Aspergillus* spp. in 35.9%. Colony counts of *Fusarium* spp. ranged from 8×10^3 to 6.7×10^9 colony-forming units (CFU) per g. These are high values when they are compared with those of the International Commission of Microbiological Specifications for Commission Foods.

Maize is frequently colonized by *Fusarium* spp. Inadequately stored grains, moisture content above 15%, and low temperatures after harvesting are probably the ideal conditions for mold colonization [5, 30–32].

F. moniliforme was the most frequent mold (82.0%) (Table 4 and Fig. 3) which agrees with foreign and Brazilian authors [2, 3, 6, 7, 17, 18, 29, 32]. This fungus colonized all the 39 samples, except one.

Table 2. Monthly distribution of 29 ELEM outbreaks, from 1988 to 1990, in four Brazilian states

Month	R. G. do Sul		São Paulo		S. Catarina		Minas Gerais		Total	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Jan	–[a]	–	–	–	–	–	–	–	–	–
Feb	–	–	–	–	–	–	–	–	–	–
Mar	–	–	–	–	–	–	–	–	–	–
Apr	–	–	–	–	–	–	–	–	–	–
May	–	–	–	–	–	–	–	–	–	–
Jun	1	7.7	3	25.0	–	–	1	50.0	5	17.2
Jul	6	46.2	4	33.3	–	–	–	–	10	34.5
Aug	3	23.1	–	–	1	50.0	1	50.0	5	17.2
Sep	3	23.1	2	16.7	1	50.0	–	–	6	20.7
Oct	–	–	2	16.7	–	–	–	–	2	6.9
Nov	–	–	1	8.3	–	–	–	–	1	3.5
Dec	–	–	–	–	–	–	–	–	–	–
Total	13	44.8	12	41.4	2	6.9	2	6.9	29	100.0

[a]– No outbreaks.

N, Number of outbreaks; (%): Relative frequency.

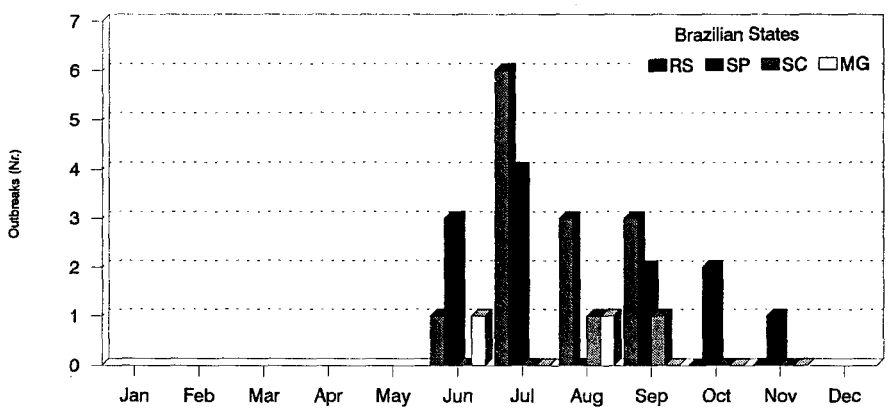

Fig. 1. Seasonal distribution of 29 ELEM outbreaks in Brazil from 1988 to 1990. Brazilian states: RS, Rio Grande do Sul; SP, São Paulo; SC, Santa Catarina; MG, Minas Gerais.

Table 3. Frequency of filamentous fungi isolated from 39 feed samples associated with 29 ELEM outbreaks in Brazil, from 1988 to 1990

Filamentous fungi	Absolute frequency	Relative frequency (%)
Fusarium spp.	38	97.4
Penicillium spp.	24	61.5
Aspergillus spp.	14	35.9

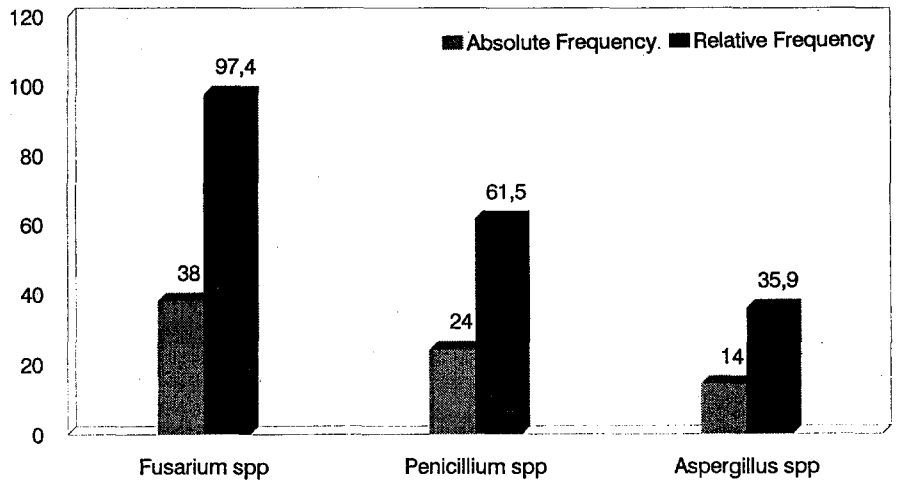

Fig. 2. Distribution of mycoflora in 39 feed samples associated with 29 ELEM outbreaks in Brazil from 1998 to 1990.

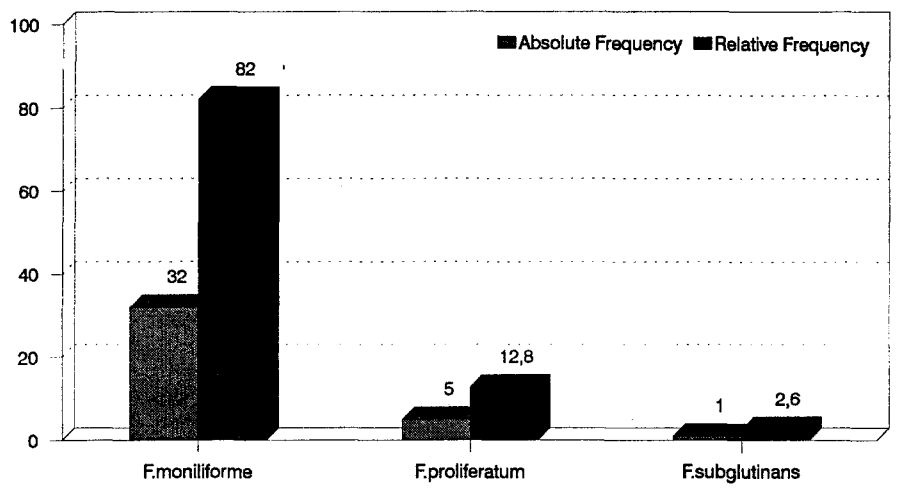

Fig. 3. Frequency of *Fusarium* spp. isolated from 39 feed samples associated with 29 ELEM outbreaks in Brazil.

Table 4. Frequency of *Fusarium* species, isolated from 39 feed samples associated with 29 ELEM outbreaks in Brazil, from 1988 to 1990

Fusarium species Isolated	Absolute frequency (F)	Relative frequency (%)
F. moniliforme	32	82.0
F. proliferatum	5	12.8
F. subglutinans	1	2.6
Absence of *Fusarium* spp.	1*	2.6
Total	39	100.0

*Rice grass sample.

The rice grass (*Echinochlo* sp.) sample was colonized only by *Aspergillus* sp. Other *Fusarium* species in the samples were *F. proliferatum* (12.8%) and *F. subglutinans* (2.6%) (Table 4 and Fig. 3), which are species not as commonly associated with the toxicosis [2–4, 24]. Besides the *F. moniliforme, F. proliferatum* is an important fumonisin producer [3, 4, 24]. One *F. subglutinans* isolated from a culture collection did not produce any detectable fumonisin [4].

Fusarium toxins are normally produced at low temperatures. The termal shock is apparently necessary to induce biosynthesis [34, 35]. *F. moniliforme* and *F. proliferatum* produced FB_1 and FB_2 in vitro, when cultivated on sterile maize. After the initial growth at 25–27 °C, fumonisin production was induced with a thermal shock of 15 °C [3, 36].

In Brazil only *F. moniliforme* isolated from feed has been associated with ELEM [15–18, 37]. The isolation of *F. proliferatum* and *F. subglutinans* has not been previously documented in Brazil from feed samples implicated as the causative fungi of the ELEM.

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Address for correspondence: Dr Benedito Corrêa, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1374-2°. andar, Cidade Universitária, 05508-900, São Paulo, SP, Brasil
Fax: (55) 11813 0845