A MYCOTOXICOSIS OF EQUIDAE CAUSED BY 
FUSARIA MONILIFORME SHELDON.
A PRELIMINARY COMMUNICATION

T. S. KELLERMAN, W. F. O. MARASAS,
J. G. PIENAAR & T. W. NAUDE

ABSTRACT

Fusarium moniliforme Sheldon was isolated from maize suspected of causing field cases of leukoccephalomalacia 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 purpura was encountered and two horses and one donkey died. Clinical signs observed in these 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 leukoencephalomalacia 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 (Mann, 1923; Melchers, 1956; Nelson & Osborne, 1956; Tuite, 1961; Tuite & Caldwell, 1971). This fungus is also common on maize in southern Africa (Doddie, 1938; Martin, Gilman & Keen, 1971) and other parts of the world (Booth, 1971; C.M.I. Distribution Maps of Plant Disease, 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, Ishib, Tsunoda, Saito, Enomoto, Ohsubo & 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-resorcyclic 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); fusaricin A, a cytotoxic compound, C_{12} H_{15} O_6 \cdot 3 H_2 O, with antitumour activity (Arai & Ito, 1970); benzoxanthrinolone pigments with antiprotozoal activity (Kjaer, Kjaer, Pedersen, Bu'Lock & Smith, 1971); fusaric acid, giberrellins and kaurene diterpenoids (Serebryakov, Simolin, Kucharov & Rosynov, 1970; Turner, 1971).

A neurotoxic syndrome in Equidae known as mouldy corn disease, cornstalk disease or leukoccephalomalacia 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 & Shibchich, 1957) and Egypt (Rudali, Abou-Youssef, Radwan, Hamdy & Hildebrandt, 1968). It was recently...
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. Incubating the were made on the three media and fungal colonies were transferred to 1,5% malt extract agar as Isolate OP-6. The resulting spore suspension prepared in sterile water. The resulting spore suspension was used to inoculate the surface of solidified 1,5% malt extract agar as Isolate OP-32. Cultures of isolate OP-6 were subcultured only twice prior to the inoculation of autoclaved maize used in toxicity trials. The next series of experiments was conducted with a culture of isolate OP-32 (Batch 5).

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 polydipsia 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 bilirubinemia (6.9 mg%, 70% conjugated).

**Horse 5**

The culture material was dosed per stomach tube to a 10 year old gelding of live mass 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, pericardium and pericardial fat, and 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.

Echymoses and pin-point haemorrhages were located primarily in both the parietal and visceral pleura, under 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 casesaccumulations 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 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 & Maronpot (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.

Acknowledgements

We are indebted to Prof. T. F. Adelaar for his unfailing interest and advice, and Mr. B. P. Maartens as well as the Staff members of the Sections Pathology and Toxicology for their competent technical assistance.

References


MYCOTOXICOsis OF EQUIDAE CAUSED By FUSARIUM MONIlIFOrME


