

EQUINE LEUKOENCEPHALOMALACIA: REPORT OF FIVE CASES

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SUMMARY: This paper reports the occurrence of 5 cases of Equine Leukoencephalomalacia associated with the ingestion of mouldy corn during the winter of 1990 in three properties of the State of São Paulo, in the municipal districts of Poá, Pirassununga and Santana do Parnaíba. The animals showed in every case the following clinical signs: blindness, ataxia, dysphagia, tendency to circle and convulsions. The necropsy revealed congestion of brain and areas of cavitation within the hemispheres of subcortical white matter. The histopathological examination showed lesions characterized by liquefactive necrosis, multifocal haemorrhages and cellular infiltration, predominantly of polymorphonuclear (neutrophils and eosinophils) associated with mononuclear cells in the white matter. Microbiological analysis showed a mycoflora composed, predominantly, by *Fusarium*

moniliforme (49.4%), followed by genera *Aspergillus* (25.8%) and *Penicillium* (24.8%).

UNITERMS: Mycotoxicoses; Food contamination, moldy corn; Poisoning; *Fusarium moniliforme*; Encephalomalacia; Horses

INTRODUCTION

Leukoencephalomalacia (LEM) is a neurotoxic disease of equidae, characterized by multifocal liquefactive necrosis predominantly of white matter in the cerebral hemispheres⁹. Several additional names that have been used for this disease entity are based on clinical signs, anatomic lesions or on the food responsible for the disease. These include blind staggers, cerebritis, leucoencephalitis, encephalomyelitis, cerebrospinal meningitis, foraging poison, corn stalk disease and mouldy corn disease^{5,14,15}.

WILSON; MARONPOT¹⁴ (1971) have shown that the fungus *Fusarium moniliforme* is the causative agent of LEM, reproducing the disease experimentally in 2 donkeys with a pure culture of the fungus isolated from contaminated maize.

Later, many metabolites of this fungus were studied, but they failed in the reproduction of the disease.

Finally, LEM was reproduced in horses by intravenous injection of fumonisin B₁, a novel mycotoxin isolated from cultures of *F. moniliforme*⁹. The signs of the disease may include inappetence, drowsiness with blindness developing in one or both eyes, partial or complete paralysis of the pharynx and twitching of the shoulder muscles, unsteadiness of gait, weakness and a tendency to circle. These signs are followed by recumbency, paddling limb movements and death¹⁴. The course of the disease is of very few hours to about a month, but death usually occurs on the second or third day¹.

Field outbreaks occur sporadically in many countries, including Brazil^{4,7,13}. The occurrence of 5 cases of LEM in the State of São Paulo - Brazil, is reported in this paper, with emphasis on the pathological and microbiological aspects of the disease.

MATERIAL AND METHOD

The clinical, epidemiological and pathological data were obtained from 5 animals from 3 outbreaks

occurring in different properties of the State of São Paulo, in the municipal districts of Poá, Pirassununga and Santana do Parnaíba. The animals were examined at the Veterinary Hospital of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, where they were submitted to clinical and laboratorial observations as well as a "post-mortem" examination. The brains were fixed in 10% neutral isotonic formaldehyde for 7 days, after which serial transversal cuts were made. Fragments from frontal, temporal and parietal lobes, thalamus and cerebellum were processed according to routine techniques for paraffin inclusion and 5-7 μ m cuts were obtained and stained by the hematoxylin-eosine method.

Samples of corn grains from the properties affected were collected for microbiological analysis. From each sample, 10 grams previously milled were diluted in 90 ml of sterile water (dilution 10^{-1}). From this, it was prepared a tenfold serial dilution until 10^{-4} , using the same diluent. Of each dilution, 1 ml was deposited in Petri dishes, and then added with 15 ml of acidified Sabouroud-dextrose agar (Difco), malted and cooled to nearly 45 °C, followed by homogeneization. After the solidification of the agar, the Petri dishes were incubated at 25 °C for five days. The identification of the fungi was performed through usual techniques^{1,3,11,12}.

RESULTS

All outbreaks occurred from June to July during the Winter of 1990. The number of deaths at the properties was variable, reaching 60% in one of them. The course of the disease was of 1 to 7 days. In every case, the animals were fed with contaminated corn, confirmed by microbiological determination, which showed high *F. moniliforme* concentrations.

The animals showed similar clinical signs, such as ataxia, uni or bilateral blindness, dysphagy, tendency to circle, twitching of the muscles and perspiration, followed by recumbency, convulsions and death. Examination of cerebrospinal fluid showed in 2 cases severe leucocytosis, mainly lymphocytic, while the other animals presented only discret pleocytosis. The necropsy of the animals revealed only brain congestion. After fixation, the transversal cuts of the brain showed, macroscopically, the presence of cavities within the subcortical white matter of the cerebral hemispheres. These lesions had irregular edges and were filled by a yellow-brown gelatinous substance. The periphery of these area showed a grayish colour and multifocal haemorrhages (Fig. 1). In every case, the wound was unilateral.

Histologically, the lesions observed were suggestive of Leukoencephalomalacia, and were characterized by liquefactive necrosis, focal haemorrhages and infiltration of the spaces of Virchow-Robin by polymorphonuclear leukocytes (eosinophils and neutrophils) associated with mononuclear cells (Fig. 2,3,4).

Other injuries observed microscopically were demyelination and hyalination of the blood vessels wall, mainly within the white matter, besides edema and gliosis. The results of the microbiological analysis showed a mycoflora composed, predominantly by *F. moniliforme* with 1.9×10^6 CFU/g (49.4%) of isolated fungi, followed by genera *Aspergillus* (25.8%) and *Penicillium* (24.8%) (Tab. 1).

DISCUSSION AND CONCLUSIONS

- 1- The course of the disease, the clinical symptomatology and the laboratorial findings observed are very similar to the ones reported in the literature^{2,6,8,9,10,13,14}.
- 2- In every case, the cerebral cavitations were unilateral, although a kind of bilateral injury similar to this has also been reported⁷. MARASAS et al.¹⁰ (1988) proposed that the intensity and distribution of the lesion may be determined by the amount of mycotoxin ingested, by the period of the disease evolution and by the individual susceptibility.
- 3- The microscopic observations revealed areas of malacia distributed close to the edges of blood vessels. These were strongly infiltrated by inflammatory cells, mainly within the white matter. These aspects are compatible with the hypothesis that the primary injury determined by the mycotoxin is on the blood vessel's wall⁷.
- 4- The outbreaks showed a seasonal distribution, concentrated in short periods of the year. The seasonal aspect is important both as a means of diagnosis and for establishing prophylactic measures.
- 5- The isolation, alone, of the mould *F. moniliforme* from the contaminated food can not be used singly to confirm the diagnosis, because this fungus is ubiquitous in stored grains. Knowledge of the role of mycotoxin fumonisin B₁, in establishing the disease, may represent an increment in this field⁹.

6- The occurrence of outbreaks of LEM in the State of São Paulo enlarges the geographical area attributed to the disease in Brazil, as the reported cases were restricted to the States of Rio Grande do Sul, Santa Catarina, Paraná and Minas Gerais. Due to its high mortality rate, and consequently, of its economic importance, LEM must be included in the differential diagnosis with other CNS affections of equidae.

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RESUMO: Relatam-se 5 casos de leucoencefalomalácia em eqüinos associados com a ingestão de milho mofado, ocorridos durante o inverno de 1990, provenientes de 3 propriedades no Estado de São Paulo, nos municípios de Poá, Pirassununga e Santana do Parnaíba. Os animais apresentaram sinais clínicos semelhantes como ataxia, cegueira, disfagia, andar em círculos e convulsões. Ao exame necroscópico, os cérebros encontravam-se congestionados, revelando ao corte áreas de cavitação ao nível da substância branca sub-cortical. O exame histopatológico exibiu lesões ao nível de substância branca, caracterizadas por necrose de liquefação, hemorragias focais e infiltrados celulares nos espaços de Virchow-Robin, compostos predominantemente por polimorfonucleares (eosinófilos e neutrófilos), associados a mononucleares. Análises microbiológicas demonstram que *Fusarium moniliforme* foi o fungo mais frequente (1.9×10^6 UFC/g de alimento).

UNITERMOS: Micotoxicoses; Alimentos, contaminação, milho mofado; Envenenamento; *Fusarium moniliforme*; Encefalomalácia; Eqüinos

REFERENCES

- 01-ARX, J.A. *The genera of fungi sporulating in pure culture*. 2.ed. Vaduz, J. Cramer, 1974.
- 02-BADIALI, L.; ABOV-YOUSSEF, M.H.; RADWAN, A.I.; HAMDY, F.M.; HILDEBRANDT, P.K. Moldy corn poisoning as the major cause of an encephalomalacia syndrome in Egyptian equidae. *Amer. J. vet. Res.*, v.29, p.2029-35, 1968.
- 03-BARNET, H.L.; HUNTER, B.B. *Illustrated genera of imperfect fungi*. 3.ed. Minneapolis, Burgess. 1972.
- 04-BRITO, L.A.B.; NOGUEIRA, R.H.G.; PEREIRA, J.J.; CHQUILOFF, M.A.G.; BIONDINI, J. Leucoencefalomalácia em eqüino associada à ingestão de milho mofado. *Arq. Esc. Vet. Univ. Fed. M. Gerais.*, v.34, p.49-53, 1982.
- 05-GRAHAM, R.; HARRIS, B. Studies on so-called corn stalk disease in horses-IV. *Vet. Med.*, v.31, p.340-6, 1936.
- 06-HALIBURTON, J.C.; BOCK, W.B.; LOCK, T.F.; VESONDER, R.F.; WILSON, B.J. Equine leukoencephalomalacia: the toxicity of three *Fusarium moniliforme* metabolites in equidae. *J. Amer. Vet. Med. Ass.*, v.179, p.262-3, 1981.
- 07-HIROOKA, E.Y.; VIOTTI, N.M.A.; SOARES, L.M.V.; ALFIERE, A.A. Intoxicação em eqüinos por micotoxinas produzidas por *Fusarium moniliforme* no norte do Paraná. *Semina*, v.9, p.128-35, 1988.
- 08-JUBB, K.V.F.; KENNEDY, P.C.; PALMER, N. *Pathology of domestic animals*. 3.ed. Orlando, Academic Press, 1985. v.1.
- 09-KELLERMAN, T.S.; MARASAS, W.F.O.; PIENMAR, J.G.; NAUDÉ, T.W. A mycotoxicosis of equidae caused by *Fusarium moniliforme* (Sheldon). A preliminary communication. *Onderstepoort J. vet. Res.*, v.39, p.205-8, 1972.
- 10-MARASAS, W.F.O.; KELLERMAN, T.S.; GELDERBLUM, W.C.A.; LOETZER, J.A.W.; THIEL, P.G.; VAN DER LUGT, V.V. Leukoencephalomalacia in a horse induced by fumonisin B₁ isolated from *Fusarium moniliforme*. *Onderstepoort J. vet. Res.*, v.55, p.197-203, 1988.
- 11-NELSON, P.E.; TOUSSOUN, T.A.; MARASAS, W.F.O. *Fusarium* species: a illustrated manual for identification. University Park, The Pennsylvania State University Press, 1983. p.193.
- 12-RAPER, K.B.; FENNELL, D.I. *The genus Aspergillus*. Baltimore, Williams & Wilkins, 1965.
- 13-RIET-CORRÊA, F.; MEIRELLES, M.A.; SOARES, J.M.; MACHADO, J.J.; ZAMBRANO, F. Leucoencefalomalácia em eqüinos associada à ingestão de milho mofado. *Pesq. vet. bra.*, v.2, p.27-30, 1982.
- Braz. J. vet. Res. anim. Sci.*, São Paulo, v.28, n.2, p.185-9, 1991.

14-WILSON, B.J.; MARONPOT, R.R. Causative agent of leukoencephalomalacia in equine animals. *Vet. Rec.*, v.88, p.1484-6, 1971.

15-WILSON, B.J.; MARONPOT, R.R.; HILDEBRANDT, P.K. Equine leukoencephalomalacia. *J. Amer. Vet. Med. Ass.*, v.163, p.1293-4, 1973.

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TABLE 1 - Frequency (CFU/g) of fungi cells isolated from samples of contaminated corn. São Paulo, Winter of 1990.

PROPERTIES	<i>Fusarium</i> sp*		<i>Penicillium</i> sp		<i>Aspergillus</i> sp		TOTAL	
	AF	RF	AF	RF	AF	RF	AF	RF
1	500,000	55.6	400,000	44.4	-	-	900,000	23.3
2	700,000	58.3	500,000	41.7	-	-	1,200,000	31.0
3	710,000	40.1	60,000	3.4	1,000,000	56.5	1,770,000	45.7

AF = Absolute frequency

RF = Relative frequency (%)

(-) = Absence of fungi

CFU/g = Colony forming units per gram of food (mean obtained in two dishes)

* = *Fusarium moniliforme*



FIGURE 1 – Transversal cut of equine brain, showing extensive area of cavitation within the subcortical white matter.

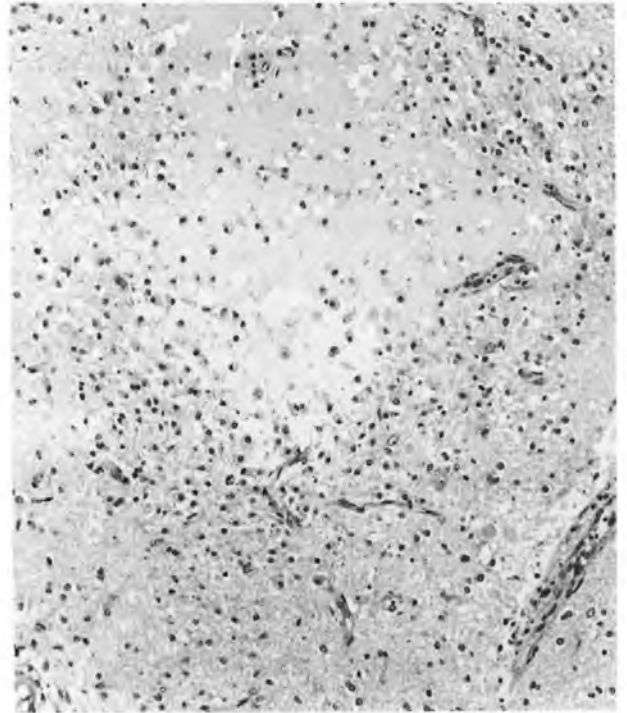


FIGURE 2 – Photomicrograph of the cerebral cortex. Note the liquefactive necrosis associated with gliosis. Observe the proximity of the process to vascular structure. HE. 165x.

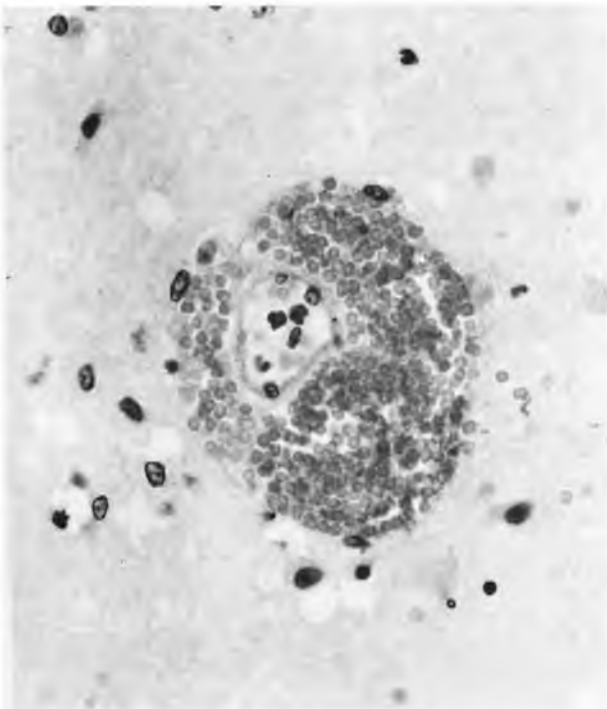


FIGURE 3 – Photomicrograph of perivascular region. Observe the presence of pronounced haemorrhage. HE. 660x.

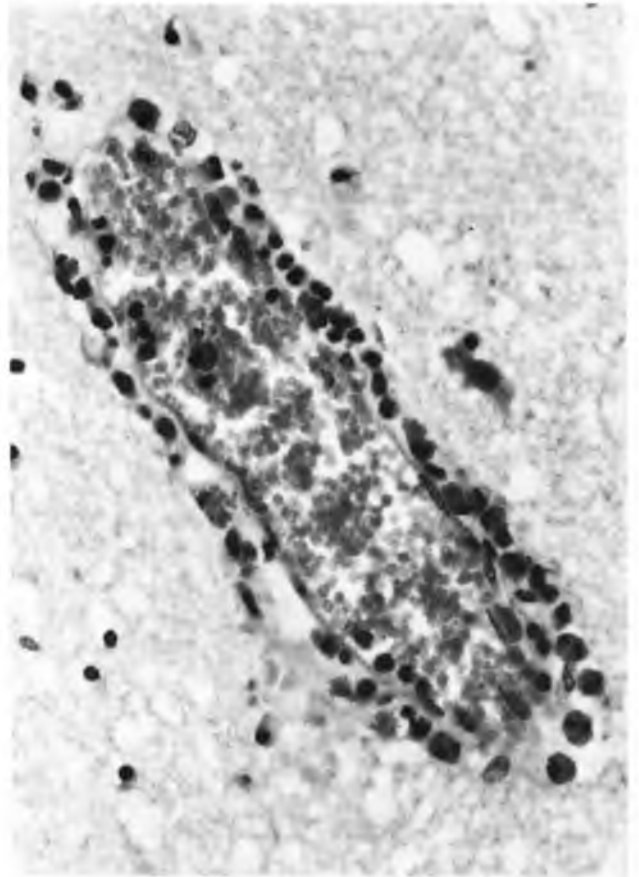


FIGURE 4 – Photomicrograph of perivascular inflammatory infiltration, composed of mononuclear and polymorphonuclear (eosinophils) cells. HE. 660x.