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INTOXICATION OF CATTLE ON KIKUYU GRASS FOLLOWING ARMY WORM *(SPODOPTERA EXEMPTA)* INVASION

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ABSTRACT

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Clinical features and pathological and mycological findings in a field outbreak of intoxication in dairy cattle grazing kikuyu grass are reported. The outbreak followed invasion of the grass by the army worm *(Spodoptera exempta)*.

Clinical signs included drooling of saliva, depression, apparent inco-ordination, sunken eyes, ruminal distension and atony, recumbency, moderate diarrhoea and "sham drinking". Seventy-seven cows (64%) were clinically affected over a period of 12 days. Of these, 37 died.

Necropsies performed on 4 affected cattle revealed necrosis of the epithelium of the forestomach, which was consistently more severe in the omasum. Light microscopy showed extensive necrosis of the epithelium of the forestomach with associated fibrinopurulent inflammation. The *stratum spinosum* and *s. granulosum* were selectively involved, but the *s. basale* was generally preserved. Electron microscopical examination of ruminal and omasal epithelium from 2 of these cattle revealed cytopathological features in the *s. spinosum* and *s. granulosum* which were consistent with stages in an acute, anoxic type of injury.

Mycological examination of the pastures revealed sparse growth of a mixed fungal population, which included *Myrothecium verrucaria*. There was no evidence of heavy fungal infestation.

Previous evidence that *M. verrucaria*, or other fungi, may be involved in the aetiology of kikuyu grass poisoning of cattle in New Zealand is addressed. It appears improbable that any of the fungi isolated in this investigation could have played an important role in the aetiology of this outbreak.

INTRODUCTION

In South Africa, kikuyu grass *(Pennisetum clandestinum)* is grown widely to support dairy cattle. Sudden, sporadic outbreaks of a disease, characterized by high morbidity and mortality, have affected cattle grazing kikuyu grass. Two of these outbreaks have been described (Bryson & Newsholme, 1978; Van Heerden, Williams, Van Rensburg & Ipland, 1978). Outbreaks of a similar disease in cattle grazing kikuyu grass have been reported in New Zealand (Busch, Harris, Coup & Cordes, 1969; Cordes, Coup, Harris, Davenport & Busch, 1969; Martinovich, Mortimer & Di Menna, 1972; Martinovich & Smith, 1973; Smith & Martinovich, 1973) and in Western Australia (Gabbedy, Gwynn, Hopkinson & Kay, 1974). Blood, Henderson & Radostits (1979) also mentioned the occurrence of the disease in southern Australia.

In the outbreaks described there are general similarities in the clinical and pathological features. Clinical signs include drooling of saliva, depression, inco-ordination, recumbency, ruminal distension and hypomotility, constipation, and evidence of colic and dehydration. A distinctive feature, which has been termed "sham drinking" by Martinovich & Smith (1973), is the congregation of affected cattle at water, but their failure to drink, even though they place their mouths on, or into, the water. The most striking pathological changes involve the forestomachs. Rumens are filled with homogeneous, sloppy contents, and necrosis of the forestomach mucosa is extensive.

The cause of the outbreaks has not been established. In South Africa, as far as we are aware, all the outbreaks have been associated with previous invasion of kikuyu grass by the army worm *(Spodoptera exempta)*, although kikuyu pastures so invaded do not invariably become toxic. Similarly, in New Zealand, a consistent association between the occurrence of outbreaks and previous invasion of the kikuyu grass by army worm *(Pseudaletia separata)* has been noted (Smith & Martinovich, 1973). In Western Australia, however, 2 outbreaks were briefly reported on kikuyu grass which apparently had not been invaded by insect pests, and the outbreaks took place in an area where the army worm, *P. separata*, apparently does not occur (Gabbedy *et al.*, 1974). Martinovich *et al.* (1972) presented evidence to suggest that the disease in New Zealand might be a mycotoxicosis. Cultures of *Myrothecium* spp., a fungus that occurs on herbage, caused disease with clinical and pathological features similar to those of kikuyu poisoning when dosed to cattle and sheep (Di Menna & Mortimer, 1971; Mortimer, Campbell, Di Menna & White, 1971). Cultures of a strain of *M. verrucaria*, collected from kikuyu grass at the time of an outbreak of kikuyu grass poisoning in cattle, also produced similar disease when dosed to sheep (Di Menna & Mortimer, 1971; Martinovich *et al.*, 1972). Martinovich *et al.* (1972) reported further that cultures of *Phoma herbarum*, another fungus found on herbage, caused disease similar to kikuyu grass poisoning when dosed to cattle and sheep. As supportive evidence that kikuyu grass poisoning may be a mycotoxicosis, these workers observed that outbreaks of the disease usually occur in warm, wet weather when maximal fungal growth on pasture would be expected.

Herein we report clinical features and pathological and mycological findings in a field outbreak of intoxication in cattle grazing kikuyu grass, following invasion by the army worm *(S. exempta)*.

HISTORY OF OUTBREAK AND CLINICAL SIGNS

The outbreak involved a dairy herd of 120 predominantly Ayrshire cattle which were grazing in camps of kikuyu grass near East London, Eastern Cape Province. The grass in these camps became heavily invaded by the army worm *(S. exempta)*, which was first recognized by the owner on 9 March 1981. All the camps were promptly sprayed with insecticides. By 2 April the army worms had disappeared from all the camps, none of their carcasses could be seen and the only remaining evidence of the invasion was the presence of a few of their faecal pellets which were scattered on the earth at the base of the sward.

The herd grazed in Camp A from 27 March to 9 April and entered Camp B on 10 April, where they grazed for 6 h. The first clinical signs were noticed early on 11

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April. The herd was promptly restricted to open yards with access to hay and water, and was not reintroduced into any of the camps.

The earliest clinical signs noticed in affected animals were that they held their heads low and that saliva trickled from their mouths. The limb weakness and apparent inco-ordination that ensued were characterized by a swaying gait, dragging of the feet and knuckling over at the phalangeal joints. Some animals congregated at the water troughs. They were observed to dip their mouths into the water but did not swallow. Colic was expressed frequently by grunting and, occasionally, by kicking at the abdomen with the hind legs. The eyes became sunken in many cases (Fig. 1) and some animals developed moderate diarrhoea. Moderate ruminal distension and ruminal atony were consistently present in affected animals. The more severely affected ones became recumbent and made occasional unsuccessful attempts to rise. They appeared to lack co-ordination, their limbs were disposed in awkward positions and their heads were turned back towards the flanks. Death occurred in sternal or lateral recumbency. Gushing of ruminal contents from the nostrils and mouth was often observed at death.

Treatment, which was commenced on 11 April, included repeated oral doses of carron oil and hypo (aqueous sodium thiosulphate solution). No clinical improvement was noted in response to treatment. The course of the disease varied from 12 h to longer than 7 days. Eighteen cattle died within the first 2 days and a further 8 died during the subsequent 2 days. By the 12th day a total of 77 animals (64%) had become clinically affected. Of these, 37 had died and 40 recovered.

Materials and Methods

Pathological and mycological investigations were undertaken on the 4th and 5th days after the outbreak commenced.

Clinical pathology

Venous blood was collected from an Ayshire cow (Case 1) which was in terminal recumbency, and the following values were determined: Packed cell volume (PVC), haemoglobin (HG) concentration, gamma glutamyl transpeptidase, total serum bilirubin (TSB), serum urea nitrogen (SUN), and serum calcium, sodium and potassium concentrations.

Gross pathology

Necropsies were performed on 3 Ayrshire cows (Cases 1–3) and a Friesland cow (Case 4), which died after showing the typical clinical signs. Cases 1, 2 & 4 were in early pregnancy and Case 3 was non-pregnant. The necropsies were commenced approximately 5 min after death in Cases 1–3, and 1 h after death in Case 4. Tissue specimens were collected for light microscopy and electron microscopy *(vide infra)*.

Light microscopy

Tissue specimens from the wall of the rumen, reticulum and omasum at various sites, including gross lesions and grossly normal areas, and specimens from the abomasum, large and small intestine, liver, kidney, brain, spleen and various lymph nodes were collected and fixed by immersion in 10% buffered formalin.

The fixed specimens were processed routinely and embedded in paraffin wax. Sections were cut at 5–7 μm thickness and stained with haematoxylin and eosin (HE). Mallory's phosphotungstic acid haematoxylin (MPAH) (Luna, 1968) was applied to selected sections. In addition, frozen sections of formalin-fixed specimens of rumen and omasum were cut at 10 μm thickness using a Reichert-Jung Cryo-Cut II microtome, and were stained with Oil Red O (ORO) (Pearse, 1961) to demonstate lipids.

Electron microscopy

Specimens of rumen and omasum mucosa were collected at various sites from Cases 1 and 2. The specimens were diced into 1 mm cubes and fixed by immediate immersion in 4% glutaraldehyde in Millonig's phosphate buffer at pH 7,3–7,4 and 4 °C (Millonig, 1961) for 24 h. Selected blocks were rinsed with phosphate buffer and then post-fixed in 2% osmium tetroxide, also in the same buffer. Following 2 or more buffer rinses, the blocks were dehydrated in a graded ethanol series, cleared in propylene oxide and embedded in Epon 812 for 48 h at 60 °C.

Thick (1–2 μm) sections were cut with glass knives on a Reichert Om V4 ultramicrotome and stained with toluidine blue (Trump, Smuckler & Benditt, 1961) for tissue orientations. Relevant blocks were trimmed to size and gold to silver sections were obtained using the above equipment.

Thin sections were picked up on copper grids, stained for 30 min in a saturated aqueous solution of uranyl acetate (Watson, 1958) and for 3–4 min in an 0,2% lead citrate solution (Reynolds, 1963). The stained grids were viewed on a Philips 301 transmission electron microscope, operated at 80 kV.

Mycology

Kikuyu grass was cut and collected at various sites from Camp A (Specimens 1–4) and from Camp B (Specimens 5–8). Faecal pellets from the previous army worm infestation were gathered from the base of the sward in both these camps.

Grass specimens and faecal pellets were examined directly for the presence of fungi with a stereomicroscope. Grass blades, cut into 1 cm long pieces, and faecal pellets were plated out on potato/carrot agar incubated for 8 days at 26 °C under intermittent radiation with daylight and near ultraviolet light, and examined for fungal growth.

Results

Clinical pathology

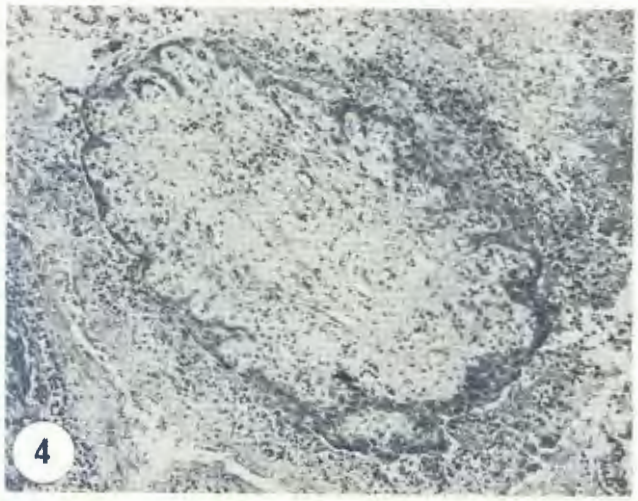
In Case 1 the only notable changes were marked increases in PCV (61%) and SUN (172,2 mg/100 mℓ), and moderate increases in serum potassium (5,87 m eq/ℓ) and serum magnesium (3,52 m eq/ℓ).

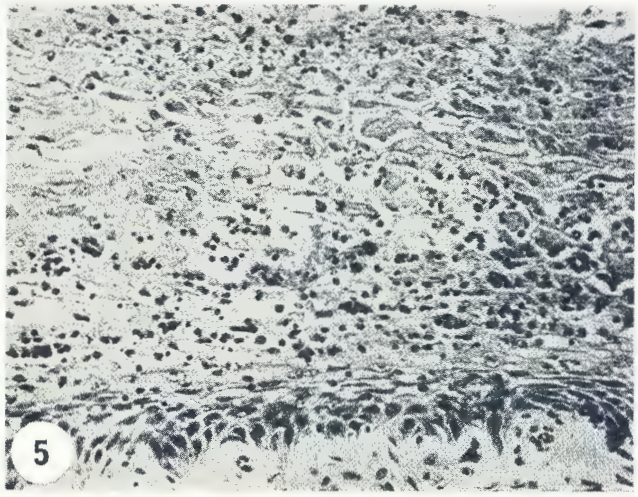
Gross pathology

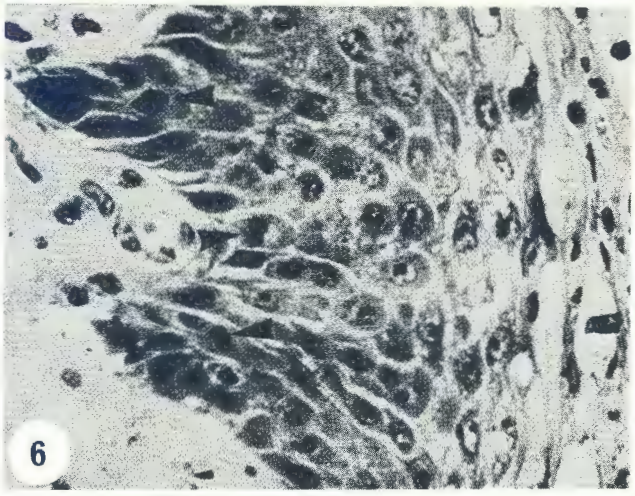
In all cases the eyes were sunken and the carcass blood was dark and viscous, suggesting dehydration.

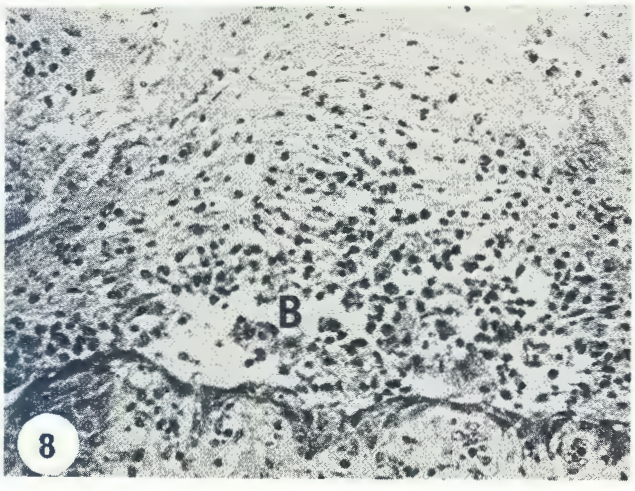
Digestive tract. The forestomachs and abomasa were consistently distended by bright green, homogeneous, sloppy ingesta. The pH of the rumen contents varied from 6,5 to 7,0. Areas of mucosal necrosis and ulceration, which varied in extent from case to case, involved the ventral sac of the rumen in 3 of the cases and were characterized by pallor and segmental loss of the mucosa with submucosal hyperaemia. Extensive mucosal necrosis of the reticulum and the reticular groove was also apparent in 1 case, in which the mucosa of reticular folds and the lips of the reticular groove were swollen and pale yellow, suggesting necrosis and/or suppuration. These changes were restricted to the reticular folds and spared the mucosa between them (Fig. 2). Similar changes, indicative of mucosal necrosis, ulceration and suppuration, were diffuse and extensive in the leaves of the omasum (Fig. 3) in all 4 cases.

FIG. 1 Sunken eye in advanced case
FIG. 2 Necrosis and suppuration of reticular mucosa
FIG. 3 Necrosis, ulceration and suppuration of omasal mucosa









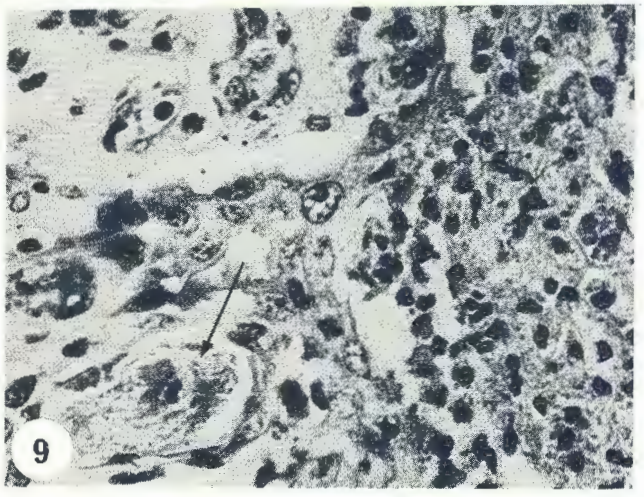


FIG. 4 Necrosis of superficial epithelium; transverse section of ruminal papilla: HE × 100

FIG. 5 Necrosis of superficial epithelium of rumen: HE × 200

FIG. 6 Preservation of ruminal *s. basale* and deep *s. spinosum*, mitotic figures in *s. basale* (arrow heads): HE × 400

FIG. 7 Necrosis of superficial omasal epithelium: HE × 100

FIG. 8 Small bulla (B) in necrotic omasal epithelium. *S. basale* is preserved: HE × 200

FIG. 9 Fibrin thrombus (arrow) within small, subepithelial blood vessel in omasum. Epithelial necrosis and inflammatory cell infiltrate to right of field: HE × 400

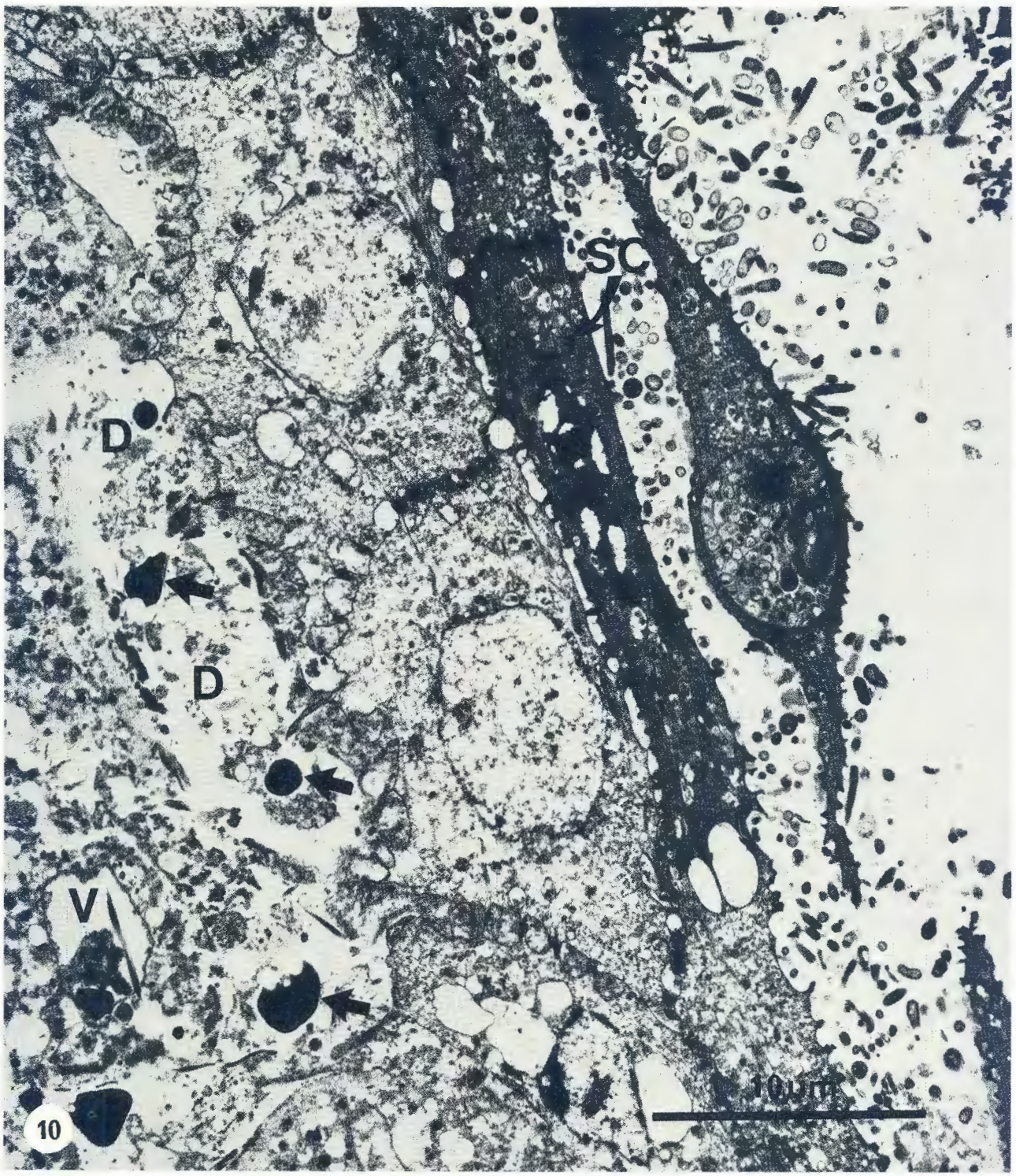


FIG. 10 Rumen epithelium; chromatin masses (arrows), and nuclear vacuolation (V) associated with disintegrated cells (D) in the *s. granulosum*. SC = *s. corneum*

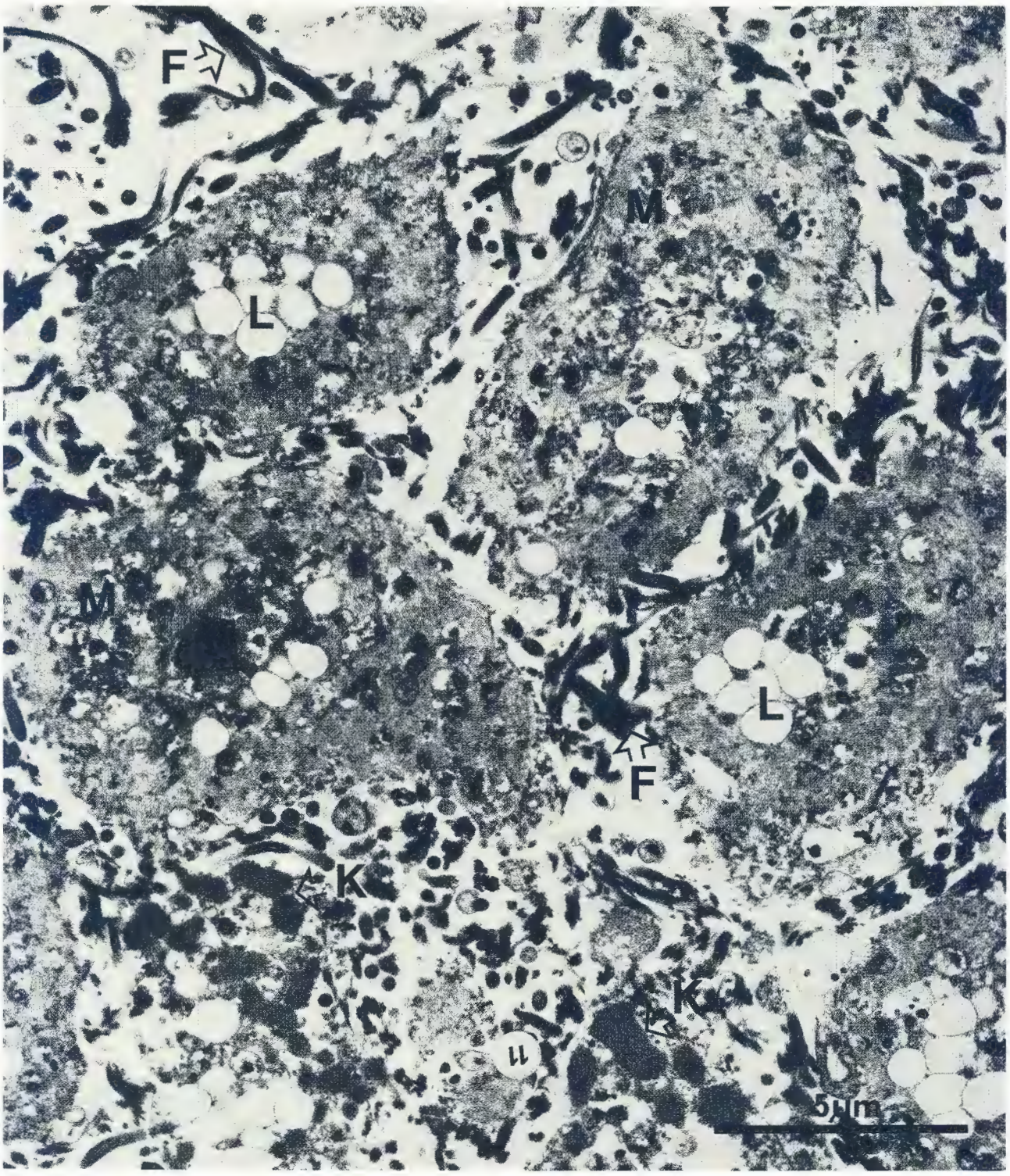
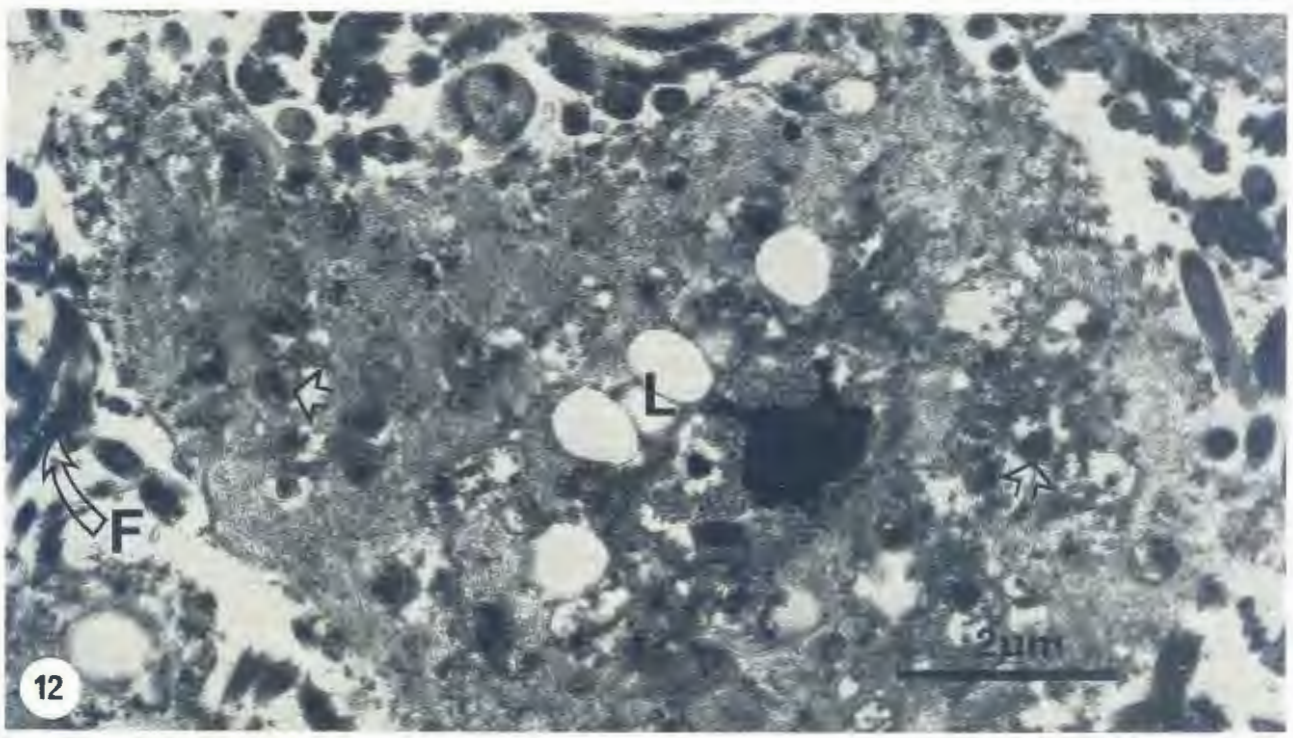


FIG. 11 Omasal *s. granulosum;* cellular separation, loss of plasmalemma and desmosomes, high-amplitude swelling of mitochondria (M) with flocculent matrical densities, cytoplasmic lipid (L) and extracellular fibrin (F) K = keratohyaline



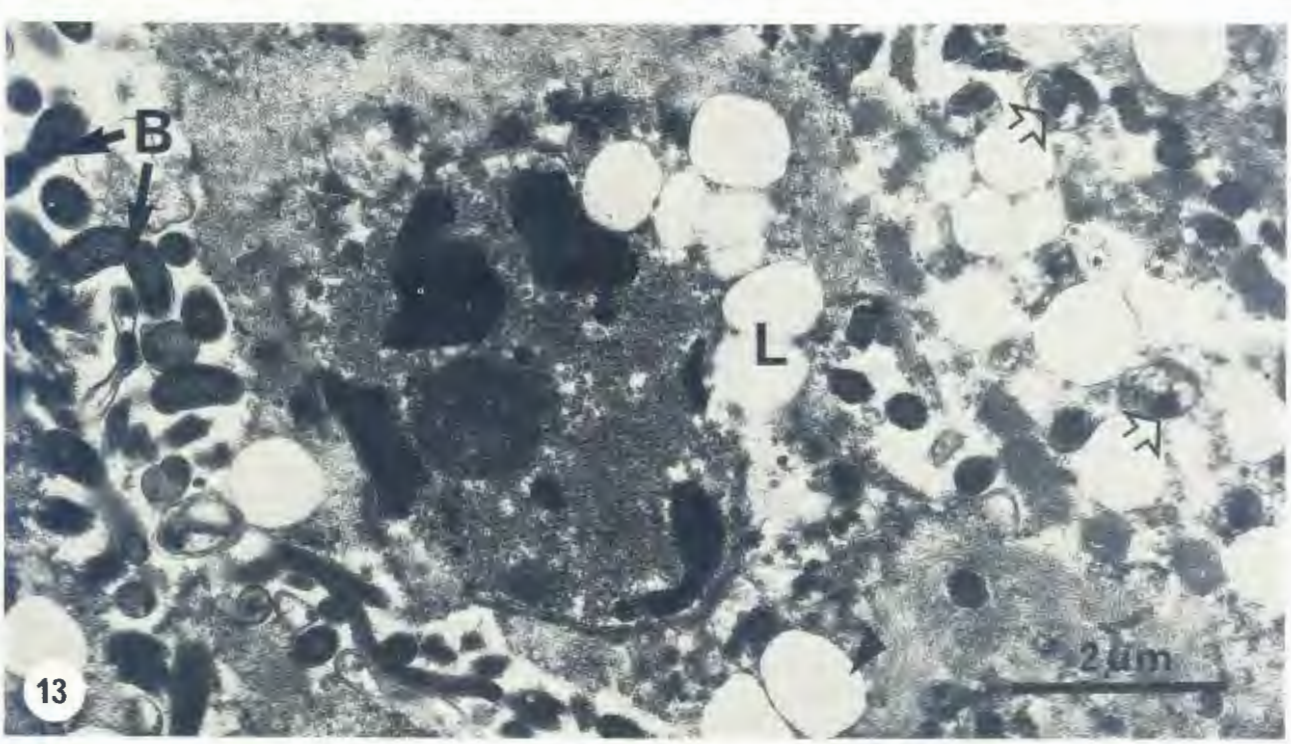


FIG. 12 Omasal *s. granulosum;* high-amplitude swelling of mitochondria with flocculent matrical densities (arrows), paranuclear lipid (L), extracellular fibrin (F), chromatin clumping and loss of plasmalemma

FIG. 13 Omasal *s. granulosum;* high-amplitude swelling of mitochondria with flocculent matrical densities (arrows), paranuclear lipid (L), peripheral clumping of chromatin, segmental loss of nuclear membrane and extracellular bacterial profiles (B)

The abomasal mucosa showed moderate to severe diffuse hyperaemia. The mucosa of small and large intestine was moderately hyperaemic and the contents of the large intestine were more liquid than normal.

Kidney. Cut surfaces bulged slightly and the cortex was slightly pale in all cases.

Light microscopy

Rumen and reticulum. Diffuse epithelial necrosis (Fig. 4 & 5) was observed in all the paraffin sections. An associated intra-epithelial, fibrinopurulent inflammatory process was evident in some of these sections.

Necrosis involved the *s. granulosum* and the more superficial cells of the *s. spinosum*. The overlying *s. corneum* was detached and often absent. Cytopathological features observed in necrotic epithelium included cytoplasmic swelling and vacuolation, increased cytoplasmic eosinophilia, karyorrhexis and karyolysis, and acantholysis, which was manifested as the separation of individual cells or cell groups, one from another. Scattered, basophilic nuclear remnants and clusters of bacteria were frequent amongst the necrotic cells. Oil Red O staining revealed numerous, small, intra- and extracellular lipid globules in the *s. granulosum* and *s. spinosum*, and also in the *s. corneum* in sections where this layer remained. Fibrinopurulent inflammation was characterized by mild to intense neutrophil infiltration within the necrotic epithelial layers, and by intra-epithelial fibrin which was demonstrable by MPAH staining in most sections.

In contrast, the cells of the *s. basale* and the deeper layers of the *s. spinosum* in most areas displayed no histopathological features, and mitotic figures were frequent in the *s. basale* (Fig. 6). The border between these cells and the necrotic cells was sharply defined (Fig. 4 & 5). Complete epithelial ulceration, evident in some sections, was restricted to small segments and was not generally extensive.

Subepithelial blood vessels were engorged. Inflammatory cell infiltration within subepithelial tissues was confined to the segments of complete ulceration.

Omasum. Widespread epithelial necrosis with preservation of the *s. basale* (Fig. 7–9) resembled the lesion in rumen and reticulum, but complete ulceration was more extensive and neutrophil infiltration was generally more intense. Small, intra-epithelial bullae, which appeared to result either from acantholysis or from loss of necrotic epithelial cells and which contained scattered necrotic epithelial cells and neutrophils, occurred frequently (Fig. 8). Subepithelial blood vessels were engorged and occassionally contained small fibrin thrombi (Fig. 9).

Abomasum. Mucosal blood vessels were moderately engorged. There were small, well demarcated ulcers in 2 cases.

Intestine. Mucosal blood vessels were moderately engorged. Necrosis was not observed in the mucosa or in the intestine-associated lymphoid tissue.

Kidney. Mild to moderate nephrosis was present in all cases, and was characterized by cytoplasmic vacuolation and increased cytoplasmic eosinophilia of proximal tubular cells, occasional detachment of these cells, moderate tubular dilatation and the presence of a few granular or hyaline intratubular casts.

Liver. Hepatic changes were confined to mild, diffuse hepatocellular swelling and slightly increased cytoplasmic eosinophilia.

Spleen and lymph nodes. Hyperaemia of some of the lymph nodes was the only change observed.

Brain. Moderate engorgement of cerebral and meningeal blood vessels was the only change observed.

Electron microscopy

Cells of the *s. granulosum* and *s. spinosum* in omasum and rumen displayed a spectrum of nuclear and cytoplasmic features consistent with acute injury (Fig. 10–13). These cells were frequently separated, one from another, and lacked plasmalemma and desmosomes (Fig. 11 & 12). Complete cellular disintegration was evident in some areas (Fig. 10). Nuclear chromatin was condensed into irregular, electron-dense masses (Fig. 11–13), segmental loss of the nuclear membrane was evident (Fig. 13) and the nucleoplasm was occasionally vacuolated (Fig. 10). Electron-dense masses, resembling condensed chromatin and scattered within areas of cellular disintegration (Fig. 10), indicated karyorrhexis. Mitochondria in many of the cells displayed high-amplitude swelling which was characterized by expansion of the inner compartment and loss of cristae. These mitochondria frequently contained flocculent, electron-dense matrical deposits (Fig. 11–13). Groups of vacuoles, which lacked a limiting membrane, suggesting that they had contained lipid, occupied the paranuclear cytoplasm in most of the affected cells (Fig. 11–13). Spaces between the separated cells were occupied by fibrin and by occasional groups of rod-shaped bacterial profiles (Fig. 11–13).

Cytopathological features were not observed in the *s. corneum* where it remained attached (Fig. 10), or in the *s. basale*.

Mycology

Few signs of fungal growth were noticed during microscopical examination of the specimens. On a few green leaves individual rust pustules *(Puccinia* sp.) were observed, as well as a few brown spots which were possibly caused by a phytopathogenic *Helminthosporium* sp. No signs of widespread colonization of the dead leaf sheaths could be seen. Faecal pellets consisted of aggregated small fragments of leaf material with occasional hyphae visible on the outside.

On plated-out specimens fungi were clearly visible after incubation (Table 1). *Myrothecium* spp. occurred on all the specimens, mostly as isolated fruiting bodies. In Specimen 3, however, *Myrothecium* had formed a large number of small fruiting bodies which were fairly uniformly distributed over specimens on the plates. The same observation, but less conspicuous, was made on Specimen 8.

Although a wide variety of fungal species developed on the specimens, there was little apparent difference from specimen to specimen in the composition of the fungal flora or in the degree of infestation for each fungal species. Specimens from Camp A and Camp B showed no conspicuous differences in the composition and frequency of occurrence of the fungal flora. Also, the amount of fungal growth observed on culturing the specimens did not differ significantly from that of specimens from a normal kikuyu pasture examined previously (Van der Westhuizen, unpublished observation, 1977).

DISCUSSION

In this outbreak the course of the disease and the clinical signs generally resembled those described previously in kikuyu grass poisoning (Bryson & Newsholme, 1978; Busch *et al.*, 1969; Cordes *et al.*, 1969; Martinovich & Smith, 1973; Van Heerden *et al.*, 1978). Dehydration, which was characterized by sunken eyes, and also by the markedly raised PCV in Case 1, has also been reported in New Zealand (Busch *et al.*, 1969; Cordes *et al.*, 1969; Martinovich & Smith, 1973). In terminal cases, Martinovich & Smith (1973) found dehydration to be very severe and considered that it may be important in bringing about death. In South Africa, however, there is less

TABLE 1 Fungi found and number of pieces of grass and faecal pellets infected with particular fungi in each specimen

Fungus	Specimens								Faecal pellets
	1	2	3	4	5	6	7	8	
	(a)								
Nigrospora sphaerica	18/30	16/30	9/20	16/30	12/30	17/30	11/30	6/10	—
Myrothecium spp. [1]	15	14	18	6	6	4	2	1	2/10
Epicoccum nigrescens	17	14	11	12	11	4	12	5	—
Cladosporium spp.	14	10	17	15	11	8	5	5	1
Helminthosporium spp. [2]	23	11	5	19	24	10	16	4	1
Phoma spp. [3]	8	9	9	8	6	10	10	8	4
Fusarium spp. [4]	21	22	19	22	10	14	14	6	6
Alternaria alternata	8	3	5	5	6	3	3	—	—
Curvularia sp.	2	—	1	—	1	2	1	1	—
Mucor sp.	2	4	—	6	1	3	5	—	—
Acremoniella atra	1	1	—	—	1	—	4	—	—
Colletotrichum sp. [5]	1	2	—	—	2	4	7	11	1
Periconia byssoides	—	1	2	3	4	4	1	1	—
Trichoderma viride	—	4	—	—	—	—	3	—	—
Metarrhizium anisopliae	—	—	4	3	2	2	5	3	—
Puccinia sp.	—	—	1	4	—	—	—	—	—
Rhinocladiella sp.	—	—	1	—	—	—	—	—	—
Choanephora sp.	—	—	—	—	1	1	—	—	—
Camarosporium sp.	—	—	—	—	—	1	—	—	—
Rhizoctonia sp.	—	—	1	—	—	—	—	—	2

(a) Number of 10 mm pieces plated out
[1] *Myrothecium verrucaria, Myrothecium* sp.
[2] *Helminthosporium bicolor, H. cynodontis, H. rostratum*
[3] *Phoma sorghina, Phoma* sp.
[4] *Fusarium moniliforme, F. solani, Fusarium* sp.
[5] *Colletotrichum graminicola*

conformity in the findings. Bryson & Newsholme (1978) reported dehydration, and the PCV was markedly raised in the 2 animals in which it was measured, whereas Van Heerden *et al.* (1978) found only mild dehydration in their cases. The severity and importance of dehydration in the disease in South Africa require further assessment.

The presence of epithelial necrosis in all sections of the rumen, reticulum and omasum that were examined microscopically indicates that the lesion was extensive and that it was not confined to the necrotic areas recognizable grossly. Although complete ulceration had occurred in some sections, necrosis was most extensive in the more superficial epithelial layers, with preservation of the *s. basale,* which suggests that the *s. spinosum* and *s. granulosum* constituted the primary site of insult.

These findings confirm previous observations in kikuyu grass poisoning (Martinovich & Smith, 1973; Van Heerden *et al.*, 1978). We are in agreement with Martinovich & Smith (1973) that necrosis of the forestomach mucosa is an extensive and consistent lesion, which is probably of primary importance in the pathogenisis of kikuyu grass poisoning. Selective involvement of the superficial epithelial layers, with sparing of the *s. basale,* is consistent with the effect of a toxic agent acting from within the lumen of the alimentary tract. Similar lesions, for example, have been described in experimental ruminal lactic acidosis (Ahrens, 1967), and also following substantial increases in rumen osmolarity, induced by dosing potassium chloride to sheep (Gemmell & Stacy, 1973). The possibility, however, of a systemic mechanism in the pathogenesis of the forestomach lesions in kikuyu poisoning cannot be completely discounted. In acute epidermal necrolysis in humans, for example, the cutaneous lesion can arise through systemic influences but, according to Graham & Koblenzer (1972), the *s. basale* is often preserved even though necrosis of the superficial epithelium is extensive. The consistently severe omasal involvement which we observed has also been reported by Martinovich *et al.* (1972) and Martinovich & Smith (1973), who have recommended omasum as the tissue of choice for histopathological confirmation of kikuyu grass poisoning.

The fungus, *M. verrucaria,* can produce toxic trichothecenes which have been shown to cause forestomach lesions in ruminants (Mortimer *et al.*, 1971) that resembled the lesions reported in kikuyu poisoning (Martinovich *et al.*, 1972). In general, trichothecenes are toxic by nature of their radiomimetic effects which include necrosis of lymphoid tissues and intestinal cryptal epithelium (Smalley & Strong, 1974). Our cattle did have forestomach lesions which resembled those resulting from experimental *M. verrucaria* intoxication (Mortimer *et al.*, 1971), but they did not show radiomimetic effects such as lymphoid necrosis or intestinal cryptal necrosis. From the limited available knowledge of the pathology of *M. verrucaria* toxicosis in ruminants it is premature to implicate this fungus in the aetiology of kikuyu poisoning in South Africa.

According to Trump, Jesudason & Jones (1978) there are 2 distinct types of acute cellular injury, namely, anoxic injury and peroxidative injury. Each type manifests a particular pattern of ultrastructural features. The ultrastructural cytopathological features that were observed in the *s. granulosum* and *s. spinosum* of the forestomach epithelium suggest stages in an acute, anoxic type of injury. Condensation of nuclear chromatin occurs at an early, reversible stage of this process, and high-amplitude swelling of mitochondria with flocculent matrical densities, karyorrhexis and cellular disintegration are features of later, irreversible stages (Trump *et al.*, 1978). None of these changes, however, betrays the identity of the injurious agent.

The cytoplasmic, lipid-like vacuoles, observed by electron microscopy, and the cytoplasmic lipid in ORO sections probably represented a normal feature. Lipid globules have been reported in normal rumen epithelium (Habel, 1959), and abundant intra- and extracellular lipid has been demonstrated in a study of the rumen wall of pregnant cattle (Cerny, 1977).

The possible influence of the invading bacteria upon the progression of the cellular and inflammatory changes could not be assessed. Bacteria in the wall of the rumen are normally restricted to the superficial layers of the *s. corneum,* where the work of Dinsdale, Cheng, Wallace & Goodlad (1980) suggests that they actively digest

these cells. It is likely that the bacterial invasion of deeper layers was consequent on the development of the epithelial lesions.

Nephrosis, which was mild to moderate, has been described previously in kikuyu grass poisoning (Martinovich & Smith, 1973). We consider that it may be related to the effects of dehydration or shock. It is unlikely that the high SUN value in Case 1 could be attributed to the effect of the relatively mild renal changes that were observed, but more probably it was related to dehydration and alimentary dysfunction. There is good evidence that SUN in cattle can become raised substantially through prerenal mechanisms (Brobst, Parish, Torbeck, Frost & Bracken, 1978; Campbell & Watts, 1970).

Since the army worms had disappeared from the camps prior to the outbreak, they cannot be incriminated directly as the cause of the intoxication. The toxic agent was more probably associated either with the grass itself or with the faecal pellets from the previous invasion, considering that only grass and faecal pellets remained at the time of the outbreak.

Of the fungal genera that grew on the plates, *Rhizoctonia, Myrothecium, Fusarium* and *Phoma* include species which are known to be potentially toxic to animals. A species of *Rhizoctonia* was isolated from the faecal pellets. *R. leguminicola*, which grows on legumes, has been associated with hypersalivation in cattle (Crump, Smalley, Henning & Nichols, 1963). Judging from the sparse growth of *Rhozoctonia* on the plates, the small number of fungal hyphae in the faecal pellets and the occurrence of most of the faecal pellets at the base of the sward where they were not readily available to cattle, it is difficult to regard this fungus as a factor in the aetiology of the outbreak. *M. verrucaria*, a known toxic species (Di Menna & Mortimer, 1971), occurred in many of the specimens after incubation. This fungus showed no visible evidence of active growth on the grass in the camps. The colonies that were noticed in the cultures probably developed from spores that adhered to the plants. Some colonies grew out of the faecal pellets but, as already considered for *Rhizoctonia*, it is unlikely that enough pellets were available to the cattle to cause intoxication. *Fusarium* spp. and *Phoma* spp. grew out of the leaf specimens and faecal pellets on the plates, but it appeared to us improbable that either of these fungi occurred in sufficient quantity on the plant material to cause a mycotoxicosis.

The mycological findings in this outbreak were similar to those in a previous outbreak of kikuyu grass intoxication near East London (Van der Westhuizen & Kellerman, unpublished records, 1977).

On the material collected from either of these 2 outbreaks there was no evidence of heavy fungal infestation. In mycotoxicoses examined in South Africa, such as lupinosis (Van Warmelo, Marasas, Adelaar, Kellerman, Van Rensburg & Minné, 1970), equine leucoencephalomalacia (Marasas, Kellerman, Pienaar & Naudé, 1976), *Aspergillus clavatus* intoxication (Kellerman, Pienaar, Van der Westhuizen, Anderson & Naudé, 1976) and diplodiosis (Mitchell, 1918), the presence of fungi was clearly visible on the toxic material. Only in the case of facial eczema did a mycotoxicosis occur without macroscopically visible signs of fungal *(Pithomyces chartarum)* infestation (Marasas, Adelaar, Kellerman, Minné, Van Rensburg & Burroughs, 1972). It may be significant that in *P. chartarum* alone it is the spores and not the mycelium or substrate that contain the toxin(s). Thus, it appears improbable that any of the fungi isolated in the present investigation could have played an important role in the aetiology of these outbreaks.

Our findings suggest that the aetiology of kikuyu grass intoxication of cattle in South Africa requires further investigation which should not be limited to mycology.

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