Onderstepoort J. vet. Res., 55, 197–203 (1988)

LEUKOENCEPHALOMALACIA IN A HORSE INDUCED BY FUMONISIN B_1 ISOLATED FROM *FUSARIUM MONILIFORME*

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ABSTRACT

MARASAS, W. F. O., KELLERMAN, T. S., GELDERBLOM, W. C. A., COETZER, J. A. W., THIEL, P. G. & VAN DER LUGT, J. J., 1988. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. *Onderstepoort Journal of Veterinary Research*, 197–203 (1988)

Each of two horses was dosed by stomach tube with culture material on maize of *Fusarium moniliforme* MRC 826. One horse developed severe hepatosis and mild oedema of the brain after 6 doses of 2,5 g of culture material/kg body mass/day in 7 days. The second horse, in a similar experiment but at a dosage rate of 1,25 g/kg/day, developed mild hepatosis and moderate oedema of the brain. In both animals the brain oedema was particularly noticeable in the medulla oblongata.

The mycotoxin fumonisin B_1 was extracted and purified from the culture material of *F. moniliforme* MRC, 826 which contained approximately 1 g/kg of this compound. A horse was injected intravenously 7 times from Day 0–Day 9 with 0,125 mg of fumonisin B_1/kg body mass/day. Clinical signs of neurotoxicosis, which appeared on Day 8, included nervousness followed by apathy, a wide-based stance, trembling, ataxia, reluctance to move, paresis of the lower lip and tongue, and an inability to eat or drink. Euthanasia was performed on the horse on Day 10 while the animal was in a tetanic convulsion. The principal lesions were severe oedema of the brain and early, bilaterally symmetrical, focal necrosis in the medulla oblongata.

This report provides experimental evidence that fumonisin B_1, produced by *F. moniliforme*, causes equine leukoencephalomalacia.

INTRODUCTION

Leukoencephalomalacia (LEM) is a neurotoxic disease of horses, donkeys and mules, characterized by multifocal liquefactive necrosis of predominantly the white matter in the cerebral hemispheres (Wilson, Maronpot & Hildebrandt, 1973; Marasas, Nelson & Toussoun, 1984; Kellerman, Coetzer & Naudé, 1988). Field outbreaks occur sporadically in many countries, including Argentina (Rodrigues, 1945; Monina, Moscotena, Ruagar, Idiart, Reinoso, Muro, Nosetto & Pons, 1981), Brazil (Brito, Nogueira, Pereira & Chquiloff, 1982; Riet-Correa, Meirelles, Soares, Machado & Zambrano, 1982), China (Iwanoff, Yuan & Fang, 1957; Pan, 1981; Xin, 1987), Egypt (Badiali, Abou-Youssef, Radwin, Hamdy & Hildebrandt, 1968), New Caledonia (Domenech, Boccas, Pellegrin, Laurent, Kohler, Magnol & Lambert, 1984; Pellegrin, Laurent, Kohler, Hameurt & Boccas, 1986), Republic of South Africa (Kellerman, Marasas, Pienaar & Naudé, 1972; Pienaar, Kellerman & Marasas, 1981), and the United States of America (Wilson & Maronpot, 1971; Wilson *et al.*, 1973; Buck, Haliburton, Thilsted, Lock & Vesonder, 1979; Haliburton, Vesonder, Lock & Buck, 1979; Wilson, Nelson & Knepp, 1985; Wilson, Nelson, Ryan, Rouse, Pittman, Neal, Porterfield & Saunders, 1985; Brownie & Cullen, 1987).

The brain lesions of equine LEM were first reproduced experimentally by feeding naturally contaminated mouldy maize in the United States by Butler (1902). The fungus *Fusarium moniliforme* Sheldon was shown to be the cause of LEM by Wilson & Maronpot (1971), who reproduced the disease experimentally in 2 donkeys with a pure culture of an Egyptian isolate of this fungus. This finding was confirmed in the Republic of South Africa by the experimental induction of LEM in 4 horses with pure cultures of 3 local isolates of *F. moniliforme* from mouldy maize (Marasas, Kellerman, Pienaar & Naudé, 1976; Kriek, Kellerman & Marasas, 1981). Cultures of these South African isolates of *F. moniliforme* were also shown to be capable of causing a fatal hepatosis in horses (Kellerman *et al.*, 1972; Kriek *et al.*, 1981; Marasas *et al.*, 1976). The *Fusarium* metabolites fusarin C and moniliformin have been found to occur naturally in maize implicated in an outbreak of LEM in the United States (Thiel, Gelderblom, Marasas, Nelson & Wilson, 1986). However, neither moniliformin nor 2 other metabolites of *F. moniliforme* (fusaric acid and 2-methoxy-4-ethylphenol) caused LEM upon intravenous injection in donkeys (Buck *et al.*, 1979; Haliburton, Buck, Lock, Vesonder & Wilson, 1981). Thus the mycotoxin produced by *F. moniliforme* which causes LEM was unknown.

Culture material on maize of *F. moniliforme* MRC 826, which was isolated from mouldy maize in the Transkei, has been shown to cause LEM in horses (Kriek *et al.*, 1981) and to be hepatocarcinogenic in rats (Marasas, Kriek, Fincham & Van Rensburg, 1984; Jaskiewicz, Van Rensburg, Marasas & Gelderblom, 1987). Culture material of this strain also exhibited cancer-promoting activity in a cancer initiation-promotion model, utilizing diethylnitrosamine-initiated rats and the induction of γ-glutamyltransferase positive foci in the liver as end-point (Gelderblom, Thiel, Jaskiewicz & Marasas, 1986). This bioassay was used in the isolation and purification of the novel mycotoxin, fumonisin B_1 (Gelderblom, Jaskiewicz, Marasas, Thiel, Horak, Vleggaar & Kriek, 1988). The structure of fumonisin B_1 (Fig. 1) has recently been elucidated (Bezuidenhout, Gelderblom, Gorst-Allman, Horak, Marasas, Spiteller & Vleggaar, 1988).

The induction of LEM in a horse by the intravenous injection of fumonisin B_1, isolated from cultures of *F. moniliforme* MRC 826, is reported in this paper.

MATERIALS AND METHODS

Cultures of F. moniliforme

The strain of *F. moniliforme* used in all the experiments was originally isolated from maize in the Transkei during 1975 and deposited in the culture collection of the South African Medical Research Council (MRC), Tygerberg, as *F. moniliforme* MRC 826 (Marasas *et al.*, 1984). This strain had previously been shown to cause LEM and hepatosis in 2 horses (Kriek *et al.*, 1981).

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Received 5 September 1988—Editor

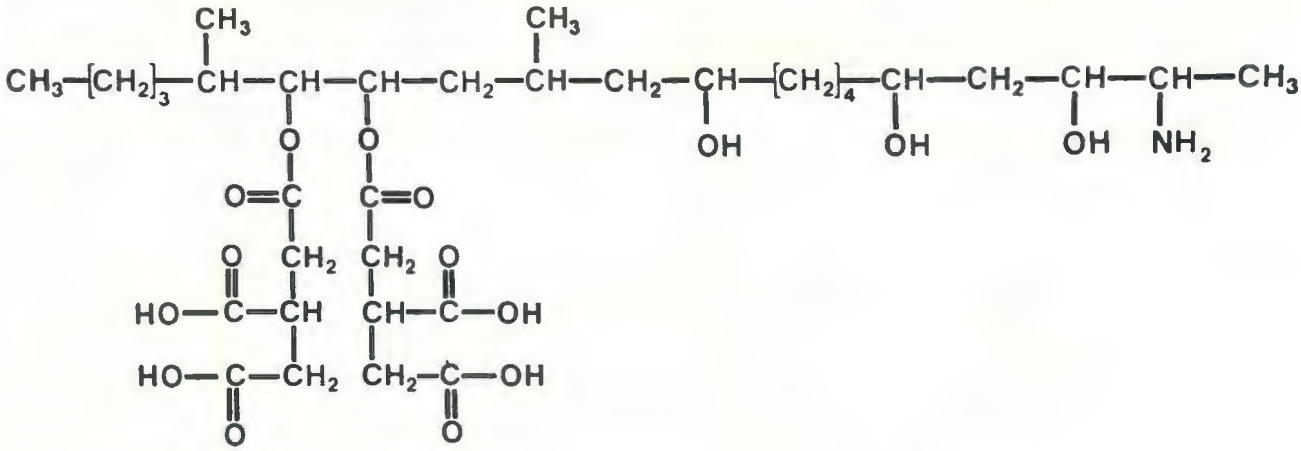

FIG. 1 Fumonisin B_1

Lyophilized conidia were used to inoculate autoclaved maize kernels, and cultures were incubated in the dark for 21 days at 25 °C. Thereafter, maize cultures were dried in an oven for 24 h at 50 °C, ground in a mill, and stored until used at 4 °C.

Isolation of fumonisin B_1

Maize cultures of *F. moniliforme* MRC 826 were extracted and fumonisin B_1 was isolated, as described by Gelderblom *et al.* (1988). The fumonisin preparation used in this investigation contained 92 % fumonisin B_1 as determined by an HPLC procedure. The maleyl-derivatized toxin was separated on a reversed phase column and the peak height observed for fumonisin B_1 by detection at 250 nm was compared with the peak heights of derivatized fumonisin B_1 standards (Gelderblom *et al.*, 1988). This method was based on a procedure devised for the quantification of host-selective phytotoxins from *Alternaria alternata* with chemical structures similar to that of fumonisin B_1 (Siler & Gilchrist, 1982).

The concentration of fumonisin B_1 in the culture material of *F. moniliforme* MRC 826 was likewise shown to be *c.* 1 g/kg.

Toxicity test in horses

Culture material of *F. moniliforme* MRC 826 was dosed by stomach tube to 2 horses (Table 1). A third horse was injected intravenously with 0,125 mg of fumonisin B_1/kg body mass/day (Table 2).

The following chemical pathological determinations were periodically done on the serum: aspartate transaminase (AST)[1], γ-glutamyltransferase (GGT)[1], lactate dehydrogenase (LD)[1] and total bilirubin (TBr) (Tietz, 1982). Enzyme activities were measured at 25 °C.

All the horses were necropsied immediately after euthanasia, carried out by the intravenous injection of an overdose of pentobarbitone sodium. The entire brain and spinal cord, and specimens of the lungs, myocardium, skeletal muscles, spleen, lymph nodes, liver, kidneys, adrenals and gastrointestinal tract were fixed in 10 % buffered formalin. After fixation, serial coronal sections *c.* 5 mm in thickness of the entire brain were cut and examined macroscopically. Selected blocks of the tissues were routinely processed, embedded in paraffin-wax, sectioned and stained with haematoxylin and eosin. Sections of the medulla oblongata were also stained by the luxol fast blue-periodic acid Schiff-haematoxylin (LFB-PAS-H) and luxol fast blue-Holmes (LFB-H) methods (Margolis & Pickett, 1956).

[1] Monotest, Boehringer Mannheim

RESULTS

Toxicity of F. moniliforme *MRC 826 culture material*

Horse 1. Six doses of 2,5 g of culture material/kg/day from Day 0–Day 7 resulted in clinical signs from Day 8 onwards (Table 1). These included apathy, weakness, anorexia, constipation and icterus.

Chemical pathological changes consistent with severe hepatic disorder, such as elevated values of TBr, AST, GGT and LD in the serum, were recorded from Day 7–Day 11 (Table 1).

Euthanasia was carried out on the horse on Day 11. The carcass showed a moderate icterus, the liver was mildly swollen and discoloured khaki-brown and the lobulation was slightly accentuated. In addition, there was mild perirenal oedema and mild oedema of the brain. Histopathological changes in the liver comprised marked cloudy swelling and hydropic degeneration, as well as mild to moderate fatty changes of hepatocytes. These were particularly prominent in the centri- and midzonal areas of the lobules. The cell membranes of the affected hepatocytes were clearly delineated. Individual hepatocytes or small groups of liver cells haphazardly distributed throughout the parenchyma were necrotic, and some of them were surrounded by aggregations of neutrophils. Isolated acidophilic bodies (necrotic hepatocytes) were also noticed in some lobules. Nuclear changes in the hepatocytes were prominent and comprised anisonucleosis (vesicular-, reniform- and bizarre-shaped), binucleation and prominent nucleoli, while a few hepatocytes showed mitosis. Mild cholestasis was also present. Moderate Kupffer cell proliferation was evident, particularly in the centri- and midzonal parts of the lobules, and these cells often contained lipofuscin and haemosiderin. Moderate numbers of neotrophils accumulated in the sinusoids. As a result of these changes the hepatic cords in the centri- and midzonal areas appeared to be somewhat disorganized. Mild to moderate oedema together with mild mononuclear cell (particularly lymphocyte) infiltration, mild fibroplasia and mild to moderate bile ductular proliferation were noted in the portal triads.

Microscopical examination showed the brain to be mildly oedematous. More severe oedematous changes were seen focally and bilaterally symmetrical in the medulla oblongata, particularly in the nuclei of the spinal tract of the trigeminal nerves and in the vestibular nuclei, as well as in the white matter tracts close to these nuclei. These changes included a moderate status spongiosus of the neuropil, the presence of variously sized, homogenous, eosinophilic globules (which gave a positive reaction with the PAS stain) in the neuropil and in many of

TABLE 1 Toxicity to horses of *Fusarium moniliforme* MRC 826 culture material

Horse				Dosing regimen (*per os*)				Clinical signs	Chemical pathological changes (maximum values)	Principal lesions
No.	Sex	Live mass (kg)	Age (yrs)	Dose (g/kg × n)	Days on which dosed	Total dose (g)	Day of euthanasia			
1	Mare	425	15	2,5 × 5 2,5 × 1	0–4 7	6375	11	Apathy, inappetence, icterus and constipation (Days 8–11)	Elevation of TBr (163 mol/ℓ), AST (2235 U/ℓ), GGT (64 U/ℓ) and LD (2685 U/ℓ) from Days 7–11	Severe hepatosis and mild oedema of the brain stem
2	Mare	385	15	1,25 × 5 1,25 × 1	0–4 7	2902	12	Slight nervousness, paralysis of lower lip, unable to walk, stands with legs awkwardly disposed. Signs appeared abruptly on Day 12: euthanised *c.* 1 h after the signs were observed	Moderate elevation of AST (340 U/ℓ from Days 8–12), GGT (22 U/ℓ from Days 9–12) and LD (1088 U/ℓ) on Day 10	Mild hepatosis and moderate oedema of the brain stem

TBr = Total bilirubin
LD = Lactate dehydrogenase (pre-dosing values: 300–504 U/ℓ)
AST = Aspartate transaminase (pre-dosing values: 104–155 U/ℓ)
GGT = γ-glutamyltransferase (pre-dosing values: 7–10 U/ℓ)

TABLE 2 Toxicity to a horse of fumonisin B_1 isolated from *Fusarium moniliforme* MRC 826 culture material

Horse				Dosing regimen (intravenous)				Clinical signs	Chemical pathological changes (maximum values)	Principal lesions
No.	Sex	Live mass (kg)	Age (yrs)	Dose (mg/kg × n)	Days on which dosed	Total dose (mg)	Day of euthanasia			
3	Mare	315	*c.* 15	0,125 × 5 0,125 × 1 0,125 × 1	0–4 7 9	276	10	Transient nervousness followed by severe apathy (doziness), reluctance to move, inco-ordination, paresis of the lower lip and tongue, dyspnoea and a convulsive seizure. Euthanised *c.* 2,25 days after the abrupt appearance of signs on Day 8	Mild elevation of AST (229 U/ℓ) and GGT (22 U/ℓ) from Days 8–10	Early, bilaterally distributed leukoencephalomalacia in the brain stem

AST = Aspartate transaminase (pre-dosing values: 95–99 U/ℓ)
GGT = γ-glutamyltransferase (pre-dosing values: 8–12 U/ℓ)

the perivascular spaces and in the cytoplasm of astrocytes. Small perivascular haemorrhages also occurred in these areas. The cytoplasm of many of the astrocytes were conspicuous and their nuclei were vesicular or occasionally reniform. Swollen axons as well as swelling of endothelial cells were evident in the more severely affected areas in the medulla oblongata. Sections stained with LFB-H and LFB-PAS-H revealed no evidence of demyelinisation.

In the kidneys, cloudly swelling and hydropic degeneration of the epithelial cells were evident in many of the convoluted tubules in the cortex; some of the cells also showed hyaline droplet degeneration and necrosis or signs of regeneration. A few of the tubules in the cortex were dilated and contained hyaline casts and isolated neutrophils.

No significant changes were noted in the other tissues.

Horse 2. Following 6 doses of 1,25 g of culture material/kg/day from Day 0–Day 7, clinical signs appeared abruptly on Day 12, i.e. 5 days after dosing had ceased (Table 1). In the morning the horse was found standing, the lower lip paralysed, and dripping saliva from the mouth. It refused to move and in an uncharacteristic display of skittishness waved its neck from side to side to avoid being touched by the handler. It stood unsteadily; once, while being bled, it almost went down on its haunches, but managed at the last minute to regain its balance. Efforts to lead the mare on a halter failed. She took one high step with a foreleg and then froze in that position, with the limb extended awkwardly in front of her.

Chemical pathological changes indicative of mild liver disorder, such as moderately elevated values of AST from Day 8–Day 12, GGT from Day 9–Day 12 and LD on Day 10, were recorded (Table 1).

Euthanasia was performed on the horse *c*. 1 h after clinical signs were first noticed on Day 12. Macroscopical lesions comprised a moderate congestion and oedema of the brain, slight accentuation of the lobulations of the liver, mild swelling and greyish-brown discoloration of the kidneys, mild intermuscular oedema among the

FIG. 2 Horse 3: Inability to eat

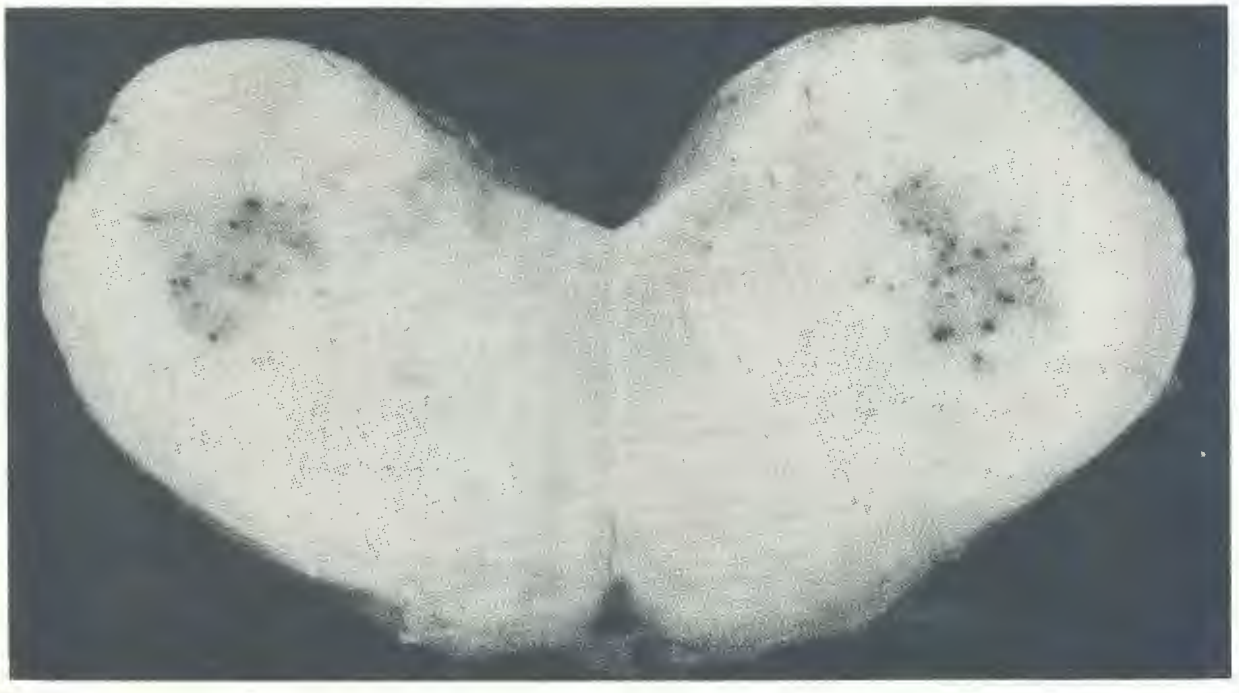

FIG. 3 Horse 3: Areas of necrosis and haemorrhage in the medulla oblongata

larger muscle groups in the hindquarters, and mild subcutaneous oedema about the udder. The principal lesions were mild hepatosis and moderate oedema of the brain. Histopathological changes in the liver of this animal were much milder than in Horse 1 and included the following: cloudy swelling and hydropic degeneration of hepatocytes, a few isolated acidophilic bodies scattered throughout the parenchyma, mild anisonucleosis of hepatocytes, mild Kupffer cell proliferation and pigmentation, and mild bile ductular proliferation.

Changes in the brain of Horse 2 were similar in nature and distribution to those in Horse 1, but more severe. None of the changes, however, were compatible with necrosis of the neutropil and glial cells and/or demyelinization. The spinal cord showed mild oedematous changes, a few swollen axons and moderate congestion of the grey matter.

No significant lesions were seen in the other tissues.

Toxicity of fumonisin B_1

Horse 3. This horse was injected intravenously 7 times from Day 0–Day 9 with 0,125 mg of fumonisin B_1/kg (Table 2). Clinical signs became apparent on Day 8. After a brief initial spell of nervousness the mare became apathetic and stood head-down, often with the feet (fore- or hind-) wide apart and the hindlegs 'tucked under', trembling intermittently and swaying gently. She was reluctant to move, walked hesitantly, took short steps, lifted the hindlegs high, and dragged the feet. Increasing paresis of the lower lip and tongue (Fig. 2) was manifested as the intoxication progressed. The horse had great difficulty in prehending, chewing and swallowing food and plugs of grass kept falling from the mouth. A watery discharge, tinged green with chlorophyl, exuded from the nostrils. Eventually, she could neither eat nor drink, and the tongue hung flaccidly from the mouth. Borborygmi became inaudible and constipation set in. Towards the end the horse was weak, trembled frequently and suffered from dyspnoea: breathing was forced, rapid and a heaveline was evident. Terminally, while being gently led about, the mare fell down in a tetanic convulsion and was put down on Day 10. The course of the intoxication, from the time that the signs were first observed to euthanasia, was *c*. 2,25 days.

Chemical pathological changes in this horse were limited mainly to mild elevations of AST and GGT from Day 8–Day 10 (Table 2).

At necropsy the brain was severely oedematous and, after fixation, greyish-brown foci *c*. 5 mm in diameter were discernible bilaterally symmetrical in the medulla oblongata (Fig. 3). The only other noteworthy gross lesions included mild congestion and oedema of the diaphragmatic lobe of the left lung, mild perirenal oedema, and numerous petechiae in the mucosa and a mild oedema of the submucosa in the caecum.

The principal microscopical lesions were a severe oedema of the brain; bilaterally symmetrical, focal necrosis in the medulla oblongata; mild hepatosis, characterized by cloudy swelling and hydropic degeneration of mostly the centrilobular and midzonal hepatocytes; a few scattered acidophilic bodies, and isolated aggregates of neutrophils in the sinusoids.

The microscopical changes in the medulla oblongata of Horse 3 had the same distribution as those in Horses 1 and 2, but were more severe, and distinct signs of necrosis of the grey and white matter were detectable. The necrotic areas were characterized by rarefaction of the neuropil, necrosis of neurons and glial cells, swelling of glial cells and axons, infiltration by neutrophils and a few macrophages with foamy cytoplasm containing LFB and PAS-positive material, and small perivascular haemorrhages (Fig. 4–6). Sections stained by the LFB-H and LFB-PAS-H methods revealed distinct loss of myelin and fragmentation and segmental ovoid swellings of axons in these areas (Fig. 7). The capillaries in and on the periphery of these focal lesions were made conspicuous by swelling and proliferation of the endothelial cells. The white and grey matter surrounding the necrotic areas showed evidence of severe oedema, such as a moderate

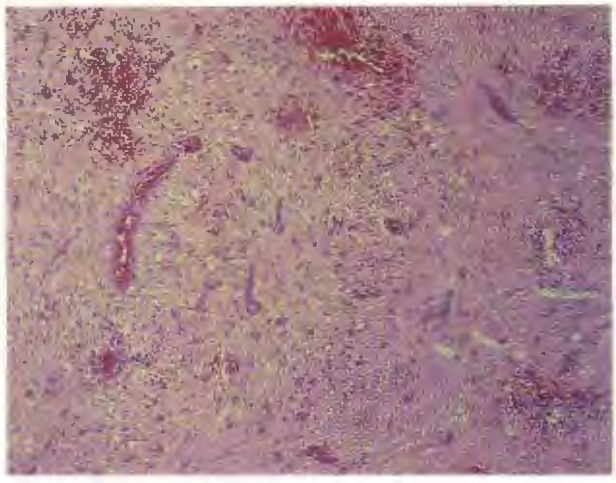

FIG. 4 Horse 3: Note necrosis of neuropil, perivascular haemorrhage and conspicuous capillaries. HE × 60

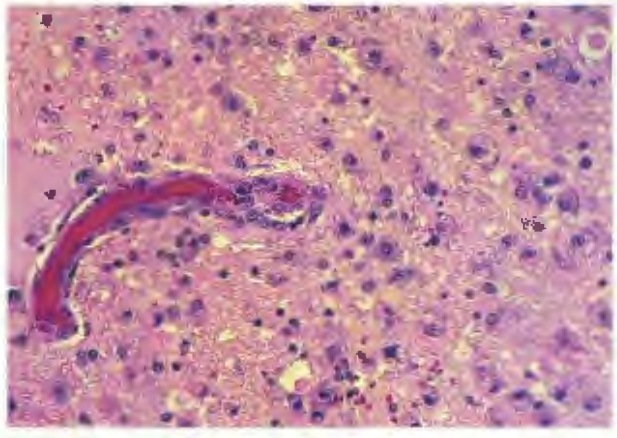

FIG. 5 Horse 3: Higher magnification of a necrotic area infiltrated by a few neutrophils and macrophages. HE × 200

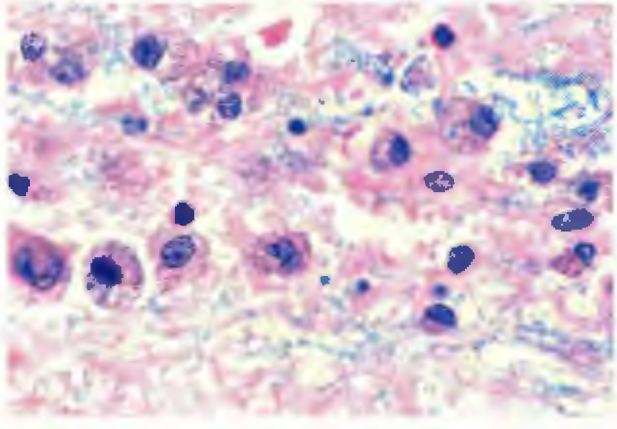

FIG. 6 Horse 3: Macrophages containing LFB- and PAS-positive material. LFB- PAS-H × 270

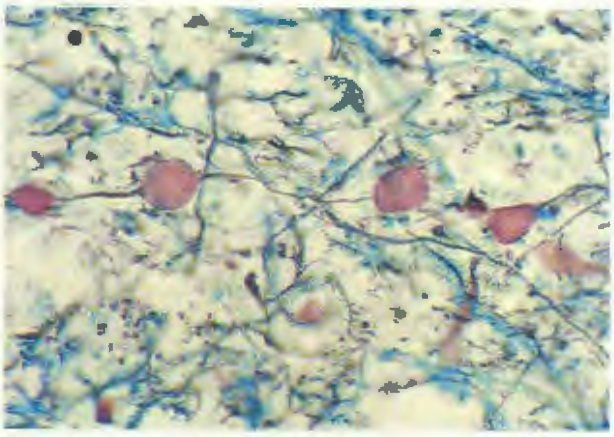

FIG. 7 Horse 3: Segmented, ovoid swellings of axons. LFB-H × 300

status spongiosus and varying sized homogeneous and eosinophilic globules (also positive with the PAS-reaction), in both the neuropil and perivascular spaces and in the cytoplasm of astrocytes (Fig. 8). The spinal cord showed congestion, as well as a few small perivascular haemorrhages and mild oedematous changes in the grey matter of the lumbar region.

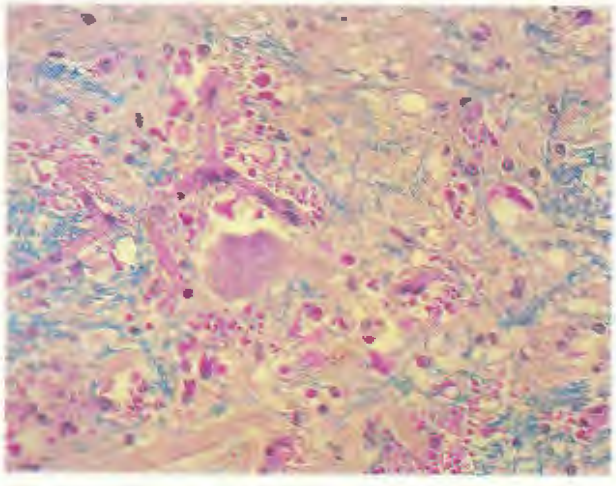

FIG. 8 Horse 3: Homogeneous, eosinophilic globules of varying sizes. LFB- PAS-H × 70

Changes in other organs included mild nephrosis, oedema of the submucosa in the large intestines, and mild congestion and oedema of the lungs. No significant changes were seen in the other tissues.

DISCUSSION

The present dosing trials with culture material of *F. moniliforme* MRC 826 confirmed a previous observation by Marasas *et al.* (1976) that a high dosage level induced a fatal hepatosis with only mild brain lesions, whereas a low level resulted in a mild hepatosis and more severe brain lesions. The lower dosage rate of 1,25 g of culture material/kg body mass/day, which induced neurological signs and moderate oedema of the brain after 6 administrations, was evidently still too high to cause LEM by Day 12, when euthanasia was performed on the horse.

The estimated concentration of fumonisin B_1 in the culture material was 1 g/kg. The intravenous dosing level (equivalent to one-tenth of the estimated concentration of fumonisin B_1 present in the daily oral dose of 1,25 g of culture material/kg) was consequently calculated as 0,125 mg fumonisin B_1/kg. The administration of 7 intravenous injections of fumonisin B_1 at this level to a horse (total dose 0,875 mg/kg), resulted in marked neurological signs on Day 8. Pathological changes consistent with early LEM were evident when this horse was necropsied on Day 10.

Although varying degrees of brain oedema were noticeable in all 3 horses in these dosing trails, the oedematous changes were always more severe in bilaterally symmetrical areas in the vicinity of the medulla oblongata. In Horse 3, the oedematous changes at this site were associated also with necrosis of the grey and white matter.

The literature on the pathology of equine LEM is scant and represents mostly reports on the findings of field outbreaks of the condition or the results of experiments where horses or donkeys received contaminated maize or culture material of the fungus for extended periods (Badiali *et al.*, 1968; Marasas *et al.*, 1976; Buck *et al.*, 1979; Pienaar *et al.*, 1981). In these instances, the lesions in the brain were mostly of long standing and striking, and characterized by distinct areas of liquefactive necrosis and cystic cavitations, particularly of the white matter in the cerebral hemispheres. It is clear, however, that the lesions in horses suffering from LEM are not only restricted to the cerebral hemispheres but could occur in other localities of the central nervous system, such as the brain stem (Schwarte, Biester & Murray, 1937; Wilson *et al.*, 1973; Buck *et al.*, 1979), cerebellum (Buck *et al.*, 1979) and spinal cord (Schwarte *et al.*, 1937; Biester, Schwarte & Reddy, 1940; Wilson *et al.*, 1973; Buck *et al.*, 1979). The term LEM that has been coined for this intoxication may also be somewhat misleading, as leukoencephalomalacia is not always present in every case and the grey matter may at times be involved (Marasas *et al.*, 1976; Buck *et al.*, 1979).

This is the first report of the induction of LEM in a horse with a chemical compound and presents experimental evidence that fumonisin B_1, produced by *F. moniliforme*, causes equine LEM.

ACKNOWLEDGEMENTS

We are indebted to Mr B. P. Maartens and other staff members of the Sections of Toxicology and Pathology of the Veterinary Research Institute, Onderstepoort, and to Mrs M. Schlechter of the Medical Research Council, Tygerberg, for competent technical assistance.

REFERENCES

BADIALI, L., ABOU-YOUSSEF, M. H., RADWIN, A. I., HAMDY, F. M. & HILDEBRANDT, P. K., 1968. Moldy corn poisoning as the major cause of an encephalomalacia syndrome in Egyptian Equidae. *American Journal of Veterinary Research*, 29, 2029–2035.

BEZUIDENHOUT, S. C., GELDERBLOM, W. C. A., GORST-ALLMAN, C. P., HORAK, R. M., MARASAS, W. F. O., SPITELLER, G. & VLEGGAAR, R., 1988. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *Journal of the Chemical Society, Chemical Communications*, 1988, 743–745.

BIESTER, H. E., SCHWARTE, L. H. & REDDY, C. H., 1940. Further studies on moldy corn poisoning (Leucoencephalomalacia) in horses. *Veterinary Medicine*, 35, 636–639.

BRITO, L. A. B., NOGUEIRA, R. M. G., PEREIRA, J. J. & CHQUILOFF, M. A. G., 1982. Leucoencefalomalacia em eqüino associada a ingestao de milho mofado. *Arquivos. Escola de Veterinara. Universidade Federal de Minas Gerais*, 34, 49–53.

BROWNIE, C. F. & CULLEN, J., 1987. Characterization of experimentally induced equine leukoencephalomalacia (ELEM) in ponies (*Equus caballus*): preliminary report. *Veterinary and Human Toxicology*, 29, 34–38.

BUCK, W. B., HALIBURTON, J. C., THILSTED, J. P., LOCK, T. F. & VESONDER, R. F., 1979. Equine leucoencephalomalacia: comparative pathology of naturally occurring and experimental cases. *Annual Proceedings of the American Association of Veterinary Laboratory Diagnosticians*, 22, 239–258.

BUTLER, T., 1902. Notes on a feeding experiment to produce leucoencephalitis in a horse with positive results. *American Veterinary Review*, 26, 748–751.

DOMENECH, J., BOCCAS, B., PELLEGRIN, F., LAURENT, D., KOHLER, F., MAGNOL, J. & LAMBERT, C., 1984. Étude de la fusariose du maïs (*Fusarium moniliforme*) en Nouvelle Calédonie et de la pathologie equine associée: la leucoencéphalomalacia équine. *Revue d' Élevage et Médicine Vétérinaire des Pays Tropicaux*, 37, 253–259.

GELDERBLOM, W. C. A., THIEL, P. G., JASKIEWICZ, K. & MARASAS, W. F. O., 1986. Investigations on the carcinogenicity of fusarin C—a mutagenic metabolite of *Fusarium moniliforme*. *Carcinogenesis*, 7, 1899–1901.

GELDERBLOM, W. C. A., JASKIEWICZ, K., MARASAS, W. F. O., THIEL, P. G., HORAK, R. M., VLEGGAAR, R. & KRIEK, N. P. J., 1988. The fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Applied and Environmental Microbiology*, 54, 1806–1811.

HALIBURTON, J. C., BUCK, W. B., LOCK, T. F., VESONDER, R. F. & WILSON, B. J., 1981. Equine leukoencephalomalacia: the toxicity of three *Fusarium moniliforme* metabolites in Equidae. *Journal of the American Veterinary Medical Association*, 179, 262–263.

HALIBURTON, J. C., VESONDER, R. F., LOCK, T. F. & BUCK, W. B., 1979. Equine leucoencephalomalacia (ELEM): a study of *Fusarium moniliforme* as an etiologic agent. *Veterinary and Human Toxicology*, 21, 348–351.

IWANOFF, X., YUAN, C. & FANG, S., 1957. Über die toxische Enzephalomalazie (moldy corn poisoning) der Einhufer in China. *Archiv für Experimentelle Veterinärmedizin*, 11, 1033–1056.

JASKIEWICZ, K., VAN RENSBURG, S. J., MARASAS, W. F. O. & GELDERBLOM, W. C. A., 1987. Carcinogenicity of *Fusarium moniliforme* culture material in rats. *Journal of the National Cancer Institute*, 78, 321–325.

KELLERMAN, T. S., COETZER, J. A. W. & NAUDÉ, T. W., 1988. Plant poisonings and mycotoxicoses of livestock in southern Africa. Cape Town: Oxford University Press, Southern Africa.

KELLERMAN, T. S., MARASAS, W. F. O., PIENAAR, J. G. & NAUDÉ, T. W., 1972. A mycotoxicosis of Equidae caused by *Fusarium moniliforme* Sheldon. A preliminary communication. *Onderstepoort Journal of Veterinary Research*, 39, 205–208.

KRIEK, N. P. J., KELLERMAN, T. S. & MARASAS, W. F. O., 1981. A comparative study of the toxicity of *Fusarium verticillioides* (= *F. moniliforme*) to horses, primates, pigs, sheep and rats. *Onderstepoort Journal of Veterinary Research*, 48, 129–131.

MARASAS, W. F. O., KELLERMAN, T. S., PIENAAR, J. G. & NAUDÉ, T. W., 1976. Leukoencephalomalacia: a mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. *Onderstepoort Journal of Veterinary Research*, 43, 113–122.

MARASAS, W. F. O., KRIEK, N. P. J., FINCHAM, J. E. & VAN RENSBURG, S. J., 1984. Primary liver cancer and oesophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme*. *International Journal of Cancer*, 34, 383–387.

MARASAS, W. F. O., NELSON, P. E. & TOUSSOUN, T. A., 1984. Toxigenic *Fusarium* species. Identity and mycotoxicology. University Park: Pennsylvania State University Press.

MARGOLIS, G. & PICKETT, J. P., 1956. New applications of the luxol fast blue myelin stain. *Laboratory Investigations*, 5, 459–474.

MONINA, M. I., MOSCOTENA, E. A., RUAGAR, J., IDIART, J. R., REINOSO, E. H., MURO, A., NOSETTO, E. O. & PONS, E. R., 1981. Leucoencefalomalacia equina. Casos registrados en el pais. *Revista Militar de Veterinaria*, 28, 13–17.

PAN, Y. G., 1981. Report of an investigation on mouldy maize toxicosis in horses. *Liaoning Xumu Shouyi*, 4, 10–12 (In Chinese).

PELLEGRIN, F., LAURENT, D., KOHLER, F., HAMEURT, J. & BOCCAS, B., 1986. Potentiel toxinogene des souches de *Fusarium moniliforme* parasitant le mais en Nouvelle Caledonie. *Microbiologie Aliments Nutrition*, 4, 157–161.

PIENAAR, J. G., KELLERMAN, T. S. & MARASAS, W. F. O., 1981. Field outbreaks of leukoencephalomalacia in horses consuming maize infected by *Fusarium verticillioides* (= *F. moniliforme*) in South Africa. *Journal of the South African Veterinary Association*, 52, 21–24.

RIET-CORREA, F., MEIRELLES, M. A., SOARES, J. M., MACHADO, J. J. & ZAMBRANO, A. F., 1982. Leucoencefalomalacia em eqüinos associada a ingestao de milho mofado. *Pesquisa Veterinaria Brasileira*, 2, 27–30.

RODRIGUES, J. A., 1945. Differenciacion entre la enfermedad de los rastrojos y la meningo encefalomielitis infecciosa de los equinas. *Anales de la Sociedad Rural Argentina*, 69, 305–307.

SCHWARTE, L. H., BIESTER, H. E. & MURRAY, C., 1937. A disease of horses caused by feeding moldy corn. *Journal of the American Veterinary Medical Association*, 90, 76–85.

SILER, D. J. & GILCHRIST, D. G., 1982. Determination of host-selective phytotoxins from *Alternaria alternata* f. sp. *lycopersici* as their maleyl derivatives by high-performance liquid chromatography. *Journal of Chromatography*, 238, 167–173.

THIEL, P. G., GELDERBLOM, W. C. A., MARASAS, W. F. O., NELSON, P. E. & WILSON, T. M., 1986. Natural occurrence of moniliformin and fusarin C in corn screenings known to be hepatocarcinogenic in rats. *Journal of Agricultural and Food Chemistry*, 34, 773–775.

TIETZ, N. W., 1982. Fundamentals of clinical chemistry. 2nd edn. Philadelphia: Saunders.

WILSON, B. J. & MARONPOT, R. R., 1971. Causative fungus agent of leucoencephalomalacia in equine animals. *Veterinary Record*, 88, 484–486.

WILSON, B. J., MARONPOT, R. R. & HILDEBRANDT, P. K., 1973. Equine leukoencephalomalacia. *Journal of the American Veterinary Medical Association*, 163, 1293–1295.

WILSON, T. M., NELSON, P. E. & KNEPP, C. R., 1985. Hepatic neoplastic nodules, adenofibrosis, and cholangiocarcinomas in male Fisher 344 rats fed corn naturally contaminated with *Fusarium moniliforme*. *Carcinogenesis*, 6, 1155–1160.

WILSON, T. M., NELSON, P. E., RYAN, T. B., ROUSE, C. D., PITTMAN, C. W., NEAL, T. P., PORTERFIELD, M. L. & SAUNDERS, G. K., 1985. Linking leukoencephalomalacia to commercial horse rations. *Veterinary Medicine*, 80, 63–68.

XIN, D. Y., 1987. Study on the aetiology of equine mouldy corn poisoning. *Acta Veterinaria et Zootechnica Sinica*, 18, 125–128 (In Chinese).