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A TREMORGENIC MYCOTOXICOSIS OF CATTLE CAUSED BY MAIZE SPROUTS INFESTED WITH *ASPERGILLUS CLAVATUS*

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ABSTRACT

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An outbreak of disease affecting a herd of 16 dairy cattle which were fed mouldy, sprouted maize is described. Eight of the cattle were affected, 5 of which died. The clinical signs included muscular tremors, hypersensitivity, ataxia, anorexia and salivation. *Aspergillus clavatus* was the only fungus isolated from the sprouts.

Clinical signs that were indistinguishable from those in the field outbreak were reproduced by dosing the mouldy maize sprouts to a steer and a sheep, and by dosing another sheep with maize inoculated with a pure culture of *A. clavatus* isolated from the mouldy maize on the farm. Light microscopical examination revealed neuronal degeneration and necrosis in the midbrain, medulla oblongata and spinal cord of all 3 of these animals.

The disease is clinically and pathologically indistinguishable from the disease caused by the ingestion of sorghum beer residue, and in certain respects it is similar to toxicoses caused by the ingestion of wheat sprouts and malt sprouts infested with *A. clavatus*.

INTRODUCTION

The saprophytic fungus, *Aspergillus clavatus*, has reportedly caused outbreaks of fatal disease in cattle which were characterized clinically by muscular tremors, ataxia and anorexia. Such outbreaks have occurred after the ingestion of mouldy, sprouted wheat, upon which *A. clavatus* was found to be the dominant fungus (Moreau & Moreau, 1960; Jaquet, Boutibonnes & Cicile, 1963), of mouldy malt sprouts from which *A. clavatus* was isolated (Minciuna, Mitroiu & Donta, 1977; Jiang, Huang, Chen, Yu, Chen & Sang, 1982) and of sorghum beer residue from which *A. clavatus* was readily isolated in pure culture (Kellerman, Pienaar, Van der Westhuizen, Anderson & Naudé, 1976). The syndrome has been reproduced in a steer by dosing it with pure cultures of *A. clavatus* isolate grown on autoclaved, non-toxic sorghum beer residue (Kellerman *et al.*, 1976).

Necropsies of the cattle which died following the ingestion of sorghum beer residue revealed degeneration and necrosis of certain of the skeletal muscles, serosal haemorrhages and evidence of gastro-intestinal stasis. Light microscopical examination of the central nervous system of these animals consistently revealed degeneration and necrosis of large motor neurons in the thalamus, midbrain, medulla oblongata and spinal cord (Kellerman *et al.*, 1976).

It is known that *A. clavatus* can form certain tremorgenic metabolites, including tryptoquivaline and tryptoquivalone (Glinsukon, Yuan, Wightman, Kitaura, Büchi, Shank, Wogan & Christensen, 1974; Clardy, Springer, Büchi, Matsuo & Wightman, 1975). Patulin, another metabolite of *A. clavatus*, has been shown to cause incoordination, paralysis, and neuronal degeneration in the cerebral cortex when injected into mice (Capitaine & Balouet, 1974). The toxic mechanisms involved in the field outbreaks of *A. clavatus* poisoning, however, are not clear. In the outbreak caused by *A. clavatus*-infested sorghum beer residue, methanol extracts of the residue proved tremorgenic when dosed to a sheep. Chromatographic investigation, however, showed that tremorgenic fractions obtained from these extracts were distinct from any of the known toxic metabolites of *A. clavatus*, and the constituents of these fractions were not identified (Kellerman *et al.*, 1976).

Herein we report an outbreak of disease in cattle characterized by hypersensitivity, tremors, ataxia and anorexia, which was caused by the ingestion of *A. clavatus* on sprouting maize.

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DESCRIPTION OF THE FIELD OUTBREAK

The outbreak involved a herd of 16 Ayrshire dairy cows, aged from 3–10 years, near Reitz in the Orange Free State. The cattle were kept in paddocks of poor quality veld grass, and each cow received daily 8–10 kg of freshly sprouted maize from a germination room. In addition, a ration of a commercially prepared milk-production meal was provided each morning when the cattle were milked.

The sprouted maize had been fed successfully from April to October each year for 8 years. The system for germinating the maize consisted of a 5 m long by 4 m wide corrugated iron germination room with a few transparent, fibreglass sheets in the walls to let in some light (Fig. 1). Heating was provided by electric elements buried in the cement floor and by an infra-red lamp mounted on one wall. The temperature varied between 10 ° and 35 °C, but in the week prior to the outbreak was between 24 ° and 25 °C. To prepare the sprouted maize, 8 kg of good quality maize was placed in a perforated container and submerged in a tank of water. Spoilt grains floating on the surface were skimmed off. The wet grain was left standing in the container on the heated floor of the germination room for 3 days and was moistened twice daily. On the 4th day the sprouting maize seeds were spread out 2 cm deep on stainless steel shelves where they were irrigated 8 times/day with 30 ℓ of water sprinkled from a perforated pipe (Fig. 1). The humidity in the unventilated room was so high that water droplets condensed on the metal roof. The sprouts were harvested after 1 week when they were about 15 cm high.

FIG. 1 Interior of germination room

FIG. 2 Cow knuckling over at hind fetlock

FIG. 3–5 Sequence showing another cow falling, with legs abnormally disposed, into sternal recumbency, and assuming a dog-sitting position while attempting to rise

In May 1983, 7 of the cattle became sick within a period of 3 days. According to the owner, the affected cattle did not eat, were hypersensitive, walked with a stiff gait and trembled. Some of them fell and remained recumbent. Two cattle were destroyed and a 3rd drowned in a dam.

We examined the herd 10 days after the onset of the outbreak. The condition of the cattle was poor, but clinical signs were not evident when they were at rest. Five of the cattle, however, displayed clinical signs when they were chased (Fig. 2–5). They tired easily, walked with the back arched, brushed with the hind feet and knuckled over at the fetlocks. Clinical signs became progressively mor marked with exertion. Eventually the hindlegs would give way and the affected animals would fall into sternal recumbency with the legs disposed abnormally. The recumbent animals then showed marked muscular tremors over the whole body, including the facial muscles. Respiratory distress was evident. The animals gaped, with the tongue protruded, and they salivated. After a rest, the animals would rise with difficulty, frequently falling back onto their haunches in a dog-sitting position. Altogether, 8 of the 16 cattle affected, 5 of which died.

FIG. 6 Mould growth at the bases of the maize sprouts

MATERIALS AND METHODS

Mycology

Two samples of mouldy, sprouted grains were collected from the shelves and were examined for the presence of fungi. Individual sprouts were removed from the bundle and those with visible signs of adhering mould growth were counted.

Small colonies of fungus were removed with a sterile needle and mounted in lactophenol (Anonymous, 1968) for microscopical examination.

Further pieces of fungal growth were placed in malt extract agar (MEA) plates (Anonymous, 1968) and incubated at 20 °C under intermittent illumination for 12 hours/day by mixed daylight-type and near ultraviolet lamps (Philips RSF 40 BLB) suspended 60 cm above the plates.

Preparation of mouldy maize sprouts

An inoculum for maize sprouts was prepared by inoculating a layer of MEA in a 500 mℓ Erlenmeyer flask with spores of *A. clavatus*, isolated from the sprouted maize and grown on MEA slants, and by incubating as above for 10 days. Ten flasks were prepared in this way.

Spore suspensions were prepared by adding to the fungal cultures in each culture flask 250 mℓ of distilled water containing 3 drops of Agapon[1] wetting agent and 6 glass beads sterilized for 15 minutes at 121 °C. By shaking gently, spores were liberated from the cultures and good suspensions were obtained. These were used to inoculate maize for feeding experiments.

To prepare mouldy maize sprouts, a procedure similar to that used on the farm was followed. The spore suspensions from 10 cultures, 2,5 ℓ in total, were added to 5 ℓ of sterilized water in an open container. Into this 8 kg of first grade yellow maize kernels, placed in a muslin bag, was immersed and allowed to soak for 15 minutes. The maize in the bag was kept in a humidified incubator at 25 °C for 3 days. Tap water was sprinkled over the maize at 3-hourly intervals during daylight hours until the maize germinated. The germinating maize was then spread in a thin layer on shelves covered with sterilized burlap under illumination by daylight-type, fluorescent tubes, was watered periodically by hand and was harvested when the sprouts had grown to about 15 cm long.

Dosing trials

Mouldy maize sprouts were collected from the germination room on the farm and were transported to the Veterinary Research Institute, Onderstepoort, where they were stored at −15 °C. The sprouts were fed daily to an 18-month-old Afrikander-type steer (Case 1) and were given daily to an adult Merino ewe (Case 2) through a ruminal fistula (Table 1).

Mouldy maize sprouts obtained by inoculating maize with *A. clavatus* spore suspensions isolated from the mouldy maize sprouts on the farm, as described above, were dosed daily to a 4-toothed Merino ram (Case 3) through a ruminal fistula (Table 1).

At the end of the trials, Cases 1 and 3 were killed by intravenous injection of pentabarbitone sodium. Case 2 had died during the trial. Necropsies were performed on each animal, and the brain, spinal cord and specimens of other selected tissues were fixed by immersion in buffered 10 % formalin. Paraffin sections of these tissues were prepared and stained with haematoxylin and eosin (HE) according to standard procedures for light microscopy.

RESULTS

Mycology

The samples of maize sprouts collected from the farm showed extensive growth of a blue-green fungus. More than 70 % of the sprouts separated from the bundle had colonies of this mould adhering to them.

The mould, the only fungus present on the sprouts, was identified as *A. clavatus* by the presence of typical clavate vesicles in the spore heads. It also grew out in pure culture from the inocula placed on the MEA plates.

Preparation of mouldy maize sprouts

The maize grain dipped in the spore suspension for preparation of sprouts germinated somewhat erratically and slowly, but reached a length of about 15 cm after 2 weeks of incubation. The bases of the green sprouts and ungerminated kernels were covered with a mat of blue-green mould growth, which was identified as *A. clavatus*.

Clinical signs

The clinical signs are summarized in Table 1. In Case 1, muscular tremors became evident in the hindquarters and flanks on Day 16 after the animal had been chased. Tremors after chasing became more marked on Days 17–19. On Days 20–28, the tremors occurred constantly when the animal was at rest and spread to involve the shoulders, neck and head. At this stage hypersensitivity was evident. On Day 28, the animal walked with stiff, extended hocks and brushed with the hindlegs. It was killed on Day 30.

In Case 2, hypersensitivity became evident on Day 12. The whole body shook when the back was tapped sharply. The animal walked with short steps and chewed intermittently. Muscular tremors began on Day 14. Chewing motions became continuous. Tremors and hypersensitivity became progressively more marked, and the animal was found dead on Day 19.

TABLE 1 Clinical signs after dosing of maize sprouts to sheep and a steer

Case	Source of sprouts	Initial live mass (kg)	Dosing regimen (g/kg/day × n*)	Total mass dosed (kg)	Duration of experiment (days)	Result
1 (Steer)	Farm	247	8 × 30	60,0	31	Tremorgenic syndrome; killed
2 (Sheep)	Farm	53	10 × 19	10,1	19	Tremorgenic syndrome; died
3 (Sheep)	Maize inoculated with *A. clavatus*	36	7,5 × 19	5,1	23	Tremorgenic syndrome; killed

* n = number of doses given

[1] Agfa-Gevaert

In Case 3, muscular tremors in the hindlegs became evident on Day 13. The animal became anorexic on Day 19. Tremors became progressively more marked and spread to involve the whole body by Day 20. The animal walked with short steps, dragged the hindlegs and knuckled over at the fetlocks on Day 20. On Day 22, it assumed sternal recumbency, with its legs abnormally disposed, and showed severe tremors. It was killed on Day 22.

Pathology

No significant changes were observed on macroscopical examination. The light microscopical changes observed were limited to the central nervous system. In all 3 cases, changes consistent with degeneration and necrosis were evident in some of the neurons of the midbrain, medulla oblongata and throughout the length of the spinal cord. These changes were similar in severity from case to case and appeared to select certain of the larger neurons, often in groups. Affected neurons showed central to complete chromatolysis, often with strong cytoplasmic eosinophilia and occasional cytoplasmic vacuolation. Neuronal neuclei were frequently flattened and displaced peripherally, and complete karyolysis was sometimes apparent (Fig. 7). The cytoplasm of some neurons was filled with a finely granular, light-brown pigment resembling lipofuscin. A few small, scattered accumulations of small glial cells were present, which were presumed to have replaced neurons which had disappeared.

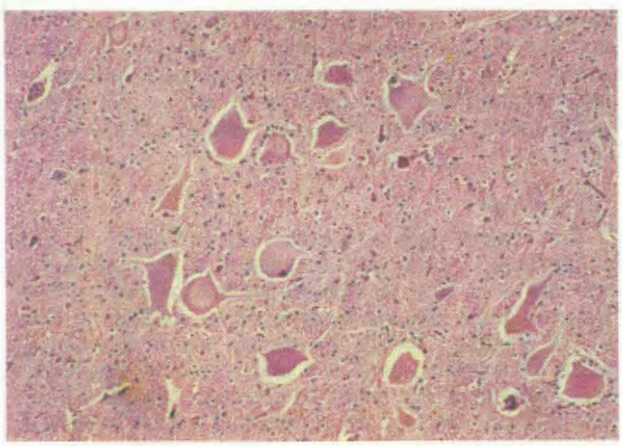

FIG. 7 Central chromatolysis and cytoplasmic vacuolation of neurons with nuclear margination and loss; medulla oblongata of steer (Case 1): HE × 400

DISCUSSION

The clinical signs in this outbreak, including tremors, hypersensitivity, ataxia and salivation, were indistinguishable from those that occurred in cattle in a disease outbreak caused by *A. clavatus*-infested sorghum beer residue (Kellerman *et al.*, 1976). These signs were also similar to those described in other outbreaks in which *A. clavatus* has been incriminated as the cause (Moreau & Moreau, 1960; Jaquet *et al.*, 1963; Minciuna *et al.*, 1977; Jiang *et al.*, 1982).

A. clavatus was the only fungus isolated from the mouldy maize in this outbreak. Clinical signs similar to those in the field outbreak were produced by dosing the mouldy maize sprouts collected from the farm and by dosing a culture of *A. clavatus*. The toxic fungal metabolite or metabolites responsible for the disease were not isolated or identified.

Degeneration and necrosis of certain of the larger motor neurons in the midbrain, medulla oblongata and spinal cord were observed in all 3 animals in the dosing trials. Neuronal changes that were similar both in nature and distribution also occurred consistently in the outbreak caused by *A. clavatus*-infested sorghum beer residue (Kellerman *et al.*, 1976). This apparently consistent association between the tremorgenic syndrome and neuronal changes suggests that the principal clinical features of *A. clavatus* poisoning result from toxic injury to the motor neurons. The reason for the selective susceptibility of certain groups of neurons remains unexplained.

This outbreak increases the known range of foodstuffs with which *A. clavatus* poisoning of cattle has been associated to include sprouted maize, and illustrates the potential danger of feeding mouldy, sprouted maize to livestock.

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REFERENCES

ANONYMOUS, 1968. Plant pathologists' pocketbook. Commonwealth Mycological institute, Kew, Surrey, England.

CAPITAINE, R. & BALOUET, G., 1974. Étude histopathologique des lésions induites chez la souris par les injections intraperitoneales et intracérébrales de patuline. *Mycopathologia et Mycologia Applicata*, 54, 361–368.

CLARDY, J., SPRINGER, J. P., BÜCHI, G., MATSUO, K. & WIGHTMAN, R., 1975. Tryptoquivaline and tryptoquivalone, two tremorgenic metabolites of *Aspergillus clavatus*. *Journal of the American Chemical Society*, 97, 663–665.

GLINSUKON, T., YUAN, S. S., WIGHTMAN, R., KITAURA, Y., BÜCHI, G., SHANK, R. C., WOGAN, G. N. & CHRISTENSEN, C. M., 1974. Isolation and purification of cytochalasin E and two tremorgens from *Aspergillus clavatus*. *Plant Foods for Man*, 1, 113–119.

JAQUET, J., BOUTIBONNES, P. & CICILE, J. P., 1963. Observations sur la toxicité d'*Aspergillus clavatus* pour les animaux. *Bulletin de l'Académie Vétérinaire de France*, 36, 199–207.

JIANG, C. S., HUANG, S. S., CHEN, M. Z. YU, Z., CHEN, L. H. & SANG, Y. Z., 1982. Studies on mycotoxicosis in dairy cattle caused by *Aspergillus clavatus* from mouldy malt sprouts. *Acta Veterinaria et Zootechnica Sinica*, 13, 247–254.

KELLERMAN, T. S., PIENAAR, J. G., VAN DER WESTHUIZEN, G. C. A., ANDERSON, L. A. P. & NAUDÉ, T. W., 1976. A highly fatal tremorgenic mycotoxicosis of cattle caused by *Aspergillus clavatus*. *Onderstepoort Journal of Veterinary Research*, 43, 147–154.

MINCIUNA, V., MITROIU, P. & DONTA, C., 1977. Severe intoxication in cows due to ingestion of mouldy malt sprouts. *Lucrarile Institutuliu de Cercetari Veterinare si Biopreparate "Pasteur"*, 13, 81–84.

MOREAU, C. & MOREAU, M., 1960. Un danger pour le bétail nourri de plantules fouragéres cultivées en germoir; la pollution d'une moisissure toxique, l'*Aspergillus clavatus*, cause des accidents mortels. *Comptes rendus de l'Académie d'Agriculture de France*, 46, 441–445.

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