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PERINATAL MORTALITY IN LAMBS OF EWES EXPOSED TO CULTURES OF *DIPLODIA MAYDIS (= STENOCARPELLA MAYDIS)* DURING GESTATION

T. S. KELLERMAN[1], L. PROZESKY[1], R. ANITRA SCHULTZ[1], C. J. RABIE[2], H. VAN ARK[1], B. P. MAARTENS[1] and ANNELIE LÜBBEN[2]

ABSTRACT

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During 1987 when cob rot was rife, perinatal losses were reported in flocks and herds that had been exposed to diplodiosis. The affected lambs or calves were either stillborn or died soon after birth. Dosing trials at the Onderstepoort Veterinary Research Institute involving 82 ewes revealed that 66 % of the offspring of dams exposed to cultures of *Diplodia maydis (= Stenocarpella maydis)* in the second trimester of pregnancy and 87 % of lambs of ewes exposed in the third trimester were born dead or died soon afterwards. A *status spongiosus* similar to that of the field cases was present in the white matter of the brains of all the affected lambs. The lambs of ewes that had shown nervous signs and those of ewes that had not shown such signs were equally affected. The experiments also clearly demonstrated that foetuses were much more susceptible to diplodiosis than the adults, e.g. in the third trimester 87 % of the lambs were affected compared to only 44 % of the dams. No perinatal mortalities were recorded in the group exposed to the culture in the first trimester.

Ewes developed significant resistance to intoxication after initial exposure to the culture and both the length of gestation and birth mass of the affected lambs were less than those of their untreated counterparts.

INTRODUCTION

Diplodiosis is a well-known endemic neuromycotoxicosis of domestic ruminants grazing on harvested maize land in winter. It is caused by the ingestion of maize infected by the common cob rot fungus, *Diplodia maydis* (Berk.) Sacc [= *Stenocarpella maydis* (Berk.) Sutton] (Van der Bijl, 1916; Mitchell, 1918; 1919; Theiler, 1927; Kellerman, Rabie, Van der Westhuizen, Kriek & Prozesky, 1985; Rabie, Kellerman, Kriek, Van der Westhuizen & De Wet, 1985). The disease is characterized by ataxia, paresis, and paralysis (Mitchell, 1918; Kellerman *et al.*, 1985; Kellerman, Coetzer & Naudé, 1988). Although mortality may be high, the prognosis is good if stock are removed from the toxic lands as soon as the first signs appear. Recovery is usually complete, without significant long-term deleterious effects. In one experiment, however, an animal became permanently paralysed and another developed an irreversible change in gait after being dosed with cultures of the fungus. A *status spongiosus* was evident in both of their brains (Kellerman *et al.*, 1985). Save for these two exceptions, no lesions have been described in diplodiosis.

Recently an apparently new aspect of diplodiosis came to light. During 1987 when cob rot was rife (Fig. 1), sporadic reports were received of perinatal losses in flocks and herds that had been exposed to diplodiosis. The affected lambs and calves were either stillborn or died soon after birth. Histopathological examination revealed a consistent, prominent *status spongiosus* of the white matter of their brains.

This study was carried out to determine whether diplodiosis could have a deleterious effect on the foetus of a ruminant.

MATERIALS AND METHODS

Inoculum

This was prepared as described by Rabie *et al.* (1985) from a toxigenic strain of *D. maydis* not associated with an authenticated outbreak of diplodiosis (MRC 2829) and one from maize collected on a field where perinatal losses of lambs had occurred (MRC 10400). The toxicity of MRC 2829 for sheep and cattle was reported on elsewhere (Kellerman *et al.*, 1985).

Culture

Sterile whole yellow maize (400 g maize + 400 mℓ water) in 2-litre wide-mouthed fruit jars was inoculated separately with MRC 2829 and MRC 10400 and incubated in the dark at 28 °C for 28 days. The culture material was subsequently dried, milled and stored for up to 5 years at −10 °C (Rabie *et al.*, 1985). In the case of the Pilot Trial MRC 2829 was cultured on commercial maize, while MRC 10400 and MRC 2829 for the Main Trail was grown on

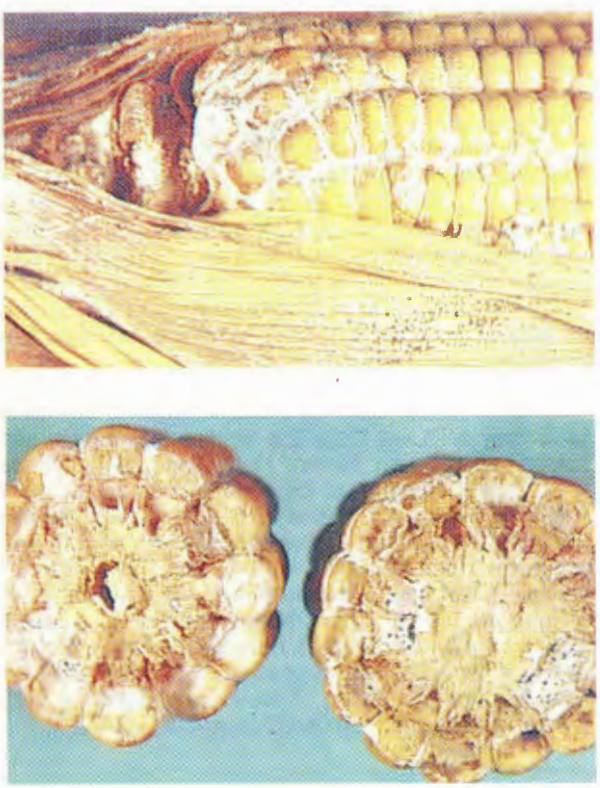

FIG. 1 Cob infected by *Diplodia maydis*: Note pycnidia, seen as black dots

[1] Veterinary Research Institute, Onderstepoort 0110, Republic of South Africa

[2] Food and Agricultural Chemicals, CSIR, P.O. Box 95, Pretoria 0001, Republic of South Africa

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seeds obtained from silos at Moroni, in an area free of *D. maydis*. Control maize was treated in the same way except that it was not inoculated.

Dosing trials

Pilot trial: Nineteen ewes in about the 3rd trimester of pregnancy were selected from a flock which had been naturally mated. The ewes were randomly divided into two groups. One group was exposed (*vide infra*) 4 times at approximately 2-weekly intervals to culture material of MRC 2829, while the other received uninoculated autoclaved maize at equivalent rates. An exposure consisted of 10 g/kg culture material administered per stomach tube on 2–4 successive days (Appendix 1 A & 1 B).

Main trial: Sixty three 2–4 tooth, non pregnant, merino-type ewes, in good condition where chosen for the experiment. The oestrus cycles of the ewes were synchronized by placing swabs impregnated with progesterone[1] in the vagina for 15 days. Each of the sheep was then injected intramuscularly with 0,5 mℓ of pregnant mare serum[2] before being artificially inseminated *c* 52 and *c* 58 h later. To ensure maximum conception, rams were put with the ewes for 4 d immediately after the last insemination. Rams were again returned for the same period 14 d after insemination to serve any of the ewes that might have failed to conceive in the groups destined to be dosed in the 2nd and 3rd trimesters. Mass was utilized as a blocking factor for Control 2, 2nd trimester and 3rd trimester ewes to ensure a relatively even range in all groups. Ewes within a certain mass range were randomly allocated to Control 2 and the treatment groups. Unequal numbers of ewes were allocated to the various groups to improve the reliability of comparisons, particularly between Control 2 and ewes exposed in the 3rd trimester, when diplodiosis was expected to exert maximum effect.

Treated sheep: Group A (9 ewes) was exposed 3 times at *c* 2-weekly intervals to culture material of MRC 10400 during the 1st trimester of pregnancy; Group B (9 ewes) was similarly exposed 3 times in the 2nd trimester; and Group C (15 ewes) 3 times in the 3rd. In a similar trial to the latter, Group D (10 ewes) was exposed 3 times at bi-weekly intervals to cultures of MRC 2829 in the 3rd trimester. An exposure consisted of 10 g/kg of culture material dosed per stomach tube on each of 3 successive days (Appendix 2 A, 2 B, 2 C & 2 D). The ewes in Groups B, C, D and Control 2 (*vide infra*) were confirmed to be pregnant by sonar scanning before commencement of dosing.

Control sheep: Control 1 was composed of 9 ewes, randomly selected from the flock immediately after insemination, i.e., before their state of pregnancy was known. They were exposed 3 times to the maize substrate in the 1st trimester (Sheep 63–71, Appendix 2 E). At the end of this trimester those that weer pregnant were included with Control 2. Control 2, comprised of 15 known-pregnant ewes, was exposed 6 times to the substrate during the 2nd and 3rd trimesters. All the control animals in the main trial received uninoculated autoclaved Moroni maize at rates equivalent to that of the culture material in the treated groups (Sheep 68–82, Appendix 2 E).

Clinical examination: All animals were regularly examined and the date of birth, birth mass (Appendix 1 & 2) and gains in mass were recorded.

Pathology: The stillborn, non-viable lambs and ewes that died were necropsied. Various organs including their brains and spinal cords were fixed in 10 % buffered formalin. After fixation, *c* 17 serial coronal sections *c* 5 mm thick of each brain and 12 sections of each spinal cord were cut. The tissue were routinely processed, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. Sections of the brains and spinal cords were also stained with Luxol fast blue-Holmes stain for myelin (Margolis & Pickett, 1956).

Definition of a positive case: A lamb was deemed affected if it was non-viable and had a *status spongiosus* in the white matter of the brain.

Identification of dams: It was not always possible to identify with absolute certainty the dams of dead lambs born during the night. A small measure of doubt therefore exists about the exact parentage of the lambs assigned to Ewes 42, 45 & 48 (Appendix 2 C).

Statistical analysis: Comparisons of frequencies were done by means of chi-squared tests. If the expected frequencies were small (Siegel, 1956) the Fisher exact probability test was used. Heterogeneous variances were evident for the comparisons of duration of pregnancy and the non-parametric Kruskal-Wallis test was used to compare the control and treatment groups. t-test (Cressie & Whitford, 1986) were utilized to compare birth masses and gains in mass of lambs. All the analyses were carried out by means of PC-programs written by H.v.A.

RESULTS

The results of the Pilot Trial are summarized in Appendix 1 and that of the Main Trial in Appendix 2.

Pilot trial

One normal lamb was born in the group that had been exposed to *D. maydis* culture compared to 14 healthy ones in the control group (Appendix 1 A & B).

Main trial

Only 5 out of 15 lambs of ewes exposed in the 2nd trimester and 5 out of 37 lambs of those exposed in the 3rd trimester survived. The lambs of ewes in the 2 control groups and those of ewes that were exposed in the 1st trimester showed no ill-effects (Appendix 2 A–E).

Combined trial

Since the conditions and results of the 2 trials were very similar, they were grouped together for statistical analysis *vide infra* (Fig. 2).

1. *Comparison of* D. maydis *isolates MRC 2829 and MRC 10400:* To determine whether the toxicity of these two isolates differed, the number of affected ewes and lambs in the 3rd trimester were compared. Since no conspicuous differences between the cultures of isolate MRC 2829 in the 2 trials could be detected, these results were pooled.

(1) Upjohn, Repromap

(2) Upjohn, Fostim

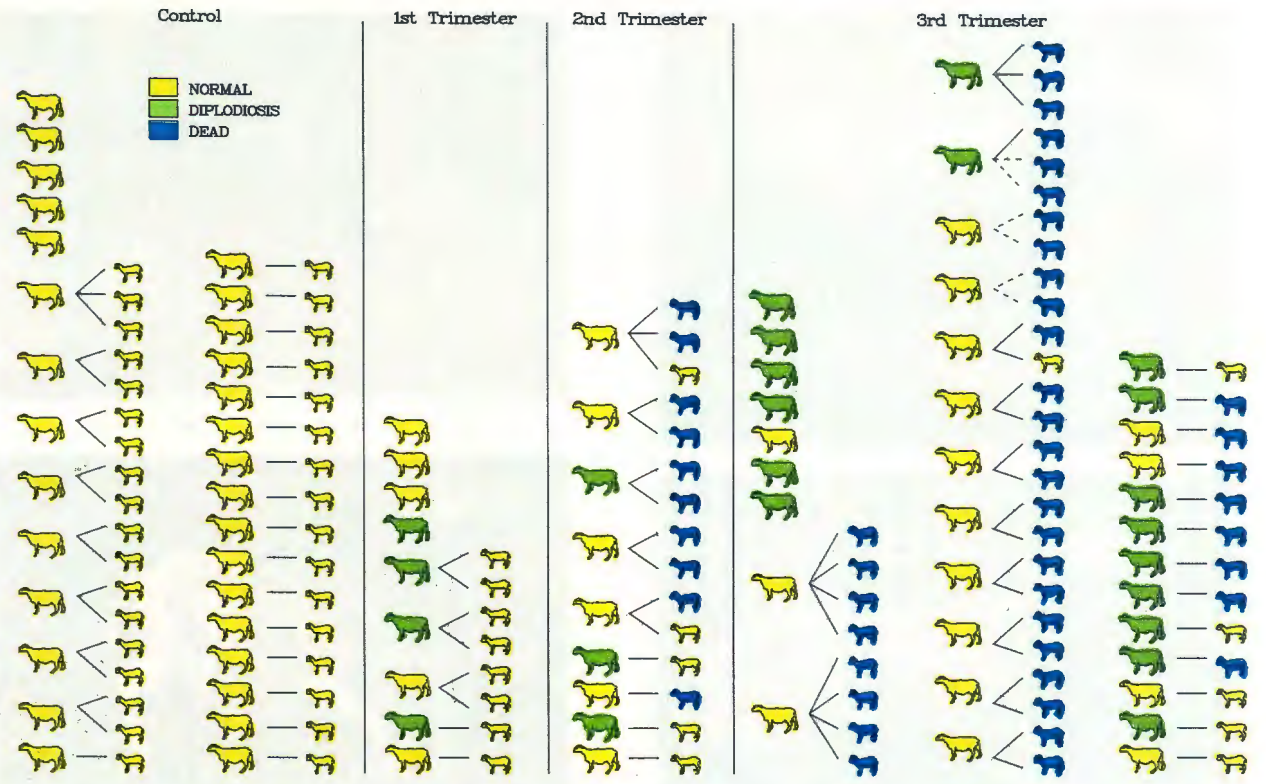


FIG. 2 Perinatal mortality in lambs of ewes dosed with cultures of *Diplodia maydis* during various stages of gestation

Ewes	*MRC2829*	*MRC10400*	
Affected	8	5	
Unaffected	5	10	$\chi^2_{(1)} = 1{,}238$ (P>0,05)
Lambs			
Dead	19	22	
Survived	1	5	$\chi^2_{(1)} = 0{,}867$ (P>0,05)

The fact that no significant difference could be found between the respective numbers of ewes and lambs affected by the isolates MRC 2829 and MRC 10400 provided further justification for pooling the results.

2. *Effect of* D. maydis *culture material on perinatal mortality of lambs:* To determine this effect, the numbers of non-viable lambs and normal lambs were compared. All ewes in the control group as well as those exposed during the 1st trimester gave birth to normal lambs and therefore statistical comparisons were only made with lambs of ewes exposed during the 2nd and 3rd trimesters.

	Control	*1st Trim.*	*2nd Trim.*	*3rd Trim.*
Non-viable lambs	0	0	10	41
Normal lambs	34	8	5	6
% non viable	0	0,0a	66,7b	87,2b

Fisher P = 0,0003 (P<0,017)

$\chi^2_{(1)} = 23{,}012$ (P<0,017)

$\chi^2_{(1)} = 2{,}037$ (P>0,017)

Percentages followed by the same letter are not significantly different.

Very significant mortality occurred in the offspring of ewes exposed to the culture in the 2nd and 3rd trimesters. Sixty-six per cent of the lambs of ewes exposed in the 2nd trimester and 87 % of the lambs of those exposed in the 3rd trimester were either stillborn or died soon after birth. Although the number of affected lambs born to ewes in the 3rd trimester group did not significantly differ from that of their 2nd trimester counterparts, perinatal losses seemed to increase with exposure late in pregnancy.

3. *Differences in lamb mortality between ewes with and without signs of diplodiosis:*

Exposure during the 2nd trimester:

	Signs	*No signs*	
No. of ewes	3	6	
Non-viable lambs	2	8	Fisher P = 0,407 (P>0,05)
Normal lambs	2	3	
% non-viable lambs	50,0	72,7	

Exposure during the 3rd trimester:

	Signs	*No signs*	
No. of ewes	12	15	
Non-viable lambs	14	27	$\chi^2_{(1)} = 0{,}090$ (P>0,05)
Normal lambs	3	3	
% non-viable lambs	82,4	90,0	

There was no significant difference in the numbers of non-viable lambs produced by ewes that had shown clinical signs (Fig. 3) and those that did not.

4. *Comparison of the number of stillborn and lame lambs:*

	Affected lambs	
Stillborn	34	$\chi^2_{(1)} = 5{,}667$ (P<0,05)
Lame lambs	17	

A significantly larger number of stillborn lambs (Fig. 4) than lame lambs (Fig. 5) were produced by the exposed ewes.

5. *Frequency of dystocia:*

	Control	*Treated*	
Dystocia	2	7	$\chi^2_{(1)} = 0{,}761$ (P>0,05)
Unassisted birth	23	29	

There was no significant differences in the incidence of dystocia between the ewes that were exposed to the culture material and the controls.

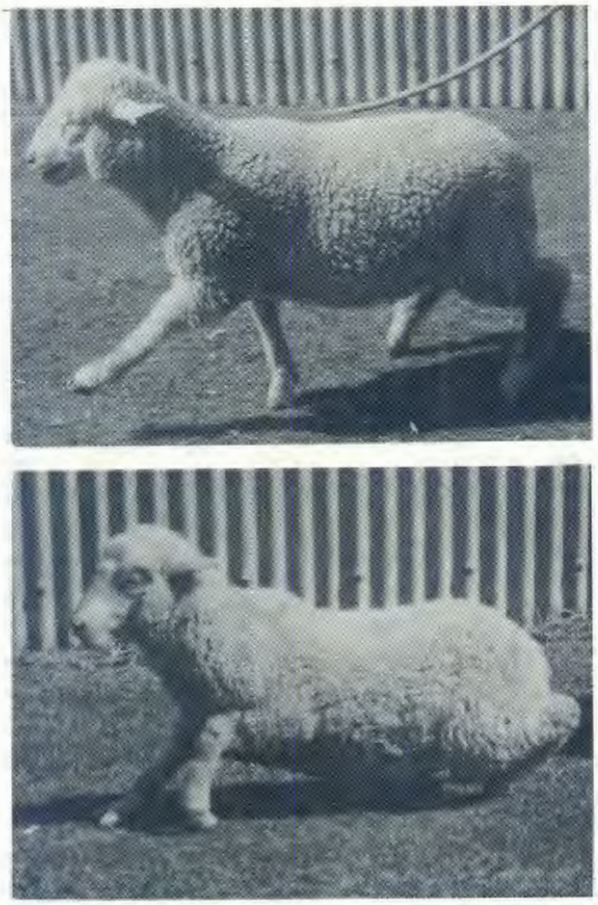


FIG. 3 Ataxia induced by diplodiosis

6. *Duration of pregnancy:* The mean gestation length of the control ewes was compared with that of treated ewes having only non-viable lambs. Length of pregnancy was recorded only in the main trial.

	Controls	*Exposed ewes*	
Mean gestation period (days)	151,5	146,9	Adj. Welsh-Aspin
Number	15	22	t = 5,914 (P<0,05)

Exposure to culture material during the 2nd or 3rd trimesters resulted in a significant shortening of the gestation period.

7. *Birth mass:* Single and multiple births were recorded in all groups. The birth masses of single lambs, twins and triplets in the control group were compared with those of affected lambs of ewes that had been exposed in the 2nd and 3rd trimesters of the Main Trial.

Single births

	Control	*2nd + 3rd Trim.*	
Mean mass (kg)	4,041	2,890	$t_{(15)} = 3,582 (P<0,01)$
No. of lambs	11	6	

Twins

	Control	*2nd + 3rd Trim.*	
Mean mass (kg)	3,038	2,646	$t_{(22)} = 2,751 (P<0,01)$
No. of lambs	3	9	

The mean mass of lambs from ewes exposed to *D. maydis* cultures during the 2nd and 3rd trimesters of pregnancy was significantly less than that of unexposed animals. The same trend appeared to be present in triplets, but only 3 sets were available for comparison.

FIG. 4 Stillborn lambs in a pen occupied by treated ewes

FIG. 5 A lame lamb

8. *The incidence of clinical signs and the development of resistance to diplodiosis during dosing trials:* Less than 50 % of the ewes in the entire experiment showed clinical signs of diplodiosis. The incidence of diplodiosis at first exposure was compared with those of later exposure. All the ewes were included, whether pregnant or not.

	Exposures		
	First	*Later*	
Clinical signs	23	7	$\chi^2_{(1)} = 10,541$
Without clinical signs	29	45	(P<0,05)
% with clinical signs	44,2	13,4	

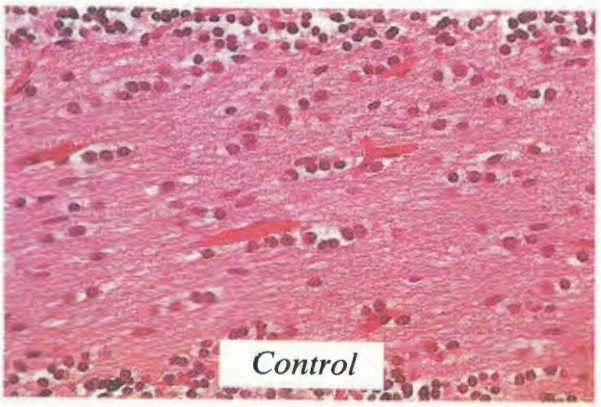
Significantly more ewes showed signs of diplodiosis at the first exposure than subsequent exposures. This apparent development of resistance is borne out by the observation in the Pilot Trial that the incidence of diplodiosis diminished after the first exposure despite the administration of progressively higher doses of culture (Appendix 1 A).

Live mass

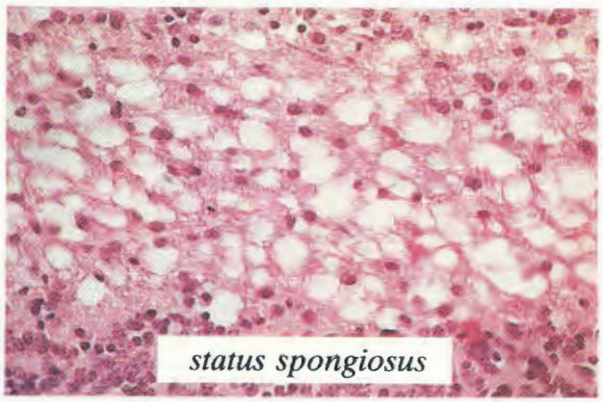
The corrected 90 day mass of the surviving lambs of ewes exposed in the 2nd and 3rd trimester did not differ significantly from the controls.

Pathological findings

Histopathological examination of affected lambs revealed spongy degeneration (*status spongiosus*) of variable degree in the central nervous system. Spongy lesions were present throughout the white matter of the brain in severely affected cases and in some of these focal areas of leukoencephalomalacia were evident. In mildly affected lambs, the lesions were focally distributed with predilection for the white matter of the cerebral and cerebellar gyri. Preliminary transmission electron microscopical studies showed that the spongy changes were attributable to widespread intramyelinic vacuoles and expansion of extracellular spaces.



Control



Status spongiosus

FIG. 6 *Status spongiousus* of the cerebellar white matter of an affected lamb

DISCUSSION

Research on diplodiosis dates back to 1918 when Mitchell experimentally reproduced the condition in cattle at the Allerton Laboratory in Natal (Mitchell, 1918). To the best of our knowledge this was the first time that a mycotoxicosis had been reproduced by feeding pure cultures of a fungus to target animals (Kellerman, 1985). Diplodiosis is not only of historical interest, however, it is also one of the commonest nervous disorders of livestock in southern Africa. Despite its importance (together with facial eczema in New Zealand and lupinosis in Australia it must rate as one of the most frequently diagnosed mycotoxicoses of ruminants in the world) there seems to be little awareness of diplodiosis abroad. This lack of awareness probably stems from the fact that, although *D. maydis* occurs wherever maize is grown (Marasas, 1977), the disease has apparently not been unequivocally diagnosed outside southern Africa.

The first question that arises is: why, in view of the frequent occurrence of diplodiosis, has perinatal losses not previously been recorded in the condition? A possible answer is that in 1987, when these losses were first noticed, the incidence of cob rot and diplodiosis was exceptionally high. Many factors are believed to have contributed to the high incidence of cob rot induced by *D. maydis* that year, including the build-up of sufficient inoculum, growing of susceptible cultivars and climatic conditions suitable for outbreaks to occur. Since the pycnidia on maize residues are the source of infection for the following crop, farming practices that favour the accumulation of plant debris on the land, such as minimum tillage, would have promoted the disease (Flett & Wehner, 1991). The high incidence and the severity of the outbreaks of diplodiosis obliged an unprecedented number of farmers to seek veterinary advice on how to deal with the problem. These 2 factors, namely the unusual severity of the disease and the involvement of veterinarians in the outbreaks, are probably the main reasons for the possible correlation between diplodiosis and the perinatal losses having come to light. Another factor that might have obscured the connection between diplodiosis and perinatal losses is the occurrence of stillbirths and lame lambs in ewes that had never shown signs of the disease. Diplodiosis was recently diagnosed in stillborn lambs of ewes in a flock that had apparently been healthy throughout gestation. According to the farmer, the ewes had eaten mouldy maize infected with *D. maydis* and histopathological examination of the brains of the dead lambs revealed characteristic lesions in the white matter. Clearly, in this case, no connection could have been made between diplodiosis and the perinatal mortality had the nature of the lesions not been known (W. S. Botha & T. S. Kellerman, Onderstepoort VRI, unpublished data, 1991).

The results of this experiment clearly show that the foetus is much more susceptible to diplodiosis than the adult sheep. In the third trimester 87 % of the lambs were affected compared to 44 % of the dams; moreover, all the lambs died while virtually all the ewes survived. It should also be pointed out that, while all the stillborn or lame lambs in this experiment had a *status spongiosus* in the brain, such lesions are extremely rare in adult animals. The only central nervous lesion previously described in diplodiosis was a laminar subcortical *status spongiosus* in the cerebrum and cerebellum of a sheep that had been paralysed for a number of days and in a steer which developed irreversible ataxia (Kellerman *et al.*, 1985). Save for these 2 rare experimental cases, microscopical lesions have not been reported in adult animals.

The reason why foetuses are susceptible to diplodiosis only in the 2nd and 3rd trimesters of pregnancy is a matter for conjecture. Neuronal multiplication in the ovine foetus occurs mainly between 40 and 80 days and glial multiplication between 95 and 130 days of gestation. During these 2 phases of rapid growth the foetus is believed to be particularly vulnerable for developmental damage (McIntosh, Baghurst, Potter & Hetzel, 1979). Myelination in turn occurs mainly during the terminal stages of pregnancy and early neonatal period (Patterson, Sweasey & Herbert, 1971). The nature of the lesions in diplodiosis, namely spongy degeneration of the white matter, suggest that the unknown toxin interferes with this myelination process. If that were the case, it would explain the vulnerability of the foetus during the latter part of pregnancy when myelin is being formed. Lesions were already evident in the cerebrums of foetuses from ewes that had died of respiratory disorders (*vide infra*) between Day 111 and 115 of gestation, i.e. 8–10 days after being dosed with cultures of *D. maydis*. This is more or less consistent with the findings of Patterson & Sweasey (1970) that myelination begins in the spinal cord on *c* Day 90 and then progresses to the brainstem on *c* Day 100, followed by the cerebrum on *c* Day 120. A more detailed description of the lesions in the foetus will be published elsewhere.

An uncommon feature of diplodiosis, revealed in this study, is the development of resistance to poisoning after initial exposure to the toxin. Whether this resistance can be ascribed to multiplication of micro-organisms which destroy the unknown neurotoxin in the rumen, or to the induction of detoxifying enzymes in the liver, is not known. Whatever the

explanation for this resistance, the phenomenon might have practical implications for the control of diplodiosis in the field.

During the course of the pilot and main trials, 6 ewes succumbed from various causes. Only 2 of the ewes died as a direct result of diplodiosis, i.e. while showing clinical signs of the disease; 1 contracted jaagsiekte; 1 showed only venous congestion; and the remainder died of respiratory problems ranging from pasteurellosis to consolidation of the lungs. Although most of the deaths were apparently unrelated to diplodiosis, it should be pointed out that the fatalities occurred only in ewes that received MRC 2829, the most toxicogenic of the 2 isolates. The possibility that diplodiosis might have been inexplicably involved in these deaths cannot be entirely disregarded.

Diplodiosis now joins the short list of plant poisonings and mycotoxicoses in southern Africa which might cause reproductive failure of livestock. The most notable plant associated with perinatal losses of stock in this area is grootlamsiekte, a disease of sheep characterized by prolongation of gestation, post-foetal maturity and dystocia. *Salsola tuberculatiformis,* the causal agent, is common on brackish soils in the southern parts of Namibia, Botswana, and the south-western and north-western Cape Province (Basson, Morgenthal, Bilbrough, Marais, Kruger & Van der Merwe, 1969). The other local plants or fungi involved in perinatal losses are relatively unimportant and only sporadically cause problems. Included amongst these are *Acacia nilotica* (Terblanche, Pienaar, Bigalke & Vahrmeijer, 1967), *Cynodon nlemfuensis* (Rodel, 1971), *Dipcadi glaucum* (Steyn, 1934) and seedheads of annual ryegrass (*Lolium* spp.) infected with nematode galls (*Anguina* sp.) colonized by a toxigenic *Corynebacterium* sp. (Schneider, 1981). *Fusarium graminearum* produces zearalenone, an oestrogenic principle not noted for inducing perinatal losses. The condition has been seen in pigs fed mouldy maize in Natal (Aucock, Marasas, Meyer & Chalmers, 1980) and the Transvaal. In Zimbabwe, skeletal deformities and post natal mortalities have been observed in lambs of ewes that had grazed full-term on heavily fertilized stargrass (*C. nlemfuensis*) pastures. The herbage of these pastures were found to be marginally deficient in iodine and rich in cyanogenetic glycosides (Rodel, 1972). The toxic principles of *D. glaucum* and *A. nilotica*, on the other hand, are not known. Pods of *A. nilotica* caused mortality and abortion, associated with methaemoglobinaemia, in goats in Natal and the Transvaal. Since the outstanding necropsy feature was the methaemoglobinaemia, the abortions were attributed to anoxic changes in the placenta and foetus (Terblanche *et al.*, 1967). No information could be found on the pathogenesis of the abortions in *D. glaucum* poisoning. The perinatal losses induced by diplodiosis can be distinguished from those of all of the others: (a) by the presence of a *status spongiosus* in the white matter of the brains of the affected lambs, (b) by the fact that these losses are known to occur only in sheep and cattle grazing on harvested maize lands in winter, and (c) because the foetuses are always carried full-term. Unlike in *A. nilotica* poisoning, methaemoglobinaemia is also lacking in diplodiosis.

The true incidence of perinatal losses induced by diplodiosis in the field is not known as previous outbreaks would either have gone undiagnosed or would have been ascribed to other causes such as vitamin A deficiency, enzootic abortion, lack of milk, predation by wild animals (as the carcases of stillborn lambs are damaged by scavengers), and so on. Now that the condition can be diagnosed by histopathological examination of brain sections, and as veterinarians become more informed of the problem, the extent of the losses will become clearer.

Perinatal mortality of lambs and calves as a result of diplodiosis has potentially severe implications for animal husbandry on the subcontinent where this mycotoxicosis is common and stock are forced to graze on mould-infected maize in winter when roughage is scarce.

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APPENDIX 1 A Perinatal effect on lambs of ewes exposed at approximately 2 weekly intervals to *Diplodia maydis* cultures (MRC 2829) in the latter part of gestation

Ewes					Partus			Lambs		
Ewe No.	Dosing regimen				Fate (Day)	Days after onset of dosing	Lamb mass (kg)	Condition of lamb	Fate	CNS lesions
	Exposure 1 10 g/kg/day × 2	Exposure 2 10 g/kg/day × 3	Exposure 3 10 g/kg/day × 4	Exposure 4 10 g/kg/day × 4						
1	Diplodiosis ++++ 7 d					np				
2	Diplodiosis ++++ 3 d		not dosed	not dosed	Died (16)					
3	Diplodiosis + 2 d					30		Stillbirth		Stat
4			Diplodiosis ++++ 6 d	not dosed	Died (39)	38	4,2	Stillbirth*		Stat
						38	3,9	Stillbirth		Stat
5	Diplodiosis + 2 d			Diplodiosis + 2 d		39	4,4	Normal		
6	Diplodiosis + 1 d					41	4,1	Lame	e	Stat
7						43	3,8	Lame	e	Stat
						43	3,0	Stillbirth		Stat
8						47	3,2	Stillbirth		Stat
9						64	—	Stillbirth*		Stat
						64	—	Lame*	e	Stat

Diplodiosis = clinical signs of diplodiosis
++++ = severity of signs
D 56–59 = duration of signs

np = not pregnant
* = dystocia

e = euthanized
stat = *status spongiosus*

APPENDIX 1 B Perinatal effect on lambs of control ewes exposed at approximately 2 weekly intervals to autoclaved maize substrate

Ewes					Partus	Lambs			
Ewe No.	Dosing regimen				Days after onset of dosing	Lamb mass (kg)	Clinical state	Fate (Day)	CNS lesions
	Exposure 1 10 g/kg/day × 2	Exposure 2 10 g/kg/day × 3	Exposure 3 10 g/kg/day × 4	Exposure 4 10 g/kg/day × 4					
10					24	—	Normal	e	—
					24	—	Normal	e	—
11					30	6,5	Normal*		
12					31	5,5	Normal		
13					30	4,4	Normal		
					30	3,3	Normal		
14					36	4,2	Normal		
15					42	4,2	Normal	d	—
					42	3,7	Normal		
16					48	3,8	Normal		
					48	2,9	Normal	d	—
17					67	3,9	Normal		
18					75	4,6	Normal		
19					105	7,3	Stillbirth*		—

Diplodiosis = clinical signs of diplodiosis
++++ = severity of signs
D 56–59 = duration of signs

* = dystocia
d = died

e = euthanized
— = no lesions

APPENDIX 2 A Perinatal effect on lambs of ewes exposed to *Diplodia maydis* culture (MRC 10400) from Day 5 to Day 34 of gestation

Ewes				Gestation (Day)	Lambs			
Ewe No.	Dosing regimen: Exposure 1 10 g/kg × 3 D 5–7	Exposure 2 10 g/kg × 3 D 18–20	Exposure 3 10 g/kg × 3 D 32–34		Lamb mass (kg)	Clinical state	Fate (Day)	CNS lesions
20				np				
21				np				
22				np				
23		Diplodiosis + D 21		np				
24	Diplodiosis ++++ D 8–14		Diplodiosis + D 35–36	147	2,7	Normal		
				147	2,6	Normal		
25	Diplodiosis ++++ D 8–11 Relapse D 15–149			149	3,5	Normal	e (149)	—
26	Diplodiosis +++ D 8–9			150	2,4	Normal		
				150	2,6	Normal		
27				152	4,0	Normal	d (153)	—
				152	3,7	Normal		
28				170	4,4	Normal		

Diplodiosis = clinical signs of diplodiosis
++++ = severity of signs
D 56–59 = duration of signs

np = not pregnant
e = euthanized
d = died
— = no lesions

APPENDIX 2 B Perinatal effect on lambs of ewes exposed to *Diplodia maydis* culture (MRC 10400) from Day 54 to Day 85 of gestation

Ewes				Gestation (Days)	Lambs			
Ewe No.	Dosing regimen: Exposure 1 10 g/kg × 3 D 54–56	Exposure 2 10 g/kg × 3 D 68–70	Exposure 3 10 g/kg × 3 D 83–85		Lamb mass (kg)	Clinical state	Fate (Day)	CNS lesions
29				146	1,9	Stillbirth		—
				146	2,5	Lame	e (146)	Stat
				146	2,3	Lame	d (148)	Stat
30	Diplodiosis ++++ D 56–59	Diplodiosis ++ D 70–71		146	3,3	Normal		
31				147	2,1	Stillbirth		Stat
				147	1,6	Stillbirth		Stat
32	Diplodiosis ++ D 57–59			147	2,2	Lame	e (148)	Stat
				147	2,2	Lame	e (148)	Stat
33				148	3,0	Lame	e (150)	Stat
34	Diplodiosis + D 57–58	Diplodiosis ++++ D 70–74		148	3,4	Normal		
35				148	2,4	Stillbirth		Stat
				148	2,9	Lame *	e (148)	Stat
36				151	2,5	Stillbirth		Stat
				151	2,6	Normal		
37				164	2,9	Normal		

Diplodiosis = clinical signs of diplodiosis
++++ = severity of signs
D 56–59 = duration of signs

* = dystocia
e = euthanized
d = died
stat = *status spongiosus*
— = no lesions

APPENDIX 2 C Perinatal effect on lambs of ewes exposed to *Diplodia maydis* culture (MRC 10400) from Day 103 to Day 133 of gestation

Ewes					Gestation (Day)	Lambs			
Ewe No.	Dosing regimen			Fate (Day)		Lamb mass	Clinical state	Fate (Day)	CNS lesions
	Exposure 1 10 g/kg × 3 D 103–105	Exposure 2 10 g/kg × 3 D 117–119	Exposure 3 10 g/kg × 3 D 131–133						
38 Stat	Diplodiosis ++++ D 105–108			e (115) Bronchitis			Dead foetus		
39					143	1,2	Stillbirth*		Stat
					143	1,5	Stillbirth*		Stat
					143	1,3	Stillbirth*		Stat
					143	1,4	Stillbirth*		Stat
40	Diplodiosis + D 106				143	2,0	Stillbirth*		Stat
					143 143	1,9 1,9	Stillbirth Stillbirth		Stat Stat
41					145	2,2	Normal	e (274)	—
					145	1,9	Lame lamb		Stat
42	Diplodiosis ++ D 106–108				147	1,1	Stillbirth		Stat
					147	2,0	Stillbirth		Stat
					147	2,0	Stillbirth		Stat
43					147	2,6	Stillbirth		Stat
44					147	2,6	Stillbirth		Stat
					147	2,6	Lame lamb		Stat
45				d (168)	148	2,6	Stillbirth		Stat
					148	2,9	Stillbirth		Stat
46					148	3,6	Normal	e (274)	—
47					148	2,8	Stillbirth		Stat
					148	2,9	Lame lamb	d (154)	Stat
48					148	2,3	Stillbirth		Stat
					148	2,3	Stillbirth		Stat
49					150	2,8	Lame lamb	d (153)	Stat
					150	3,1	Lame lamb	d (152)	Stat
50	Diplodiosis ++++ D 111–115				150	2,9	Normal	d (163)	—
51	Diplodiosis + D 106				151	4,2	Normal	e (305)	—
52					169	4,0	Normal	e (374)	—

Diplodiosis = clinical signs of diplodiosis
++++ = severity of signs
D 111–115 = duration of signs

* = dystocia
e = euthanized
d = died
stat = *status spongiosus*
— = no lesions

APPENDIX 2 D Perinatal effect on lambs of ewes exposed to *Diplodia maydis* culture (MRC 2829) from Day 103 to Day 133 of gestation

Ewes					Gestation (Day)	Lambs			
Ewe No.	Dosing regimen			Fate (Day)		Lamb mass (kg)	Clinical state	Fate (Day)	CNS lesions
	Exposure 1 10 g/kg × 3 D 103–105	Exposure 2 10 g/kg × 3 D 117–119	Exposure 3 10 g/kg × 3 D 131–133						
53	Diplodiosis + D 106			d (111) pulmonary congestion			Dead foetus		Stat
							Dead foetus		Stat
54	Diplodiosis + D 106			d (113) pulmonary congestion			Dead foetus		Stat
							Dead foetus		Stat
55				d (114) Jaagsiekte			Dead foetus		Stat
56	Diplodiosis ++++ D 105–115			d (115) Decomposed			Decomposed foetus		
57	Diplodiosis + D 106–107				144	3,1	Stillbirth		Stat
58					146	2,8	Stillbirth*		Stat
					146	2,0	Stillbirth*		Stat
59	Diplodiosis + D 106				147	3,2	Lame lamb	d (149)	Stat
60					147	2,0	Stillbirth*		Stat
					147	2,0	Stillbirth*		Stat
					147	2,6	Stillbirth*		Stat
					149	3,6	Lame lamb*	e (149)	Stat
61	Diplodiosis ++++ D 105–108		Diplodiosis + D 134		147	2,4	Lame lamb	e (147)	Stat
62	Diplodiosis + D 106				147	2,9	Stillbirth		Stat

Diplodiosis = clinical signs of diplodiosis
++++ = severity of signs
D 111–115 = duration of signs

* = dystocia
e = euthanized
d = died
stat = *status spongiosus*

APPENDIX 2 E Perinatal effects on lambs of control ewes exposed to autoclaved maize substrate from Day 5–34, Day 5–133 or Day 54–133

Ewes										Gestation (Days)	Lambs			
Ewe No.	Dosing regimen: Repeated exposures to 10 g/kg × 3										Lamb mass (kg)	Clinical state	Fate (Day)	CNS lesions
	Day 5–7	Day 18–20	Day 32–34	Day 54–56	Day 68–70	Day 83–85	Day 103–105	Day 117–119	Day 131–133					
63	×	×	×							np				
64	×	×	×							np				
65	×	×	×							np				
66	×	×	×							np				
67	×	×	×							np				
68	×	×	×	×	×	×	×	×	×	147	3,3	Normal		
69	×	×	×	×	×	×	×	×	×	149	3,2	Normal		
70	×	×	×	×	×	×	×	×	×	152	4,8	Normal		
71	×	×	×	×	×	×	×	×	×	167	4,5	Normal		
72				×	×	×	×	×	×	148	1,7	Normal		
										148	2,8	Normal		
										148	2,8	Normal		
73				×	×	×	×	×	×	149	3,8	Normal		
74				×	×	×	×	×	×	149	2,9	Normal		
										149	3,1	Normal	d (151)	–
75				×	×	×	×	×	×	149	2,4	Normal	d (150)	–
										149	3,2	Normal		
76				×	×	×	×	×	×	149	3,3	Normal		
77				×	×	×	×	×	×	150	3,1	Normal	d (153)	–
										151	3,6	Normal	e (168)	–
78				×	×	×	×	×	×	151	2,9	Normal		
79				×	×	×	×	×	×	152	4,4	Stillbirth		–
80				×	×	×	×	×	×	153	4,5	Normal		
81				×	×	×	×	×	×	153	5,3	Normal		
82				×	×	×	×	×	×	154	4,4	Normal		

np = not pregnant
× = dosed
e = euthanized
d = died
– = no lesions

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