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A MYCOTOXICOSIS OF EQUIDAE CAUSED BY *FUSARIUM MONILIFORME* SHELDON. A PRELIMINARY COMMUNICATION

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ABSTRACT

KELLERMAN, T. S., MARASAS, W. F. O., PIENAAR, J. G. & NAUDÉ, T. W. A mycotoxicosis of Equidae caused by *Fusarium moniliforme* Sheldon. A preliminary communication. *Onderstepoort J. vet. Res.* 39(4), 205–208 (1972).

Fusarium moniliforme Sheldon was isolated from maize suspected of causing field cases of leucoencephalomalacia in horses in South Africa. It was cultured on autoclaved maize and dosed to three horses and three donkeys.

One horse and one donkey did not develop any sign of toxicosis; in another donkey an unexplained transient pruritis was encountered and two horses and one donkey died. Clinical signs observed in those animals that died included subcutaneous oedema and icterus. The gross pathological lesions consisted of severe cardiac haemorrhages; petechiae and ecchymoses in various organs; oedema; icterus and liver damage.

Histopathological lesions included diffuse fatty changes in the liver; fibroplasia around the central veins and portal tracts with bile duct proliferation; increased numbers of mitotic figures in the hepatocytes; megalocytosis and biliary stasis. The only brain lesions found were small perivascular haemorrhages.

This is in contrast with previous findings on this fungus where leucoencephalomalacia was the characteristic lesion. The liver damage and haemorrhagic syndrome caused by these isolates of *F. moniliforme* are discussed in the light of the prevalence of this fungus on maize.

INTRODUCTION

Fusarium moniliforme Sheldon, the conidial state of *Gibberella fujikuroi* (Sawada) Ito apud Ito & Kimura, is the most prevalent fungus on maize (*Zea mays* L.) kernels in the United States of America and has been isolated from 100% of the seed in some samples (Manns, 1923; Melchers, 1956; Nelson & Osborne, 1956; Tuite, 1961; Tuite & Caldwell, 1971). This fungus is also common on maize in southern Africa (Doidge, 1938; Martin, Gilman & Keen, 1971) and other parts of the world (Booth, 1971; C.M.I. Distribution Maps of Plant Diseasse, Map No. 102, Ed. 4, 1972). Moreover, extreme susceptibility to kernel infection by *F. moniliforme* has recently been demonstrated in maize with Texas (T) cytoplasm (Warmke & Schenk, 1971) and in high-lysine inbred lines (Ullstrup, 1971). In view of the prevalence of *F. moniliforme* on maize, knowledge concerning the mycotoxins produced by this fungus is vitally important to human and animal health.

Van der Walt & Steyn (1943) described a field outbreak in the western Transvaal of a neurotoxic syndrome in horses characterized by brain lesions, liver damage, oedema and haemorrhage. They suspected that this disease was caused by sugar bean (*Phaseolus vulgaris* L.) hay heavily contaminated by *F. moniliforme* and other fungi. Nelson & Osborne (1956) reported that *F. moniliforme* was the predominant fungus on mouldy maize implicated in deaths of swine in North Carolina. The causative role of *F. moniliforme* was not proved in either of these cases. Conflicting results have been reported regarding the toxicity of pure cultures of *F. moniliforme* to experimental animals. Extensive toxicity trials in rabbits produced negative results (Steyn, 1933, 1950; Van der Walt & Steyn, 1946), while acute toxicity to chickens, ducklings, mice and rats was reported by several investigators (Scott, 1965; Van Rensburg, Purchase & Van der Watt, 1971; Martin *et al.*, 1971; Ueno, Ishikawa, Nakajima, Sakai, Ishii, Tsunoda, Saito, Enomoto, Ohtsubo & Umeda, 1971; Marasas & Smalley, 1972).

Relatively little information is available concerning the chemistry of the toxic metabolites produced by *F. moniliforme*. An emetic principle and a toxic factor produced in liquid defined medium have been partially purified (Prentice, Dickson & Dickson, 1959; Prentice & Dickson, 1968). Mirocha, Christensen & Nelson (1969) reported that *F. moniliforme* produces the oestrogenic metabolite, F-2, 6-(10-hydroxy-6-oxo-trans-1-undecenyl) β-resorcylic acid lactone, but conflicting results were reported by Caldwell & Tuite (1968) and Caldwell, Tuite, Stob & Baldwin (1970). *F. moniliforme* is known to produce a compound F-3 that is closely related to the oestrogenic metabolite F-2 and is also suspected of having oestrogenic activity (Mirocha, Christensen & Nelson, 1968a, 1968b; Mirocha *et al.*, 1969). This compound, together with *F. moniliforme*, has been found in feed suspected of causing abortion or infertility in dairy cattle (Mirocha *et al.*, 1968a; 1969). Other metabolites produced by *F. moniliforme* include malonic acid (Nakamura, Shimomura & Ono, 1958); fusariocin A, a cytotoxic compound, $C_{23}\ H_{24}\ O_5 \cdot \frac{1}{2}\ H_2O$, with antitumour activity (Arai & Ito, 1970); benzoxanthentrione pigments with antiprotozoal activity (Kjaer, Kjaer, Pedersen, Bu'Lock & Smith, 1971); fusaric acid, gibberellins and kaurane diterpenoids (Serebryakov, Simolin, Kucherov & Rosynov, 1970; Turner, 1971).

A neurotoxic syndrome in Equidae known as mouldy corn disease, cornstalk disease or leucoencephalomalacia and characterized by focal necrosis of the white matter of the cerebrum, has been reproduced experimentally by feeding naturally contaminated mouldy maize in the United States of America (Butler, 1902; Schwarte, Biester & Murray, 1937), China (Iwanoff, Chang-kuo & Shih-chieh, 1957) and Egypt (Badiali, Abou-Youssef, Radwan, Hamdy & Hildebrandt, 1968). It was recently

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established by Wilson & Maronpot (1971) that this disease is caused by *F. moniliforme*.

Own Observations

During July 1970 a field outbreak of suspected leucoencephalomalacia occurred near Potchefstroom, western Transvaal, in horses fed maize chaff. Fungi were isolated from this chaff by placing small amounts of material by means of sterile forceps on either potato dextrose agar containing sodium novobiocin, malt salts agar or Czapek-Dox agar containing tergitol and incubating at 25°C. A total of 100 such direct platings were made on the three media and *F. moniliforme* was isolated from 100% of the platings, together with a few colonies of other fungi. One colony of *F. moniliforme* was isolated in pure culture and maintained on slants of 1,5% malt extract agar as Isolate OP-6.

During October 1971 approximately 100 horses died of an unknown disease at various riding schools at Kliprivier near Johannesburg. The mortality was, however, later proved not to be due to a mycotoxicosis. Fungi were isolated as described above from the following three feed samples obtained from two different riding schools: Sample A- maize meal; Sample B- maize cob meal mixed with molasses; Sample C- cracked maize. *F. moniliforme* was the predominant fungus isolated from all three samples. One colony of *F. moniliforme* was isolated in pure culture and maintained on slants of 1,5% malt extract agar as Isolate OP-32.

Inocula of both isolates (OP-6 and OP-32) of *F. moniliforme* were prepared as follows: spore suspensions prepared in sterile water from stock slant cultures were used to inoculate the surface of solidified 1,5% malt extract agar in 1 litre flasks (100 ml agar/flask). The cultures were incubated at 25°C until profuse sporulation occurred and the spores from each flask harvested in 1 litre of sterile water. The resulting spore suspension was used to inoculate mass cultures for use in toxicity trials. This procedure ensures that primary isolates from the original mouldy maize were subcultured only twice prior to the inoculation of autoclaved maize used in toxicity trials.

Cultures of the respective isolates on maize were prepared as follows: yellow maize kernels were autoclaved for 20 minutes at 121°C and 1,03 bar in 1 litre glass fruit jars (200 g maize kernels in 120 ml distilled water/jar). The autoclaved kernels in each jar were inoculated by means of a hypodermic syringe with 10 ml of spore suspension prepared as described above. Cultures of isolate OP-6 were incubated in a growth room at 25 to 28°C for 10 days, followed by 4 weeks of incubation at 20°C. Cultures of isolate OP-32 were incubated outside under a roof for 3 weeks (day temperatures: 23 to 31°C; night temperatures: 13 to 18°C). Following the specified incubation periods the contents of the jars were minced in a meat mincer, air-dried in flat metal pans at room temperature and stored at 4°C until used.

Initially the dry, milled culture material was fed mixed in a standard concentrate ration (1:3 m/m). Later when it was found that the mixture was not fully acceptable to the animals the culture material was dosed per stomach tube. Dosing was carried out daily, excluding week-ends.

The first series of experiments was conducted with cultures of Isolate OP-6 (Batch 1 to Batch 4).

Horse 1, a 2 year old mare of live mass *c.* 250 kg, readily consumed 23 kg of Batch 1 in 23 days without developing signs of toxicosis.

Donkey 2, a 3 year old stallion of *c.* 210 kg live mass, consumed 7 kg of Batch 2 in 10 days. A transient unexplained pruritis developed on the 13th day which affected the head, neck and withers. The lesion was bilaterally symmetrical, no peripheral zone of reaction was visible and skin scrapings were negative for parasites. After about 24 h the attack of pruritis ceased.

In a trial started about one month later the same donkey consumed 16 kg of Batch 3 in 37 days without ill effects.

Donkey 3, a 9 year old stallion of live mass 231 kg readily consumed 13 kg of Batch 4 in 15 days and then lost its appetite. In the 6 days that followed before it died, the donkey ate only 1 kg of the material. In all, 14 kg of culture material was ingested over a period of 21 days. The following clinical signs were observed on the 21st day:

The donkey stood head down, ears drooping and legs tucked under. It was reluctant to move and when forced to do so, reeled drunkenly. The lips and nose were swollen, severe icterus was present and a few petechial haemorrhages could be seen in the conjunctiva. Breathing was abdominal and rapid. The heart rate was increased. Later the tachycardia and polypnoea worsened, cyanosis set in and the donkey assumed sternal recumbency with the head upright and nose pressed to the ground. It died shortly afterwards.

When the first clinical signs were noticed the toxicosis was already far advanced. The duration of the toxicosis could not be determined but the time that elapsed between observation of the first signs and death was 4 h. The most conspicuous change in the blood chemistry was a marked bilirubinaemia (6,9 mg%, 70% conjugated).

Donkey 4, a 5 year old stallion of live mass 228 kg reluctantly consumed 1 kg of Batch 4 in 6 days. As the material was obviously unacceptable the remainder was dosed per stomach tube at 1 kg/day for 8 days and 1,25 kg/day for 6 days. This amounted to a total of 16,5 kg of culture material taken in over 25 days. No visible signs of toxicosis were produced.

The next series of experiments was conducted with a culture of Isolate OP-32 (Batch 5).

Horse 5: The culture material was dosed per stomach tube to a 10 year old gelding of live mass *c.* 300 kg. It received 1 kg/day for 3 days, 2 kg/day for 3 days, 3 kg/day for 2 days and 4 kg/day for 4 days. In all, accounting for week-ends, this amounted to a total of 31 kg administered in 16 days.

On the 17th day the horse was found dead, having died during the night without clinical signs being observed.

Horse 6: The same culture material (Batch 5) that had been stored for 3 months was dosed to a 7 year old mare of live mass 302 kg. She received 2 kg daily for 9 days which amounted to a total of 18 kg in 11 days.

On the 12th day the following signs were observed: The horse was somnolent and docile. There was slight oedema of the lips, supraorbital fossae and hind legs; the shoulder muscles trembled; the animal was weak and a few petechial haemorrhages were seen in the vulval mucosa. Respiration was rapid but the breathing, unlike that of Donkey 3, was not abdominal, no marked icterus was present and no tachycardia was observed.

The mare became progressively weaker; she lay down, lateral recumbency followed on sternal recumbency, and she died 18 h after showing the first signs.

Pathology

In the three animals that had died (Donkey 3, Horse 5 & Horse 6) the gross lesions were characterized by severe haemorrhages in the heart, petechiae and ecchymoses in various other parts of the carcass, localized oedema of the subcutis and, in two cases, liver damage and icterus.

Very extensive subendocardial haemorrhages, which were particularly marked in the left ventricle, occurred in all three animals. These large haemorrhages merged to form irregular suggillations around the base of the ventricle. In some areas the endocardium was raised 2 to 3 cm by the accumulation of blood underneath it. Smaller haemorrhages were scattered under the endocardium over the rest of the ventricles, especially on the papillary muscles. Numerous subendocardial petechiae were concentrated along the coronary grooves. Microscopic examination revealed oedema of the myocardium with small focal areas of hyaline necrosis and accumulation of lipid droplets within the heart muscle cells. The latter change was most marked in Donkey 3. Frequent intramyocardial haemorrhages also occurred in the heart muscle underlying the endocardium.

Ecchymoses and pin-point haemorrhages were located under both the parietal and visceral pleura, underneath the peritoneum of the abdominal wall, in the mucosa of the stomach, the small intestine, and (in one case) the bladder. In Donkey 3 these haemorrhages in the small intestine and stomach were very numerous, the mucosa was markedly congested and free blood was present in the lumen of the gut. This phenomenon was less pronounced in Horse 5 while in Horse 6 only the stomach showed congestion and numerous pin-point haemorrhages with very few haemorrhages in the remainder of the intestinal tract. In addition, Donkey 3 and Horse 6 showed oedema of the mucous membrane of the caecum with pin-point haemorrhages.

In all three cases accumulations of gelatinous straw-coloured fluid, associated with small haemorrhages, were present in the subcutaneous connective tissue and in the intermuscular fascia. The accumulations were localized over the region of the supraorbital fossae, base of the neck, lower part of the ribs and chest and, in Donkey 3, also in the muzzle and lower lip. In addition, oedema of the mediastinum and the perioesophageal connective tissue accompanied by a very marked hydropericardium (1 600 ml) and a prominent stasis of the subepicardial lymphatics were evident in Horse 6.

On histopathological examination diffuse fatty changes, seen as large lipid droplets in the hepatocytes, were prominent in the livers of Donkey 3 and Horse 5. Other microscopic features in these two cases were areas of early fibroplasia around the central veins and in the portal tracts with bile duct proliferation. The fatty changes were most marked in the liver of Donkey 3, which also showed an increased number of mitotic figures in the hepatocytes, while megalocytosis and bile stasis were observed in the liver of Horse 5, but with no increase in mitotic figures. In Horse 6 small lipid droplets occurred in the hepatocytes throughout the liver lobules but none of the more chronic changes observed in the livers of the other two cases were present.

Serial coronal sections of the brains revealed no gross lesions. Except for small perivascular haemorrhages, mainly in the white matter, there were no other obvious microscopic changes in the central nervous system.

Discussion

Our findings differ from those of Wilson & Marompot (1971) in that no lesions were found in the nervous system. In this investigation the cultures of *F. moniliforme* appeared primarily to affect the liver.

Van der Walt & Steyn (1943) recorded icterus, cirrhosis and fatty changes of the liver associated with nervous disturbance in horses grazing sugar bean hay infected with *F. moniliforme* and other fungi. Iwanoff *et al.* (1957) confirmed that icterus may occur in field outbreaks of equine mouldy corn toxicosis associated with encephalomalacia

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