LEUKOENCEPHALOMALACIA IN TWO HORSES INDUCED BY ORAL DOSING OF FUMONISIN B_1

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ABSTRACT

KELLERMAN, T. S., MARASAS, W. F. O., THIEL, P. G., GELDERBLOM, W. C. A., CAWOOD, M. & COETZER, J. A. W., 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. *Onderstepoort Journal of Veterinary Research* 57, 269–275 (1990).

Leukoencephalomalacia (LEM) was induced by the oral administration of fumonisin B_1 (FB_1) to 2 horses: a filly received 59,5 mg/kg of a 50 % preparation of FB_1, administered in 21 doses of 1,25–4 mg/kg over 33 days; a colt, 44,3 mg/kg of 95 % pure FB_1 in 20 doses of 1–4 mg/kg in 29 days. Both animals developed nervous signs such as apathy, changes in temperament, inco-ordination, walking into objects, and one showed paralysis of the lips and tongue. Characteristic lesions of LEM were present in the brains. These trials proved conclusively that FB_1 can induce LEM in horses.

INTRODUCTION

The equine neurotoxicosis, leukoencephalomalacia (LEM), was first reproduced experimentally with naturally contaminated mouldy maize in the United States of America by Butler (1902), with pure cultures of the fungus *Fusarium moniliforme* Sheldon by Wilson & Maronpot (1971), and with a pure compound, fumonisin B_1 (FB_1) isolated from cultures of *F. moniliforme,* by Marasas, Kellerman, Gelderblom, Coetzer, Thiel & Van der Lugt (1988). In the experiment with FB_1, a horse was injected intravenously 7 times with 0,125 mg of FB_1/kg live mass/day (total dose: 276 mg of FB_1). Typical clinical signs of LEM, including nervousness followed by apathy, a wide-based stance, trembling, ataxia, reluctance to move, paresis of the lower lip and tongue, and inability to eat or drink, appeared on Day 8. Euthanasia was performed on the horse on Day 10 while the animal was in a tetanic convulsion. The principle lesions were severe oedema of the brain and early, bilaterally symmetrical, focal necrosis of the medulla oblongata (Marasas *et al.,* 1988). Advanced brain lesions of LEM (Badiali, Abou-Youssef, Radwin, Hamdy & Hildebrand, 1968; Wilson & Maronpot, 1971; Marasas, Kellerman, Pienaar & Naudé, 1976; Buck, Haliburton, Thilsted, Lock & Vesonder, 1979; Haliburton, Vesonder, Lock & Buck, 1979; Pienaar, Kellerman & Marasas, 1981; Marasas, Nelson & Toussoun, 1984; Kellerman, Coetzer & Naudé, 1988; Wilson, Nelson, Marasas, Thiel Shephard & Sydenham, 1990a) were not present in this horse.

The induction of the typical clinical signs and characteristic brain lesions of LEM in 2 horses by the oral administration of FB_1, is reported in this paper.

MATERIALS AND METHODS

Fumonisin B_1 preparation

FB_1 was isolated from maize cultures of *F. moniliforme* MRC 826 as previously described (Gelderblom, Jaskiewicz, Marasas, Thiel, Horak, Vleggaar & Kriek, 1988) with some modifications (M. Cawood, W. C. A. Gelderblom, R. Vleggaar, P. G. Thiel & W. F. O. Marasas, unpublished data). The

purity of FB_1 was determined by high performance liquid chromatography (HPLC) according to the method described by Alberts, Gelderblom, Thiel, Marasas, Van Schalkwyk & Behrend (1990). The FB_1 preparation used in the pilot trial (Horse 1) had a purity of *c.* 50 %, the other 50 % being inorganic matter that co-eluted during the purification on silica gel (Gelderblom *et al.,* 1988). The FB_1 preparation used in the second experiment (Horse 2) had a purity of 95 to 98 %, and is referred to as pure FB_1 in this paper.

Dosing regimens

The dosing regimens for Horse 1 and 2 are summarized in Table 1 and Fig. 1.

Horse 1. In a pilot trial, the FB_1 preparation (8 925 mg) dissolved in water was administered per stomach tube to a 9-month-old filly of 150 kg live mass. Based on the purity of the FB_1 preparation (50 %), the effective total dose was 29,7 mg FB_1/kg live mass.

Horse 2. In a follow-up experiment, pure FB_1 (8 417 mg) was similarly administered to a 14-month-old colt of 190 kg live mass. Based on a purity of 95 % for FB_1 used in this experiment, the total dose was 42,1 mg FB_1/kg live mass.

Clinical observations and chemical pathology

The horses were kept under close observation and all clinical signs noted. In order to monitor the effects of FB_1 administration on liver function, the following chemical pathological determinations were performed on serum samples collected periodically during dosing: aspartate transaminase (AST)[1], gamma glutamyl transferase (GGT)[1], lactate dehydrogenase (LD)[1] and total bilirubin (Tietz, 1982). Enzyme activities were measured at 25 °C.

Pathology

The horses were necropsied immediately after euthanasia, carried out by the intravenous injection of an overdose of pentobarbitone sodium. The 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 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.

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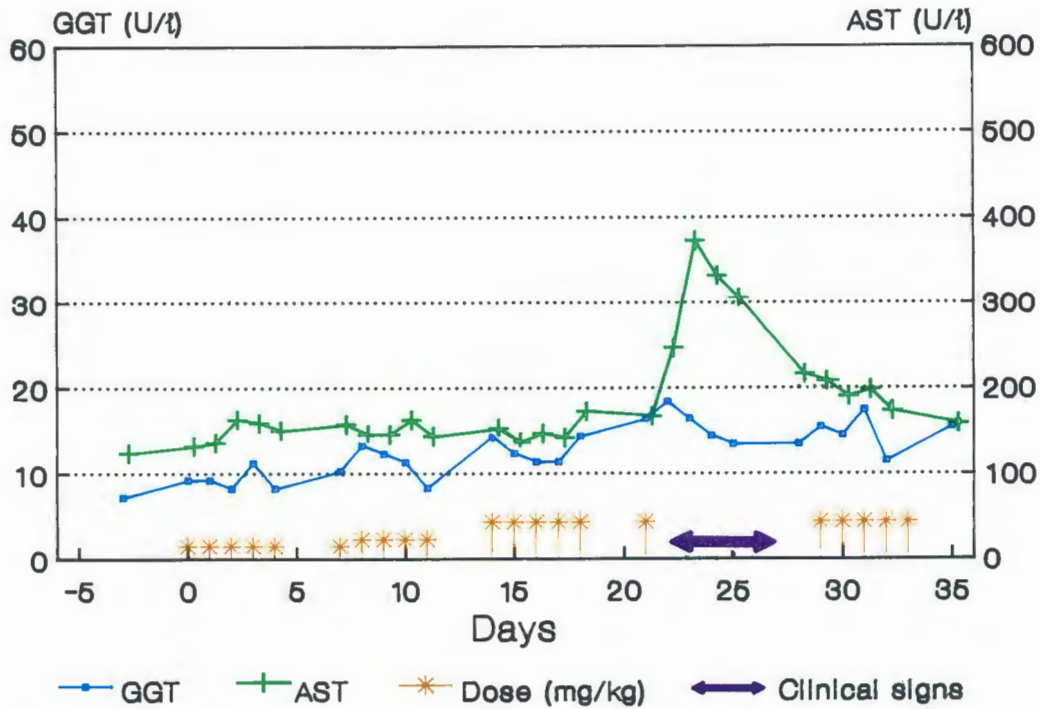[1] Monotest, Boehringer Mannheim

FIG. 1 Clinical pathological changes in the serum of Horse 1 dosed *per os* with a preparation (50 % pure) of fumonisin B₁
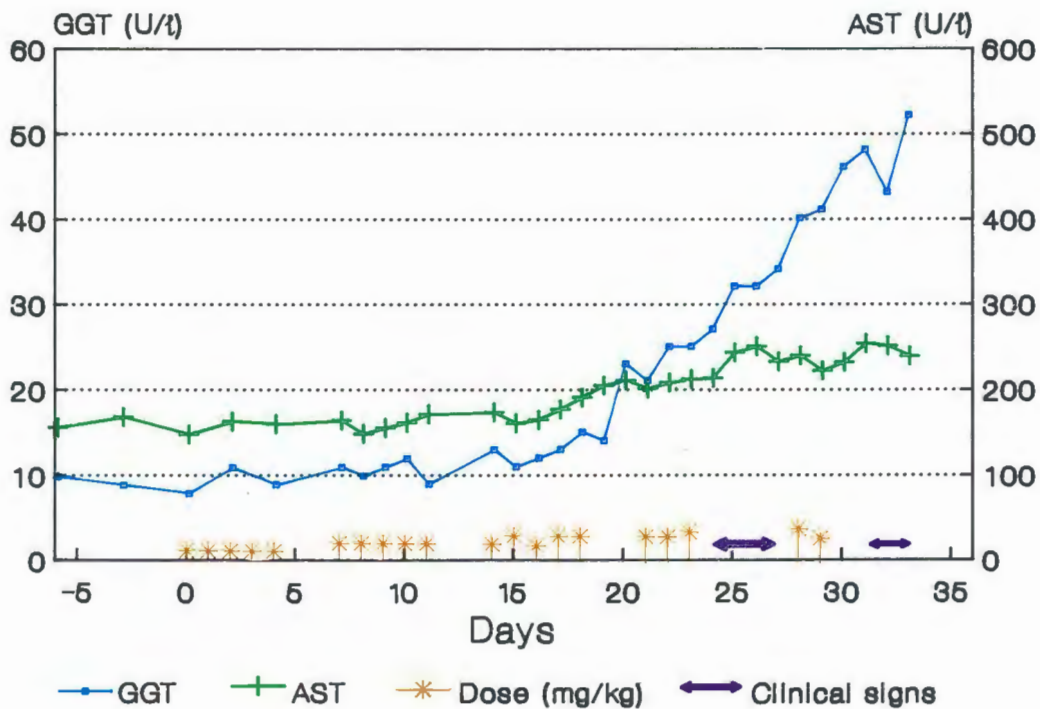

FIG. 2 Clinical pathological changes in the serum of Horse 2 dosed *per os* with 95 % pure fumonisin B₁

RESULTS

Clinical signs

Horse 1. Clinical signs were manifested from Day 22 to 27 (Table 1) and consisted of a change in temperament such as apathy and apparent stupidity; the filly stood wide-eyed, with ears pricked, staring uncomprehendingly at a person directly in front of it, or would almost walk into an obstacle before veering at the last moment to avoid collision. It was incoordinated, walking with a shuffling gait, hocks flexed and rump down, taking short steps, apparently lame in the left fore limb, occasionally stumbling and sometimes dragging the feet and knocking-on. When forced to turn suddenly it would lift both fore feet off the ground and pivot clumsily on the hind-limbs. It also had difficulty in judging distances and would sometimes bump its mouth against the crib while attempting to eat. Eventually it could neither eat or drink; hay hung unchewed from the mouth, and it could not swallow water.

The nervous signs improved progressively from about the 2nd day after they were first noticed until by the 6th day (Day 27) the foal was essentially normal. Despite the administration of 5 further doses of 4 mg/kg of FB_1 on Days 29–33, this apparent state of normality, during which the foal was perhaps slightly wilder and more clumsy than before, lasted until euthanasia was carried out on Day 35.

Horse 2. Clinical signs were manifested from Day 24 to 26 and again from Day 31 to 33 (Table 1). On the first occasion, Horse 2 stumbled and fell while getting up from the sternal position. Mild tremors were evident in the hind quarters, flanks and shoulders, and it made sporadic pawing motions with the right fore limb. It appeared to have undergone a subtle change in temperament, becoming uncharacteristically apathetic and docile, even allowing humans to come close before it moved away.

After a 4-day period of apparent normality, and 2 doses of FB_1 on Days 28 and 29, the signs reappeared, this time lasting until Day 33 (Table 1). At first the colt was merely apathetic and stood motionless with the head down and neck extended, showing little interest in the environment. If disturbed, it walked about restlessly without purpose, stumbling over the watertrough or bumping against the railings of the circular pen in which it was kept. The animal appeared rapidly to loose cognisance of its surroundings. It walked into objects with increasing frequency, and once, after a minor collision, reeled back and fell. Eventually it stood motionless for long periods, sometimes with its head pressed against the railings. Although the horse at this stage could still be led by the halter, it would stand immobile immediately upon release, as if incapable of voluntary movement. Its gait was not noticeably affected. Euthanasia was carried out on Day 33, when it could no longer eat or drink.

Chemical Pathology

The chemical pathological changes in Horse 1 and Horse 2 are illustrated in Fig. 1 and 2, respectively. In Horse 1 dosed with the 50 % pure preparation of FB_1, there was an elevation of AST activity in the serum between Days 22 and 31, with a maximum of 365 U/ℓ on Day 23. In contrast, there was a marked elevation of GGT activity in Horse 2 dosed with pure FB_1 between Days 20 and 33, with a maximum of 52 U/ℓ on Day 33.

Pathological changes

Horse 1. At necropsy a sunken area, *c.* 2 cm in diameter, was evident in the lateral part of the anterior frontal lobe of the left cerebral hemisphere (Fig. 3). The cerebrospinal fluid beneath the meninges in this area was slightly increased and tinged yellowish-brown. On the cut section of this focus the white matter was softer than normal and reddish-brown (Fig. 4). No lesions were seen in other parts of the brain. The synovial fluid was slightly increased in both carpal joints.

Microscopically, the focal lesion in the brain revealed liquefactive necrosis of the white matter, numerous macrophages (Fig. 5), aggregates of mineralization, and a few small haemorrhages. Some of the blood vessels in and at the periphery of the necrotic area showed marked hypertrophy and hyperplasia of endothelial cells, fibrinoid changes of their cell walls and mild perivascular mononuclear cell infiltration. Mild to moderate fibrosis was evident around some of these blood vessels (Fig. 6) and a fibrin mural thrombus occurred in a blood vessel in

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TABLE 1 Induction of leukoencephalomalacia by the oral administration of a preparation of fumonisin B_1 and pure fumonisin B_1 to horses

No.	Sex	Live mass (kg)	Age (yrs)	Dose (mg/kg × n)	Days on which dosed	Total dose (g)	Day of euthanasia	Clinical signs	Clinical pathology	Results
1	Filly	150	0,75	1,25 × 5* 1,25 × 1* 2,00 × 4* 4,00 × 5* 4,00 × 1* 4,00 × 5*	0–4 7 8–11 14–18 21 29–33	8,925*	35	Apathy, stupidity, inco-ordination, walking into objects, paralysis of lips and tongue (Day 22–27) followed by apparent recovery (Day 28–35)	Marked elevation of AST (maximum 365 U/ℓ) activity in the serum between Days 21 and 30	Leukoencephalomalacia, left frontal lobe
2	Colt	190	1,3	1,00 × 5 2,00 × 5 2,00 × 1 3,00 × 1 1,84 × 1 3,00 × 2 3,00 × 2 3,56 × 1 4,00 × 1 2,90 × 1	0–4 7–11 14 15 16 17–18 21–22 23 28 29	8,417	33	Apathy, docility, tremors, pawing motions (Day 24–26). Recovery or remission (Day 27–30). Apathy, bumping into objects, soporiferousness, inability to eat or drink (Day 31–33)	Marked elevation of GGT (maximum 52 U/ℓ) activity in the serum between Days 20 and 33	Leukoencephalomalacia, left frontal to occipital lobes

* Preparation of fumonisin B_1 (50 % pure)

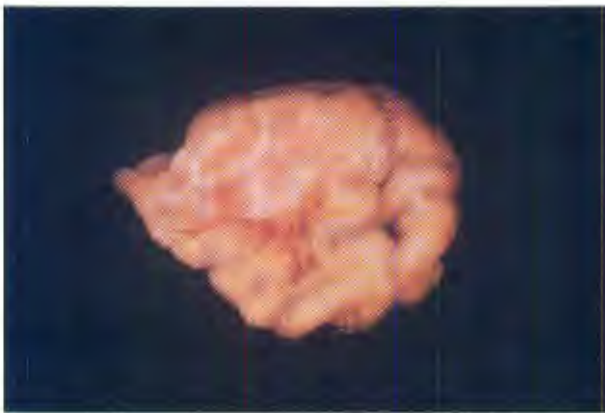FIG. 3 Horse 1: Sunken malacic area in frontal part of the left cerebral hemisphere

FIG. 6 Marked perivascular fibrosis in a necrotic area in the brain

and *c.* 4 cm in length, in the subcortical white matter of the left dorsal frontal lobe, extended posteriorly from just anterior of the middle of the hemisphere to the occipital lobe (Fig. 7). Apart from a small gelatinous focus, c. 1 cm in diameter, in the white matter of the right occipital lobe, no other lesions were seen in the brain and spinal cord.

FIG. 4 Horse 1: Coronal section of malacic area

FIG. 7 Horse 2: Severe swelling of left cerebral hemisphere and liquifactive necrosis of subcortical white matter

FIG. 5 Numerous macrophages arranged arround an affected blood vessel in the brain of Horse 1. Not fibrinoid changes in the vascular wall.

the malacic area. The white matter in close proximity to the focal lesion revealed mild status spongiosus and mild to moderate proliferation of astrocytes.

Apart from diffuse cloudy swelling and hydropic degeneration of hepatocytes no other lesions were evident in the tissue collected for examination.

Horse 2. The left cerebral hemisphere was diffusely swollen and the gyri were somewhat flattened. On cut section a yellowish-brown, less-dense, gelatinous, irregularly-shaped focus, *c.* 1–2 cm in width
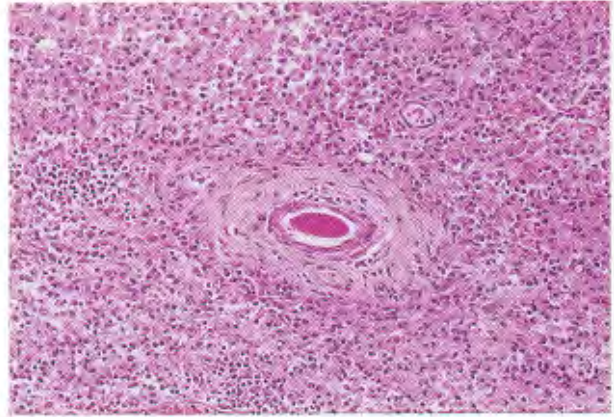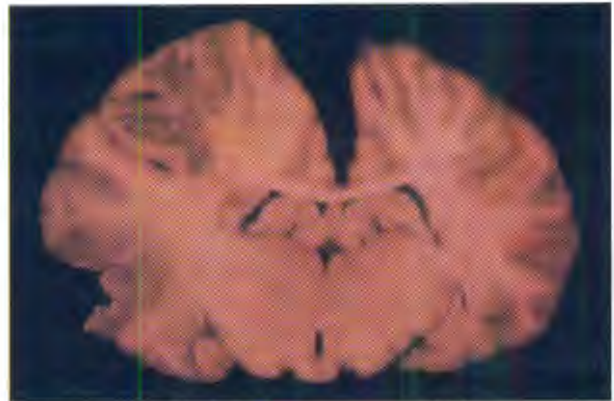
The kidneys were moderately swollen and diffusely greyish-yellow. The other organs and tissues showed no significant macroscopic lesions.

Microscopically, the grossly discernible focal lesions revealed moderate to severe rarefaction of the neuropil, partial loss of cellular detail of the white matter, swelling and proliferation of astrocytes, infiltration of moderate numbers of macrophages, and the presence of swollen axons (Fig. 8). Many of the blood vessels in and on the periphery of these foci revealed moderate hyperplasia and hypertrophy of endothelial cells, and haemorrhage and/or accumulation of oedematous fluid or homogeneous, eosinophilic droplets of varying size in dilated perivascular spaces (Fig 9).

Apart from these focal lesions the white and grey matter of the remainder of the left side of the brain showed changes referable to a moderate oedema.
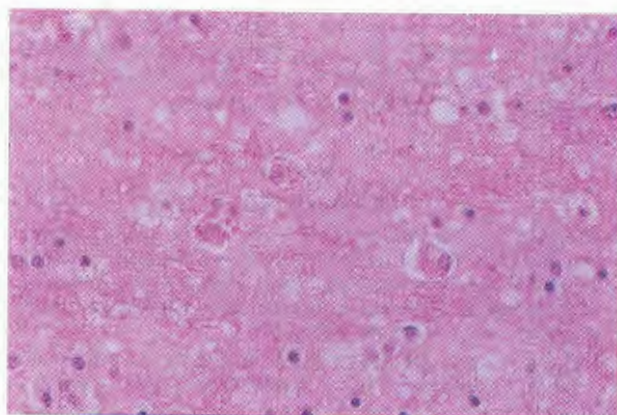
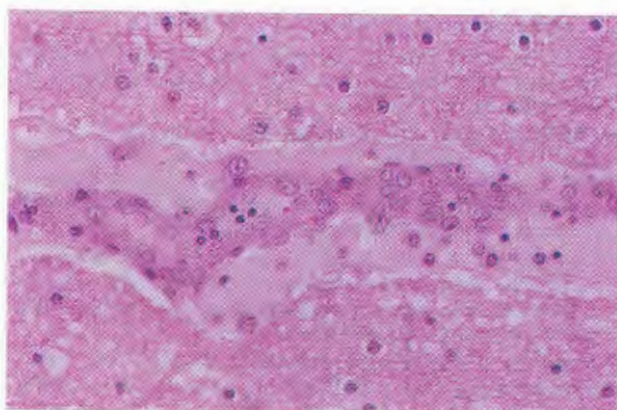FIG. 8. Rarifaction of white matter with infiltration by a few macrophages

FIG. 9. Marked endothelial hyperplasia and perivascular oedema in an area of rarifactin in the brain

The right side of the brain showed only a mild oedema.

The epithelium of the proximal convoluted tubules in the kidneys revealed cloudy swelling and hydropic degeneration.

DISCUSSION

Natural outbreaks of LEM in South Africa have, as far as can be established, been recorded only in adult horses. Experimental evidence reported in this study that a 9-month-old foal contracted the condition may, therefore, be of some diagnostic significance. Knowing that young horses are susceptible may also have bearing on the selection of animals for future research. Young animals are good subjects, easy to handle and require less FB_1 for the induction of LEM. Since LEM can be induced by either the oral or the intravenous routes (Marasas *et al.*, 1988), injection of fumonisins by the latter route would result in a further reduction of the dose. The fact that grossly discernible malacic areas were not evident in the brain of that horse injected intravenously with FB_1 (Marasas *et al.*, 1988), was probably related to the level, route and rate of administration of the toxin. In the case of the present 2 orally-dosed horses, the courses of the toxicoses were remarkably similar with respect to the onset of clinical signs (Day 22–24) and the number of dosages (16 to 18). However, the total dose administered prior to the

appearance of the first clinical signs differed widely, i.e. 19,7 and 31,6 mg/kg live mass in Horse 1 and Horse 2, respectively. Possible reasons for this discrepancy may be the differences in sex, age and individual variation between the 2 horses. The clinical signs of LEM may vary according to the size and siting of the lesion in the brain. The commonest signs observed in experimental cases induced with either culture material of *F. moniliforme* or FB_1 were changes in temperament, ranging from frenzy to depression with or without locomotory disturbance, walking into objects and paralysis of the lips and tongue. It should be borne in mind that the nervous signs are not always associated with liquefactive necrosis of the brain: experimental cases have been produced in which only oedema of the brain was evident (Marasas *et al.*, 1988). The apparent recovery of both horses after the first manifestation of signs may have been due to relief of intracerebral pressure after the diminution of brain oedema. Horse 1 recovered remarkably well despite the presence of a focal necrotic lesion in its left cerebral hemisphere. These observations indicate that LEM may not always be fatal and that horses may function reasonably well even with severe cerebral damage.

Poisoning of horses with FB_1 is unusual in that both the brain and liver lesions are caused by the same toxin. Considerable evidence has been produced that small doses of culture material over long periods culminate in LEM, whereas high doses over shorter periods result in hepatosis (Kellerman, Marasas, Pienaar & Naudé, 1972; Marasas *et al.*, 1976; Marasas *et al.*, 1988). LEM is not easy to reproduce experimentally as too high doses might lead to acute fatal liver failure while too small doses have no effect. This fact is illustrated by the failure of Laurent, Platzer, Kohler, Sauviat & Pellegrin (1989) to induce LEM in a horse despite the *per os* administration of 11,4 g of "macrofusine", a compound evidently similar to FB_1. The difficulties encountered in inducing LEM are exacerbated by the lack of reliable parameters for measuring nervous damage. As nervous signs are often proceded by elevations in the activities of liver enzymes such as AST and GGT in the serum, small but progressively higher doses of FB_1 were given until the activities of one or both of these enzymes started to rise. This indicated that a neurotoxic dose had possibly been given. Extreme care must nevertheless be exercised in the interpretation of chemical pathological data as nervous signs have been known to appear 5 days after dosing had ceased, without notable elevations of liver enzymes having been recorded (Marasas *et al.*, 1988). The effects of the interrupted administration of FB_1, compared to the continuous ingestion of maize naturally contaminated with FB_1, and other fumonisins, on the pathogenesis of LEM, remain to be determined.

Analytical techniques to detect and quantify FB_1 and fumonisin B_2 (FB_2), in feeds have been developed recently (Shephard, Sydenham, Thiel & Gelderblom, 1990; Wilson, Ross, Rice, Osweiler, Nelson, Owens, Plattner, Reggiardo, Noon & Pickrell, 1990b). This has made it possible to compare the dosage rates of FB_1 employed in the present study with the levels of fumonisins in naturally contaminated feeds implicated in field outbreaks of LEM. The conclusion that FB_1 is a causative factor of LEM has been substantiated by the fact that this compound, together with FB_2, have been detected in every incriminated feed sample thus far analyzed. Norred, Plattner, Voss, Bacon & Porter (1989)

detected both FB_1 and FB_2 in a sample of maize screenings associated with a field outbreak of LEM in the USA. This finding was confirmed by Voss, Norred, Plattner & Bacon (1989), who detected both FB_1 and FB_2 in two maize samples implicated in separate field outbreaks of LEM in the USA. Although these authors confirmed the presence of FB_1 and FB_2 in the naturally contaminated feed samples by mass spectrometry, the levels were not quantified.

In South Africa, Shephard et al. (1990) detected 8,85 and 3,00 µg/g FB_1 and FB_2, respectively, in a commercial mixed feed sample associated with a field outbreak of LEM. Thiel, Shephard, Sydenham, Marasas, Nelson & Wilson (1990) reported that 14 feed samples from confirmed cases of LEM in the USA contained FB_1 and FB_2 at levels ranging from 1,30 to 27,00 µg/g (mean = 7,70 µg/g) and 0,10 to 12,60 µg/g (mean = 3,10 µg/g), respectively. It was not possible to determine what proportion of the diet of the horses that developed LEM was contributed by the contaminated feeds. Consequently the dosage rates of naturally occurring fumonisins required to induce LEM under field conditions could not be calculated.

Wilson et al. (1990b) found both FB_1 and FB_2 in 3 samples of maize screenings which formed part of the diet of 66 horses. Of these 18 contracted LEM and 14 died. The fumonisins were present in damaged kernels and cob parts but not in undamaged kernels. The 3 samples contained 37, 56 and 122 µg/g FB_1 and 2, 11 and 23 µg/g FB_2, respectively. These authors calculated a daily dose rate of FB_1 for each of 13 of the horses that died by estimating the daily intake of contaminated maize screenings, assuming an average concentration of 72 µg/g FB_1 in the maize screenings and using the known live mass of each individual animal. Their estimates of the daily intake of FB_1 varied from 0,6 to 2,1 mg/kg/day. These dosage rates agree well with the dosage rates used in this study (1,25–4,00 mg/kg/day) to induce LEM in horses by per os administration of FB_1, especially since the possible contribution of FB_2 to the toxigenic potential of the feeds was not taken into account by Wilson et al. (1990b) in estimating the daily dosage rates. No information is available on the toxicity of FB_2 to horses, but it may be similar to that of FB_1. Until data become available, a similar toxigenic potential will have to be assumed for FB_1 and FB_2 in risk assessment, especially since FB_2 invariably forms a sizeable part of the total fumonisin concentration in contaminated feeds implicated in outbreaks of LEM (Shephard et al., 1990; Thiel et al., 1990; Wilson et al., 1990b). When comparing the daily dosage rates used to induce LEM experimentally in this investigation (Horse 1: 0,625–2,0 and Horse 2: 1,0–4,0 mg/kg/ day) with those estimated by Wilson et al. (1990b) (0,6–2,1 mg/kg/day), it should also be kept in mind that lower rates might also induce the disease. This investigation was, however, not designed to assess to lowest experimental dosage rates needed to induce LEM.

The study unequivocally proves that FB_1 can induce LEM in horses. Comparison of dosage rates furthermore indicates that the levels of FB_1 and FB_2, detected in feeds associated with confirmed cases of LEM, could account for the development of the disease in affected animals.

ACKNOWLEDGEMENTS

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