

INDUCTION OF DIPLODIOSIS, A NEUROMYCOTOXICOSIS, IN DOMESTIC RUMINANTS WITH CULTURES OF INDIGENOUS AND EXOTIC ISOLATES OF *DIPLODIA MAYDIS*

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ABSTRACT

KELLERMAN, T. S., RABIE, C. J., VAN DER WESTHUIZEN, G. C. A., KRIEK, N. P. J. & PROZESKY, L., 1985. Induction of diplo-diosis, a neuromycotoxiosis, in domestic ruminants with cultures of indigenous and exotic isolates of *Diplodia maydis*. *Onderstepoort Journal of Veterinary Research*, 52, 35-42 (1985)

Diplo-diosis, a neuromycotoxiosis, principally of cattle, which is characterized by ataxia, paresis and paralysis, was induced in 13 cattle, 16 sheep and 3 goats, by dosing them with *Diplodia maydis* [= *D. zeae* (Schw.) Lév.] cultured on sterilized maize seeds.

The results of these experiments confirmed the findings of earlier workers that diplo-diosis is a mycotoxiosis caused by *D. maydis*.

The intoxication was induced with cultures of South African isolates of *D. maydis* obtained from local maize, one of which was associated with a suspected field outbreak, and with cultures of isolates from maize imported from the United States of America and Argentina. Other findings emerging from the experiments were, *inter alia*, that cultures incubated for less than 8 weeks were seemingly non-toxic, that there was little individual variation in response of cattle to cultures of the different toxic isolates or batches of the isolates, that apparent relapses of clinical signs can occur several weeks after dosing had ceased and that a small percentage of animals can show permanent locomotory disturbance. Light microscopical examination revealed no lesions in acutely affected animals, but an extensive laminar subcortical *status spongiosis* was evident in the cerebrum and cerebellum of a sheep that had been long paralysed and a steer that had permanent locomotory disturbance.

INTRODUCTION

Diplo-diosis is a common neurotoxicosis of cattle and more rarely of sheep grazing on harvested maize fields in winter. The disease, which is characterized by ataxia, paresis and paralysis, has never been reported outside southern Africa (Marasas, 1977). Mitchell (1918) first reproduced diplo-diosis experimentally by feeding either naturally mouldy cobs or pure cultures of *Diplodia maydis* to cattle. Later, his findings were experimentally confirmed in cattle and/or sheep by Theiler (1927), Shone & Drummond (1965), and Van Warmelo & Naudé (unpublished data, 1967). Affected stock usually recover rapidly after being removed from the source of intoxication, but new cases can still appear up to 10 days afterwards (Mitchell, 1918, 1919; Theiler, 1927; Steyn, 1934, 1949; Adelaar, 1958). The literature on diplo-diosis has recently been competently reviewed by Marasas (1977; 1978a; 1978b).

This investigation was prompted by the paucity of experimental data on diplo-diosis and the difficulties encountered in the artificial reproduction of the disease. Diplo-diosis, as far as we are aware, was the first mycotoxiosis to be experimentally induced by feeding pure cultures of a fungus to the target animal. According to published reports, however, only 3 oxen and 5 sheep have been successfully intoxicated by pure cultures in over 65 years.

MATERIALS AND METHODS

D. maydis (Isolate 2407) was isolated from maize collected in a field during an outbreak of suspected diplo-diosis in the western Transvaal. During the manual harvesting of maize on this field, the mouldy cobs had either been left on the stalks or discarded onto the ground. The fungus was also isolated from maize associated with an unspecified disease of cattle in South Africa (Isolates 2828, 2829) and from grain samples drawn from the holds of 2 ships laden with maize from the United States of America (Isolate 3153) and Argentina (Isolate 3209).

Cultures of the various *D. maydis* isolates were prepared by incubating sterile, moistened maize seeds inoculated with conidia for 3-8 weeks at 28 °C. The inoculated maize seeds were then dried at 40-45 °C for 24 h in a force-draught oven, milled, and tested for toxicity in ducklings (Rabie, Kellerman, Kriek, Van der Westhuizen & De Wet, unpublished data, 1984). Only those cultures which caused high mortality in ducklings were dosed per stomach tube to farm animals (Tables 1-6). A portion of Culture 2829 was made into a slurry with water at 75 °C, subjected to 75 °C for 48 h, and dried as described above. The heated and unheated portions were then dosed separately (Table 5).

Apart from the pure cultures, milled, naturally infected maize cobs (Fig. 1 & 2) from the supposedly toxic field were fed to a heifer.

All the animals were examined daily, and periodically the following routine chemical pathological determinations were done on their blood: packed cell volume, haemoglobin concentration, serum gamma-glutamyl transpeptidase and serum aspartate aminotransferase activities, serum urea and serum sodium, potassium, calcium and magnesium concentrations.

Selected animals (Bovine 10, Bovine 12, Sheep 5, Sheep 13 and Sheep 17) were killed for *post-mortem* examination. Specimens of the brain and spinal cord as well as of various organs were fixed in 10 % buffered formalin. Paraffin sections were prepared and stained with haematoxylin and eosin according to routine methods.

RESULTS

The results are summarized in Tables 1-6.

Experimentally induced diplo-diosis in cattle: Thirteen out of the 16 cattle that received culture material developed typical signs of diplo-diosis, but none died of the intoxication (Tables 1, 4 & 5). The dose needed to elicit signs varied between about 10-30 g/kg. The first signs appeared 1-8 d after commencement of dosing. Affected cattle usually recovered within a day or 2, but in exceptional cases the signs could last for almost a week. Steer 15 suffered from an apparent transient relapse 32 d after ostensibly recovering from experimental intoxication and 22 d after last being dosed (Table 4). The relapse occurred while the steer had steadfastly refused to eat a ration containing excessive monensin for a few days.

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FIG. 1. Maize cobs infected with *D. maydis*FIG. 2. Pycnidia of *D. maydis*

The clinical signs of only a few hours' duration were consistent with those of diplodiosis (Table 4) and not with those of monensin poisoning.

Newly affected cattle sometimes stood apathetically with their heads low, backs arched and tails tucked in. They were weak and mostly reluctant to move (Fig. 3 & 4), often assumed a wide-based stance (Fig. 4) or leaned slightly to one side in the hindquarters, apparently unable to abduct a hindleg to maintain balance. If induced to move, they walked unsteadily, usually swaying slightly in the rear or even tottering crab-wise, characteristically taking short steps with a stiff-legged (especially in the hindlimbs) high-stepping or saw-horse gait (Fig. 5 & 6), while sometimes knuckling over. Affected cattle tended to fall either in sternal recumbency or laterally in opisthotonus with the legs extended (Fig. 7). They could rise only with great difficulty, in the process frequently sinking onto their haunches in a dog-sitting position or going down either sternally or laterally. All eventually stood up, even after many hours of recumbency (Fig. 8). Some salivated mildly and, with the exception of Bovine 15, which had diarrhoea, all were constipated. Bovine 10 developed a permanent, involuntary high-stepping gait of the hindlimbs (Table 1). Permanent ataxia in a forelimb was also manifested by another steer (Steer 11) subsequent to the formation of abscesses in the bruised parts (Table 1).

Experimentally induced diplodiosis in sheep: Sixteen out of 25 sheep developed clinical signs (Tables 2, 5, 6). The dose required to induce diplodiosis varied from 10–130 g/kg, with the disease manifesting itself 2–16 d after commencement of dosing. Over 80% of the sheep reacted within 2–4 d of receiving 10–25 g/kg culture. Sheep 5, Sheep 13 and Sheep 17 were sacrificed for necropsy, the former in the acute phase of intoxication after being sick for 2 d and the latter 2 in paralysis after showing signs for 7 and 4 d respectively. One sheep died inexplicably overnight without signs being observed, and another was fatally asphyxiated as a result of mis-dosing. None of the sheep succumbed to diplodiosis.

The clinical signs of sheep, which included a wide-based stance, an arched back, a stiff-hindlegged gait with short steps and hopping with the hindlegs together like a rabbit, falling on knees and sternum, and recumbency, closely resembled those of cattle. Two sheep became apparently permanently paralysed, lying either laterally or sternally with the legs abnormally disposed.

Experimentally induced diplodiosis in goats: Three out of 3 goats developed diplodiosis 2–3 d after the commencement of a dosing programme, in which a total of 15 g/kg culture was administered to each. The clinical signs resembled those of cattle and sheep (Table 3).

The effect of length of incubation on the toxicity of culture: Clinical signs were induced only with cultures which had been incubated for 8 weeks. Cultures incubated for shorter periods, i.e. 3 and 5 weeks respectively, failed to induce diplodiosis even at a dose double that of the toxic 8-week culture (Table 4).

The effect of heat on toxicity: Part of a culture subjected to 75 °C for 48 h failed to cause intoxication at twice the toxic dose of the non-heated portion (Table 6).

The toxicity of milled, naturally mouldy cobs: A 2.5-year-old heifer with an initial live mass of 230 kg consumed 377 kg of ground cobs from the 'toxic' field in 115 days without ill effect. The daily intake was c. 3.25 kg per day and the total dose c. 681 g/kg milled cobs.

Chemical pathological findings: No notable clinical pathological changes were observed in any of the species other than occasional slight to moderate elevations of aspartate aminotransferase activity, especially in goats (74–300 U/l). These elevations appeared to arise from muscular damage sustained during falling episodes.

Pathological changes: In both acute and chronic cases no macroscopic lesions were observed. Histopathologically, no changes were observed in acute cases (Bovine 12, Sheep 5, Sheep 17), whereas an extensive laminar subcortical *status spongiosus* was evident in the cerebrum and cerebellum of a sheep that had long been paralysed (Sheep 13) and in a steer that had permanent locomotory disturbance (Bovine 10).

DISCUSSION

This investigation has confirmed the historic findings of Mitchell (1918) that diplodiosis is a mycotoxicosis caused by *D. maydis*. The difficulties encountered in the experimental reproduction of diplodiosis—only 3 cattle and 5 sheep having been artificially intoxicated in 65 years—and the apparent absence of the disease in other maize-producing regions of the world where *D. maydis* is present (Van der Byl, 1916; Mitchell, 1918; Theiler, 1927; Shone & Drummond, 1965; Marasas, 1977), suggested that a re-examination of the aetiology of the intoxication was necessary. Any doubts about the aetiological role of *D. maydis* in diplodiosis, however, has now been dispelled by the induction of the condition with pure cultures in 13 cattle, 16 sheep and 3 goats.

The isolation of toxigenic *D. maydis* strains from maize imported from North and South America is puzzling in the light of the apparent absence of diplodiosis in both of these continents. Although *D. maydis* is encountered wherever maize is grown, diplodiosis has never been reported outside southern Africa (Marasas, 1977). Marasas (1977) suggested that the absence of recorded outbreaks of diplodiosis elsewhere could be sought in a

TABLE 1 Toxicity of local *D. maydis* cultures for cross-bred cattle

No.	Bovine animal			Dosing regimen						Duration of experiment (days)	Fate	Result
	Sex H=Heifer B=Bull S=Steer	Age (Years)	Initial live mass (kg)	Culture No.	Batch No.	Dose (g/kg × n)	Period dosed (days)	Total dose (g/kg)	Total dose (kg)			
1	H	2	196	2407	1	5 × 3	0-2	15	2,940	8	Survived	Back arched, head low; walks with stiff-legged, high stepping gait of hindlimbs, taking short steps (Day 3 and Day 5)
2	H	2	270	2407	1	2,5 × 6	0-7	15	4,050	15	Survived	Arched back; walks with stiff hindlimbs and high-stepping gait, knuckles over; staggers and falls down, leans against fence for balance; salivation (Day 8-12)
3	S	2	195	2407	1	2,5 × 5 5 × 1	0-4 7	17,5	3,415	17	Survived	Arched back, wide based stance; walks with stiff-legged, high-stepping saw-horse gait, knuckles over, leans sideways, lateral recumbency (Day 8-14)
4	S	2	189	2407	2	5 × 4	0-4	20	3,780	8	Survived	Sternal recumbency, legs abnormally disposed, rises with difficulty, falls in dog-sitting position; unsteady, stiff-legged, saw-horse gait, lifting legs high, salivation (Day 4-7)
5	B	2	257	2407	2	5 × 6	0-5	30	7,70	7	Survived	Arched back, hindquarters lean sideways, walks with stiff-legged, high-stepping, saw-horse gait (Day 5-6)
6	S	2	245	2407	3	5 × 5	0-4	25	6,125	8	Survived	Sways in hindquarters, stiff hind legs, short steps, salivation (Day 6-8)
7	S	2	121	2407	4	5 × 5	0-4	25	3,025	8	Survived	High-stepping gait
8	S	0,8	138	2407	M*	5 × 5 5 × 8	0-4 7-14	65	8,970	15	Survived	Staggers; stiff-legged, high stepping gait (Day 5-7)
9	S	0,5	86	2407	M*	5 × 5 5 × 4	0-10	35	3,810	10	Survived	No signs
10	H	2	245	2407	5	5 × 3	0-2	15	3,675	43	Destroyed	Arched back, leans sideways in hindquarters; staggers crab-like with stiff-legged, high-stepping, saw-horse gait; falls repeatedly, rises with difficulty; lame from trauma (Day 2-13); Permanent involuntary, high-stepping gait of hindlimbs (Day 14-43)
11	H	1,5	252	2828	1	5 × 3	0-2	15	3,780	62	Survived	Falls frequently, rises with difficulty, back arched, steps short lame (trauma), salivation (Day 2-7). Abscesses formed in bruised hindquarters, shoulder and brisket. Permanent ataxia; left foreleg thrown outward and forward.
12	H	1,5	228	2829	1	5 × 3	0-2	15	3,420	3	Destroyed	Arched back; tremors, muscular spasms; incoordinated, high-stepping, stiff-legged, saw-horse gait; falls down repeatedly, lateral recumbency (Day 2-3)

* Mixed culture containing components of batches 2, 3, 4

TABLE 2 Toxicity of local *D. maydis* cultures for Merino sheep

No.	Sheep			Initial live mass (kg)	Culture No.	Batch No.	Dosing regimen				Duration of experiment (days)	Fate	Result
	Sex	Age					Dose (g/kg × n)	Period dosed (days)	Total dose (g/kg)	Total dose (kg)			
1	F	2t		38	2407	1	5 × 5 10 × 4 5 × 1	0-4 5-10 11	70	2,66	13	Died	Died overnight without signs being observed
2	F	mt		44	2407	1	5 × 10 10 × 2	0-11 14-15	70	3,08	21	Survived	No signs
3	F	mt		45	2407	1	5 × 10 10 × 2	0-11 14-15	70	3,15	21	Survived	No signs
4	W	mt		49	2407	1	5 × 10 10 × 2	0-11 14-15	70	3,43	21	Survived	No signs
5	W	mt		32,5	2407	1	5 × 5 10 × 5 15 × 3	0-4 7-11 14-16	120	3,25	17	Destroyed	Posterior paresis, wide-based stance, walks with rump tucked in and high-stepping gait, falls repeatedly, lateral recumbency with legs extended (Day 16-17)
6	W	mt		34	2407	1	5 × 5 10 × 5 15 × 3 10 × 1	0-4 7-11 14-16 17	130	4,42	21	Survived	Reluctant to stand, walks with rump tucked in and hocks flexed (Day 16-18)
7	W	mt		25	2407	2	5 × 5 10 × 5 15 × 3 10 × 1	0-4 7-14 14-16 17	130	3,25	21	Survived	Arched back, hindlegs tucked under, walks with shuffling gait and short steps (Day 16-18)
8	W	mt		35	2407	2	5 × 5 10 × 5 15 × 3	0-4 7-14 14-16	120	4,20	21	Survived	Transient diarrhoea (Day 17)
9	W	mt		33	2407	2	5 × 5 10 × 5 15 × 3	0-4 7-14 14-16	130	4,29	21	Died	Asphyxia
10	F	mt		18	2828	1	5 × 2	0-1	10	0,18	7	Survived	Back slightly arched, walks with stiff hindlegs and short steps (Day 2-3)
11	F	mt		21	2828	1	5 × 2	0-1	10	0,21	7	Survived	Wide-based stance, walks with stiff hindlegs and short steps, tremors (Day 2-3)
12	F	mt		21,5	2828	1	5 × 3	0-2	15	0,321	7	Survived	Arched back, wide-based stance, walks with stiff hindlegs and short steps, incoordination between hind and forelimbs, falls on sternum and slides forward on brisket (Day 3-4)
13	W	mt		21,5	2829	1	5 × 2	0-1	10	0,215	10	Destroyed	Wide-based stance, walks with stiff hindlegs and short steps, incoordinated staggers, falls down onto knees and slides forward on sternum, head tremors, lateral recumbency (Day 3-10)

TABLE 2 (Continued)

Sheep			Dosing regimen					Duration of experiment (days)	Fate	Result		
No.	Sex	Age	Initial live mass (kg)	Culture No.	Batch No.	Dose (g/kg × n)	Period dosed (days)				Total dose (g/kg)	Total dose (kg)
14	W	mt	23	2829	1	5 × 2	0-1	10	0,230	7	Survived	Weak in hindquarters, unsteady, walks with short steps (Day 2)
15	F	mt	19	2829	1	5 × 2	0-1	10	0,190	7	Survived	Wide-based stance, walks with stiff hindlegs, short steps and high-stepping gait (rear), incoordinated, falls on sternum (almost somersaults), head tremors (Day 2-3)

mt = milk tooth
2t = 2 tooth

F = female
W = wether

TABLE 3 Toxicity of local *D. maydis* cultures for Boer goats

Goat			Dosing regimen					Duration of experiment (days)	Fate	Result		
No.	Sex	Age	Initial live mass (kg)	Culture No.	Batch No.	Dose (g/kg × n)	Period dosed (days)				Total dose (g/kg)	Total dose (kg)
1	F	2t	41	2829	1	5 × 3	0-2	15	0,615	6	Survived	Stiff hindlegs hops with hindlegs together like a rabbit (Day 3-4),
2	F	6t	41	2829	1	5 × 3	0-2	15	0,615	6	Survived	Stands with head and neck propped against wall for balance; ataxic, walks with stiff-legged gait, hind and forelimbs unsynchronized, falls on brisket with forelegs folded under chest, has difficulty in abducting hindlegs to maintain balance (Day 3-4)
3	F	fm	45	2829	1	5 × 3	0-2	15	0,65	7	Survived	Stiff-legged, crab-like gait (Day 2-3)

2t = 2 tooth
fm = full mouth

TABLE 4 The effect of length of incubation on the toxicity of *D. maydis* cultures to cattle

Bovine animal			Dosing regimen					Duration of experiment (days)	Fate	Result		
No.	Sex	Age (years)	Initial live mass (kg)	Culture No.	Incubation length (weeks)	Dose (g/kg × n)	Period dosed (days)				Total dose (g/kg)	Total dose (kg)
13	S	1,5	228	2407	3	5 × 10	0-11	50	11,000	20	Survived	No signs
14	S	1,5	210	2407	5	5 × 10	0-11	50	11,300	20	Survived	No signs
15	B	0,75	126	2407	8	5 × 5 5 × 4	0-4 7-10 32	25 20	3,150 2,320	32	Survived	Diarrhoea and lateral recumbency with legs extended, tries to rise but falls (Day 5) Stiff hindlegs, falls (Day 8-9) Transient relapse: pronounced stiff-legged, high-stepping, saw-horse gait, salivation (Day 32)

S = steer
B = bull

TABLE 5 The toxicity of cultures of *D. maydis* isolated from maize imported from the United States of America (USA) and Argentina (Arg.)

Experimental animal		Dosing regimen						Duration of experiment (days)	Fate	Result	
No.	Sex	Age (year: teeth)	Initial live mass (kg)	Culture No.	Source of maize	Dose (g/kg × n)	Period dosed (days)				Total dose (g/kg)
B16	H	1,2	165	3153	USA	5 × 2 5 × 7	0-1 4-11	35	5,775	Survived	Stiff hindlegs, high-stepping gait (hindlegs) (Day 1, 2 & 4)
S16	W	mt	46	3153	USA	5 × 4	0-3	20	0,920	Survived	No signs
S17	W	mt	40	3153	USA	5 × 2	0-1	10	0,480	Destroyed	Walks with stiff hindlegs and short steps, sometimes hops with hindlegs together like rabbit; lateral recumbency (Day 2-6)
S18	W	2t	43	3153	USA	5 × 5	0-4	25	1,075	Survived	Arched back, hindlegs placed well forward under body; walks with stiff hindlegs, taking short steps (Day 4-5)
S19	W	mt	38	3153	USA	10 × 2	0-1	20	0,760	Survived	Hindquarters sway, walks with stiff hindlegs, taking short steps (Day 2-6)
S20	W	2t	42	3209	Arg.	5 × 5	0-4	25	1,050	Survived	Arched back, hindlegs placed well forward under body; walks with stiff hindlegs taking short steps (Day 3-4)
S21	W	mt	59	3209	Arg.	5 × 4	0-3	20	1,180	Survived	Arched back, walks with stiff hindlegs taking short steps, hops with hindlegs together like rabbit (Day 3)
S22	W	mt	49	3209	Arg.	10 × 1	0	10	0,49	Survived	Walks with stiff-legged saw-horse gait, taking short steps, staggers, knuckles over (Day 3-4)

mt = milk tooth
2t = 2 tooth
H = heifer
W = wether

TABLE 6 The toxicity of a local culture of *D. maydis* for Merino sheep after being made into a slurry and exposure to 75 °C for 48 hours

Sheep			Dosing regimen					Duration of experiments (days)	Fate	Result	
No.	Sex	Age	Initial live mass (kg)	Culture No.	Heat treated (H)	Dose (g/kg × n)	Period dosed (days)				Total dose (g/kg)
23	F	mt	22	2829	—	5 × 3	0-2	15	0,330	Survived	Lateral recumbency making ineffectual paddling motions, cannot rise unaided (Day 3), hindquarters lean over to one side, staggers, movement hind- and forelimbs unsynchronized, walks with hocks in flexion, falls on sternum with forelegs extended (Day 4)
24	F	mt	21	2829	H	5 × 4	0-3	20	0,420	Survived	No signs
25	F	mt	20	2829	H	5 × 6	0-5	30	0,600	Survived	No signs

mt = milk tooth
F = female

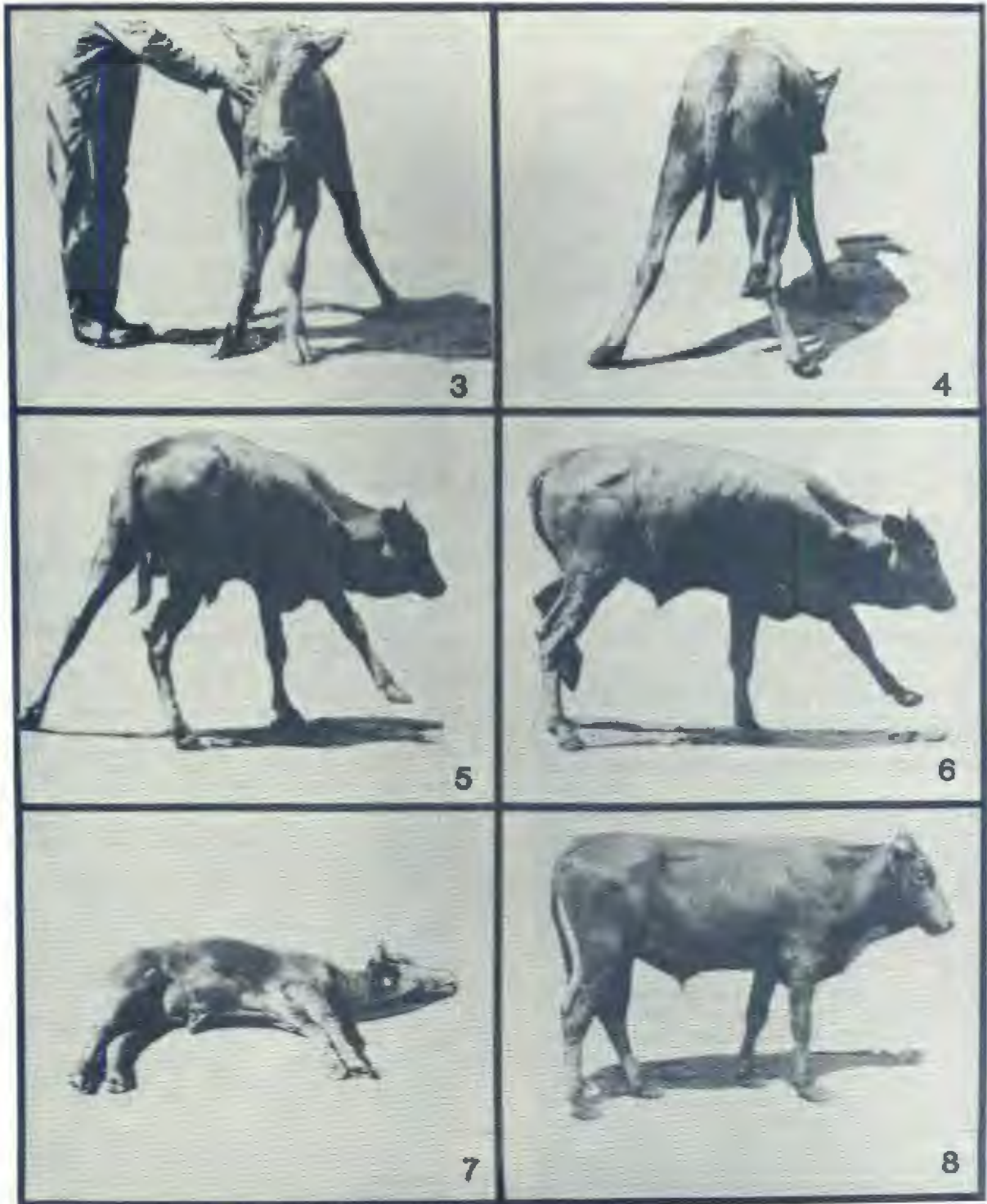


FIG. 3 & 4 Unsteadiness and wide-based stance

FIG. 5 & 6 Stiff-legged, high-stepping, saw-horse gait

FIG. 7 Lateral recumbency in opisthotonus with legs extended

FIG. 8 Recovery

combination of factors, *inter alia*, agricultural practices, variability in the toxicity of naturally infected maize, of pure cultures and of batches of cultures, the amount of culture consumed and differences in the susceptibility of individual animals. Variability in toxigenic potential between strains of different geographical areas may also play a role.

Previous workers (Van der Byl, 1916; Mitchell, 1918, 1919; Theiler, 1927; Shone & Drummond, 1965) reported considerable variation in the toxicity of naturally infected maize samples and of pure cultures of the fun-

gus. In our present study, cattle reacted fairly uniformly to the administration of different toxic cultures or to batches of the cultures, most being clinically intoxicated by doses of c. 20 g/kg (Tables 1, 4, 6). There were strong indications, however, that sheep were less susceptible to Culture 2407 than cattle (Table 2). Cattle developed diploidiosis at doses of 15–30 g/kg of this particular culture, whereas sheep needed at least 120 g/kg to do so. No such marked species differences were evident in any of the other cultures that intoxicated cattle and sheep at 15–35 g/kg and 10–25 g/kg respectively.

Since over 80 % of the sheep that reacted had received 10–25 g/kg culture, the majority of them cannot be said to be conspicuously more resistant than cattle.

Not all isolates of *D. maydis* are neurotoxicogenic (Van der Byl, 1916). Recently, 63,2 kg (or c. 200 g/kg) of a culture known to be toxic for ducklings (Rabie, Van Rensburg, Kriek & Lüben, 1977) failed to induce ill effects in a 320 kg cow (Rabie & Kellerman, unpublished data, 1980). Mortality in ducklings thus need not necessarily be correlated with nervous signs in cattle. This lack of correlation between mortality in ducklings and diplodiosis in ruminants casts doubt on the suitability of ducklings as a model for the bioassay of chemical fractions during extraction of the unidentified neurotoxin.

Part of the difficulties experienced in reproducing diplodiosis over the years probably stemmed from failure to culture the fungus for 8 weeks. As the cultures used in the successful reproduction of diplodiosis (Mitchell, 1918; Theiler, 1927; Shone & Drummond, 1965) had all been incubated for at least 2 months, Marasas (1977) contended that length of incubation period could be an important factor in determining their neurotoxicity. Our results support this contention (Table 4). Evidence has been submitted that cultures causing mortality of ducklings and rats also must be incubated for 6–8 weeks before they become toxic (Rabie *et al.*, 1977).

Our experience that mouldy maize from a suspected outbreak was non-toxic to a heifer is not unique. Other workers, too, at times fed supposedly toxic maize to ruminants with negative results (Van der Byl, 1916; Shone & Drummond, 1965). In the light of our present knowledge, failure to induce intoxication in cattle should be attributed to the low toxicity of the mouldy maize rather than lack of susceptibility on the part of the animals.

Perhaps it would be worthwhile to mention that not one case of diplodiosis has yet been brought to our attention in cattle feeding on maize that had been processed in any way, e.g. maize milled on the cob or ground maize incorporated into rations. The reason for this possibly chance observation is not known. Subjecting mouldy maize to heat, however, has now been shown to reduce its toxicity.

This study has possibly also shed additional light on the observation of previous workers (Theiler, 1927; Adelaar, 1958; Steyn, 1949) that new cases of diplodiosis can occur up to 10 d after stock had been removed from the source of intoxication. One steer ostensibly suffered a transient relapse more than 4 weeks after having apparently recovered from diplodiosis and 22 d after dosing had ceased (Table 4). If this steer had indeed suffered a relapse, some of the new cases appearing after stock had been removed from the toxic fields may have been animals suffering from such relapses.

Although no consistent lesions have been reported in diplodiosis, catarrhal enteritis and hyperaemia of the kidneys and lungs have been described in bovine diplodiosis (Mitchell, 1918). In this investigation, a laminar subcortical *status spongiosus* was evident in the cerebrum and cerebellum of a sheep that had been paralysed for a number of days and a steer with irreversible ataxia. This is the first account of histopathological lesions in diplodiosis. No conspicuous gross changes were noticed in any of the animals and, save for the 2 instances mentioned, no microscopical lesions were discernible.

The most important objective of research on diplodiosis must now be to isolate and identify the major toxic metabolite(s) that cause nervous signs in ruminants. A toxic component, diploidiatoxin, has been isolated from *D. maydis* cultures (Steyn, Wessels, Holzappel, Potgieter & Louw, 1972), but it has never been administered to ruminants.

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