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EXPERIMENTAL *ALBIZIA VERSICOLOR* POISONING IN SHEEP AND ITS SUCCESSFUL TREATMENT WITH PYRIDOXINE HYDROCHLORIDE

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ABSTRACT

GUMMOW, B., BASTIANELLO, STELLA, S., LABUSCHAGNE, LEONI & ERASMUS, G. L., 1992. Experimental *Albizia versicolor* poisoning in sheep and its successful treatment with pyridoxine hydrochloride. *Onderstepoort Journal of Veterinary Research*, 59, 111–118 (1992).

Five sheep developed severe nervous signs after being drenched with *Albizia versicolor* pod-material. Four of these sheep were treated with pyridoxine hydrochloride (a vitamin B_6) when the symptoms of toxicity became life-threatening. All the treated sheep recovered dramatically and completely after treatment while the untreated one died 2 h after receiving pod-material. A therapeutic dose of 20–25 mg pyridoxine hydrochloride/kg body mass given twice with an 8 h interval is the recommended treatment regimen. The route of administration will depend on the severity of symptoms. Chemical pathology and post-mortem findings are discussed.

INTRODUCTION

Ingestion of the pods of *Albizia versicolor* and *A. tanganyicensis* are associated with hypersensitivity, intermittent tetanic convulsions and mortality of cattle in southern Africa (Kellerman, Coetzer & Naudé, 1988). *A. versicolor* is a medium to large tree, usually *c.* 10 m in height, with a rounded or spreading crown. *A. tanganyicensis* is a medium-sized, sparingly branched, more upright tree usually only *c.* 3–8 m tall, with characteristic brownish-red, thin, papery bark. The bark often peels in broad papery strips revealing a pearly, creamy-grey undersurface giving it the common name "paper bark". *A. versicolor* and *A. tanganyicensis* are fairly well represented in the subcontinent north of the Limpopo, and occur together in the far northern Transvaal and Zimbabwe. *A. versicolor* has a more easterly distribution, stretching along the eastern Transvaal through Swaziland into Zululand and northern Natal. *A. tanganyicensis,* is predominant in the north-western parts of the Transvaal (Kellerman *et al.*, 1988).

Outbreaks of albiziosis in cattle usually occur in late winter or early spring when the pods are blown from the trees by strong winds (Needham & Lawrence, 1966., Kellerman *et al.*, 1988). Young pods are the most toxic with the toxin being concentrated in the pod cases and to a lesser extent in the seeds. As little as 0,57–1,14 kg of *A. versicolor* pods have proved fatal to cattle with body masses of *c.* 230 kg, and sheep that experimentally received *A. versicolor* developed severe clinical signs and died (Needham & Lawrence, 1966).

In 1987 Steyn, Vleggaar & Anderson elucidated the structure of two neurotoxins from *A. tanganyicensis.* Since the principal toxin, 4-methoxy-pyridone, was similar in structure to that of the B_6 vitamins, the hypothesis was made that this neurotoxin may be an antagonist to vitamin B_6. In 1990 Gummow & Erasmus showed that pyridoxine was an effective treatment for *A. versicolor* toxicity in the guinea-pig and postulated that this treatment should be effective in ruminants as well. The purpose of the current study was to investigate this postulate.

Two experiments were conducted. The first was a pilot trial carried out with a single adult ewe to establish a toxic dose of *A. versicolor,* to determine the symptomatology and to establish if pyridoxine was an effective treatment. The second experiment was aimed primarily at confirming that pyridoxine was an effective treatment for *Albizia* poisoning in sheep.

MATERIALS AND METHODS

Pilot trial: Extrapolating from guinea-pig trials (Gummow & Erasmus, 1990) a dose of 5 g *A. versicolor* pod-material/kg body mass in 2,5 ℓ water, was dosed by stomach tube to a healthy full mouth Dorper ewe of 60,5 kg (pilot sheep, Fig. 1). The podmaterial consisted of seed pods of *A. versicolor* collected in the young semi-green state from a tree in Pretoria North which had been air dried for several weeks before being ground into a fine powder.

A therapeutic dose of 10 mg/kg pyridoxine HCl[1], estimated to be sufficient to counter the intoxication, was administered when the signs of intoxication appeared life-threatening.

Blood (with and without heparin) was collected from the jugular vein at 30 min intervals beginning with the first sample just prior to the dosing of pod-material and ending with the last sample at 6 h post-dosing. A further 3 blood samples were taken at 22,25, 26,5 and 50,5 h after the dosing of pod material. At the same time as the blood was collected the temperature, respiratory and pulse rates, rumen movements and habitus of the animal were recorded. The blood samples were analyzed for creatine phosphokinase (CK)[2], lactate dehydrogenase (LDH)[2], aspartate aminotransferase (AST)[2], glucose, haematocrit (Ht), haemoglobin (Hb) and plasma protein levels.

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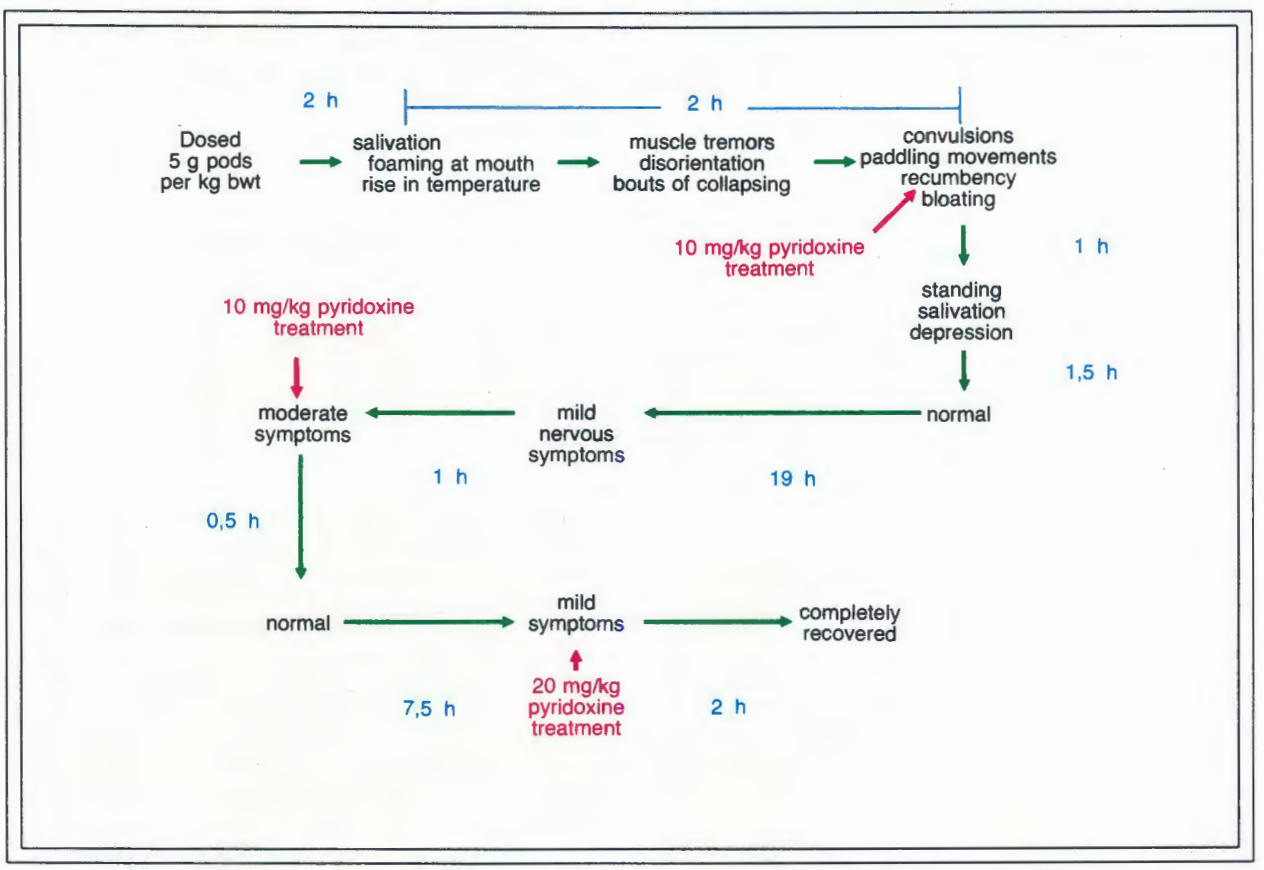

FIG. 1. Symptomatology and treatment of pilot sheep dosed with *Albizia versicolor* pods

Experiment 2: Five healthy male Dorper weaner lambs with live masses between 26 and 28 kg were used in this experiment (Lambs 1–5, Fig. 2). Four of these lambs (Lambs 1–4, Fig. 2) were drenched with 10 g/kg pod-material in 2 ℓ water (i.e. double the dose of pod material used in the pilot trial). The first lamb (Lamb 1, Fig. 2) was left untreated to determine whether the dose of pod-material was lethal. The other 3 lambs (Lambs 2–4, Fig. 2) were treated with pyridoxine at an initial dose of 20 mg/kg. The 5th animal (Lamb 5) received pyridoxine at the same dose and time as the treatment group but no plant material.

The animals were bled just prior to the dosing of pod material and then at hourly intervals until 6 h post-dosing. Further blood samples were taken at 12, 25, 31, 49, 55 and 169 h post-dosing. Due to the rapidity with which the symptoms occurred only the habitus and temperature of the animals could be recorded during the course of this experiment. The blood samples were analyzed for the same parameters described in the pilot trial. In addition CK isoenzymes were analyzed on the blood taken at 4 h post-dosing. Cerebral spinal fluid (CSF) was collected together with the first blood samples, but insufficient clean CSF could be obtained for meaningful analyses. A post-mortem examination was conducted on the animal that died (Lamb 1). The surviving animals (Lambs 2–5) were not slaughtered after the trial.

RESULTS

Symptomatology

Pilot trial (Fig. 1)

Clinical signs began 2 h after the animal received pod material and consisted initially of salivation, foaming at the mouth and a sharp rise in temperature (39,8 °C). These symptoms progressed to muscle tremors, apparent disorientation and intermittent sudden bouts of collapse with convulsions and paddling movements. After 2 h of progressively worsening signs the animal could no longer rise and began having violent spasms. Slight bloating together with rumen stasis was also noted and a temperature of 40,2 °C and pulse of 195 were recorded. At this stage of severe intoxication 10 mg/kg pyridoxine was administered intravenously (i.v.). The clinical signs improved within 10 min of treatment and by 30 min all convulsions and tremors had ceased. An hour after treatment the animal was standing, but continued to salivate and remain depressed. Two and a half hours post treatment it appeared to be clinically normal. No further symptoms were seen until approximately 19 h after the first treatment when the animal again suffered a relapse. After showing nervous signs for 1 h, it was again treated with 10 mg/kg pyridoxine subcutaneously (s.c.) and the animal recovered gradually over the next half an hour. Mild symptoms, including frothing at the mouth, depression and loss of appe-

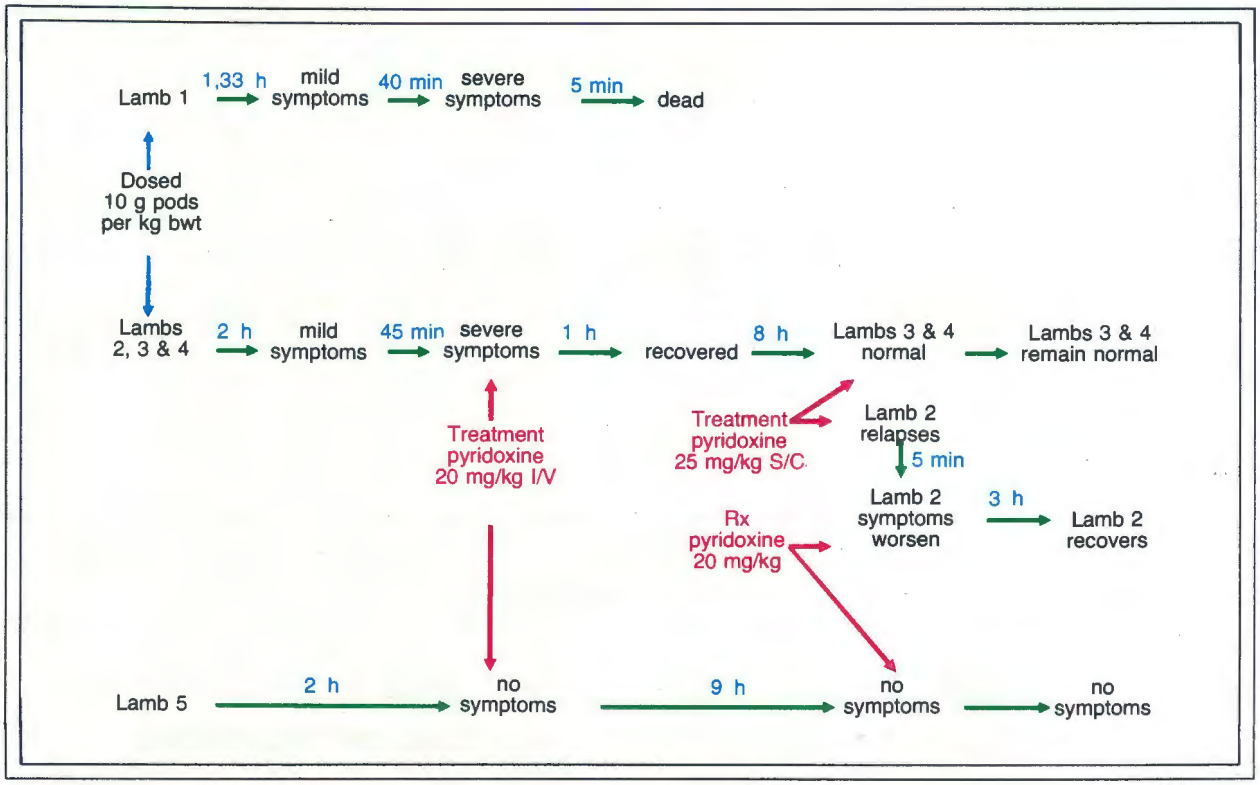

FIG. 2. Symptomatology and treatment of lambs dosed with *Albizia* pods

tite recurred 7,5 h later. A double dose of 20 mg/kg was then given s.c. and after 2 h no further symptoms were seen.

Experiment 2 (Fig. 2)

All four lambs (Lambs 1–4) that received pod material began showing signs of intoxication within 1,3–2 h. These signs were very similar to those described in the pilot trial with a rise in temperature being one of the first indicators of toxicity (Fig. 3). Approximately 2–3 h after receiving pod material the signs had become very severe and 3 animals (Lambs 2–4) were given 20 mg/kg pyridoxine i.v. The untreated control animal (Lamb 1) died 2 h after receiving pod-material with the only additional terminal symptom being a grinding of the teeth. The 3 treated animals recovered rapidly and, apart from a depressed habitus which took a few hours to disappear, were standing up within 30 min and were apparently fully recovered 1 h after treatment. About 8 h after treatment Lamb 2 again developed severe symptoms. Twenty-five mg/kg pyridoxine was then administered s.c., which had no immediate effect. The condition of the animal continued to deteriorate and fearing that it would die, a further 20 mg/kg was given [half intramuscularly (i.m.) and half i.v.] 5 min after the s.c. treatment. The latter treatment rapidly stabilised the animal's condition and within 30 min it was standing, although for several hours it continued to show symptoms of disorientation, circling, head pressing and intermittent muscle tremors. The other 2 animals in the treated group (Lambs 3 & 4) did not develop further signs of intoxication but were still given a second treatment of 25 mg/kg s.c. at 8 h. These 2 animals showed no further signs throughout the trial apart from a depressed habitus in one case.

The pyridoxine treatment produced no adverse effects in the control animal (Lamb 5) which received pyridoxine HCl at the same time intervals as Lambs 2 & 4 (Fig. 2).

Chemical Pathology

The changes in temperature (Fig. 3) and chemical pathology parameters followed the same pattern in both experiments. The rise in temperature preceding and during the period of symptoms was accompanied by a rise in blood glucose levels, with the exception of the untreated animal (Lamb 1) which became hypoglycaemic just prior to death (Fig. 4). The treated control animal (Lamb 5) also showed some elevation in blood glucose levels.

A rise in activity of CK was recorded in the lambs which were dosed pod material (Lambs 1–4). This reached a peak about 30 h after the first signs of intoxication were noted (Fig. 5) and was followed slightly later by elevations in AST (Fig. 6) and LDH (Fig. 7) activities. The CK isoenzyme analysis showed that the MM (CK_3) fraction made up 75–100 % of the total CK activity with the remaining fraction being mainly BB (CK_1). Only trace levels of the MB (CK_2) fraction were seen. All the other chemical pathology parameters monitored in both experi-

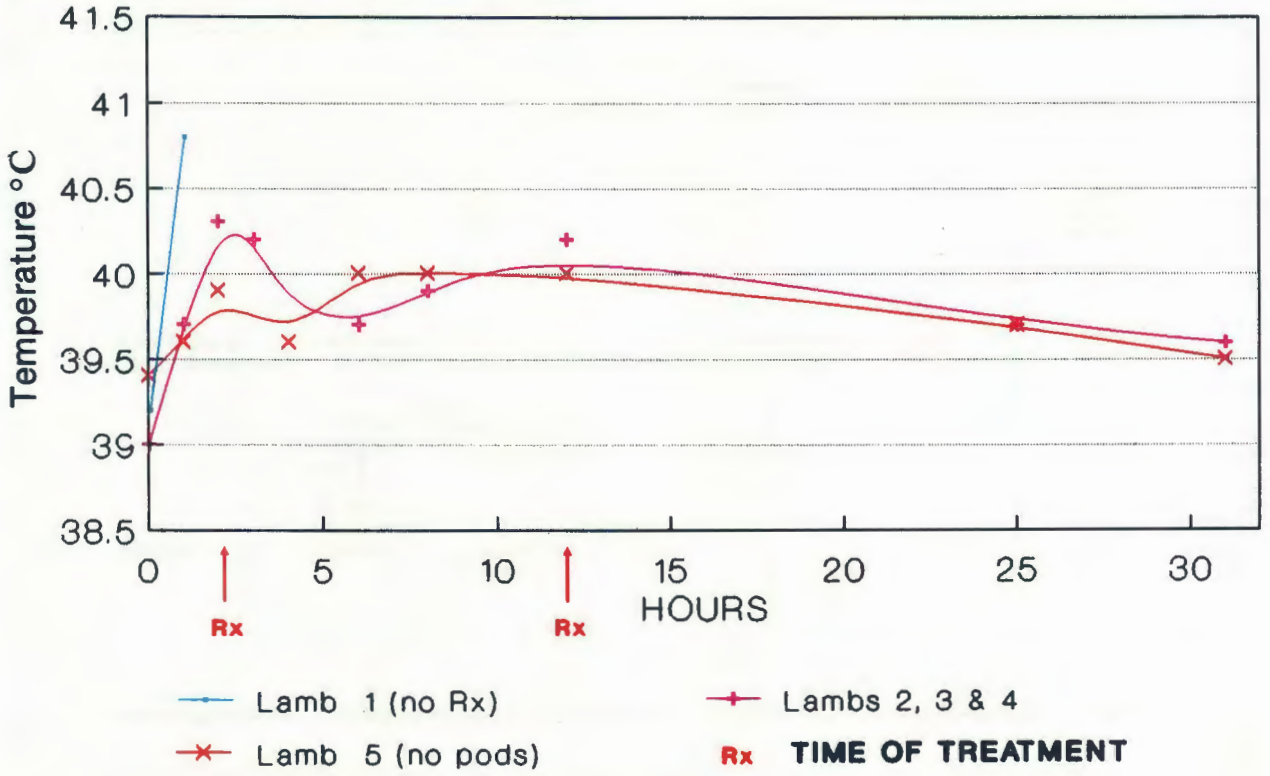

FIG. 3. The temperature of lambs dosed with *A. versicolor* pods over time

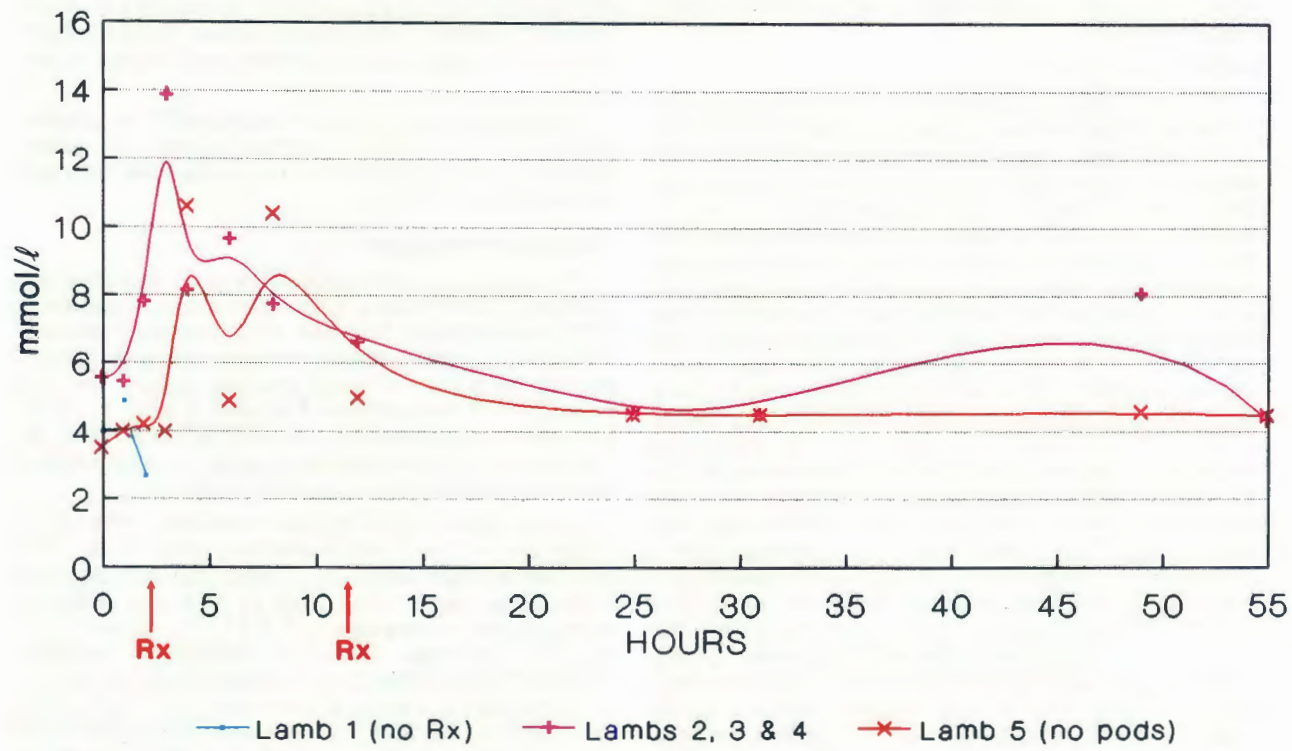

FIG. 4. Blood glucose levels of lambs dosed with *A. versicolor* pods over time

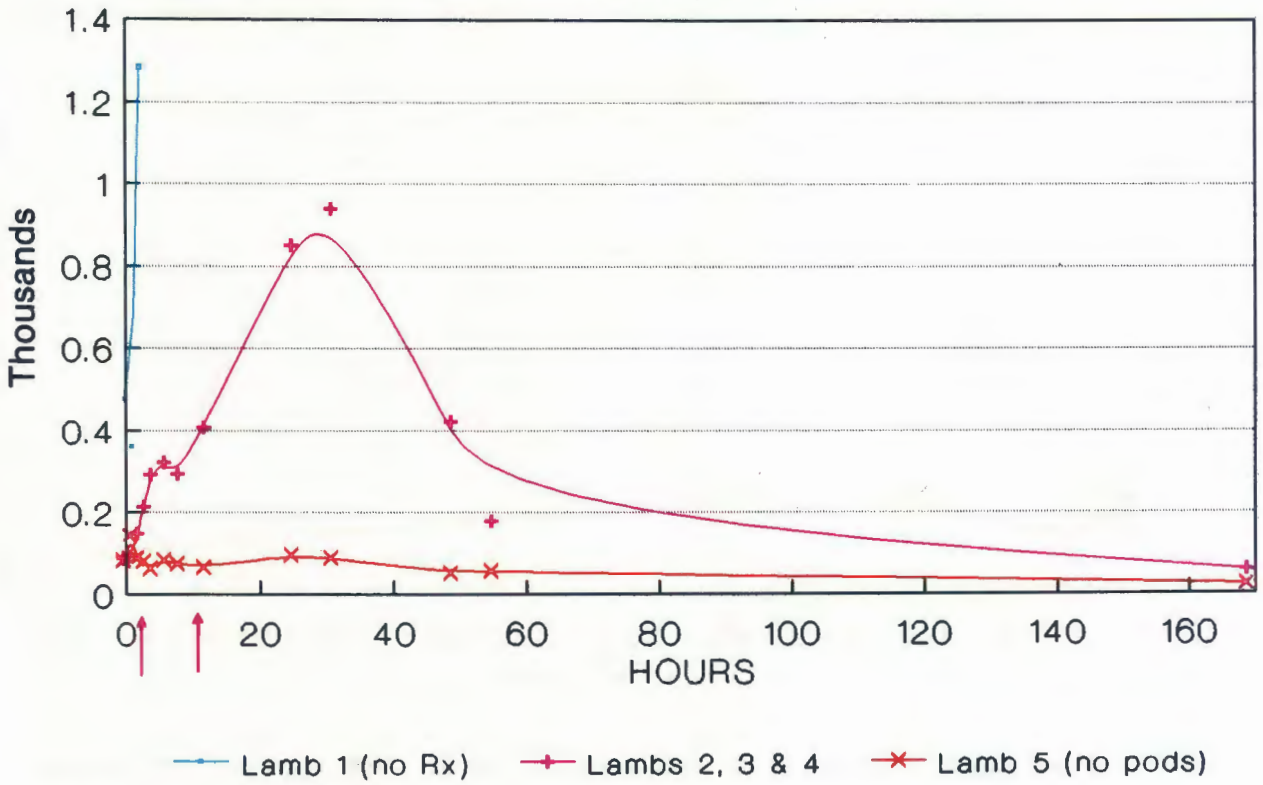

FIG. 5. Serum CK levels of lambs dosed with *A. versicolor* pods over time

ments remained normal throughout the course of the experiments.

No rise in the activity of CK, AST or LDH was recorded in the lamb which did not receive any pod material (Lamb 5, Fig. 5–7).

Pathology

Macropathology: The carcase and internal organs of Lamb 1 showed severe diffuce congestion and evidence of cyanosis. There were widespread petechiae, ecchymoses or suggillations in the subcutis, intermuscular tissues, pleurae, lungs, trachea, thymus and cervical lymph nodes and in the loose connective tissues around the trachea and oesophagus. Large suggillations and haematomas were evident in the muscles and connective tissues over the cisterna magna and lumbosacral regions and in the perirenal adipose tissue. The meningeal vessels and choroid plexus were extremely congested. Besides congestion and haemorrhages the lungs also revealed focal emphysema along the ventral borders. The CSF within the cisterna magna was dark-red in colour. There were focally disseminated pinpoint to pinhead-sized white foci throughout the mucosa of the small intestines.

Histopathology: The histopathological examination confirmed the generalised congestion and widespread haemorrhages observed on macroscopic examination. Subdural haemorrhages were noted in the ventral region of the midbrain and lumbar spinal cord whilst focal microhaemorrhages were present in the ventral horns of the gray matter, ventrolateral tracts of the lumbar spinal cord and in the corona radiata in the vicinity of the caudate nucleus.

The intestines revealed a moderate diffuse granulomatous enteritis with scattered eosinophils and severe focal aggregates of coccidial protozoa in various stages of development.

Discussion and Conclusions

It was established in the pilot trial that 5 g *A. versicolor* pod material/kg body mass caused severe nervous and related signs in a sheep and that these symptoms could be reversed by administering 10 mg/kg pyridoxine. The relapses seen after treatment are probably explained on the basis that pod material remained in the rumen for a period exceeding the half-life of pyridoxine and/or its metabolites thus enabling the toxin to continue working after the effect of the drug had worn off. Further treatment was thus required to protect the animal until all the toxin had been excreted or metabolised or had been sufficiently diluted to render it harmless.

The symptomatology in Experiment 2 was similar to that of the pilot trial despite the fact that a double dose of toxic pod material had been given. Therapeutic dosages appeared to be linked to the toxic dose as more pyridoxine was required to counter the effects of the higher doses of pods. The treatment was nevertheless highly successful even in cases with severe signs.

The rise in temperature seen in both experiments

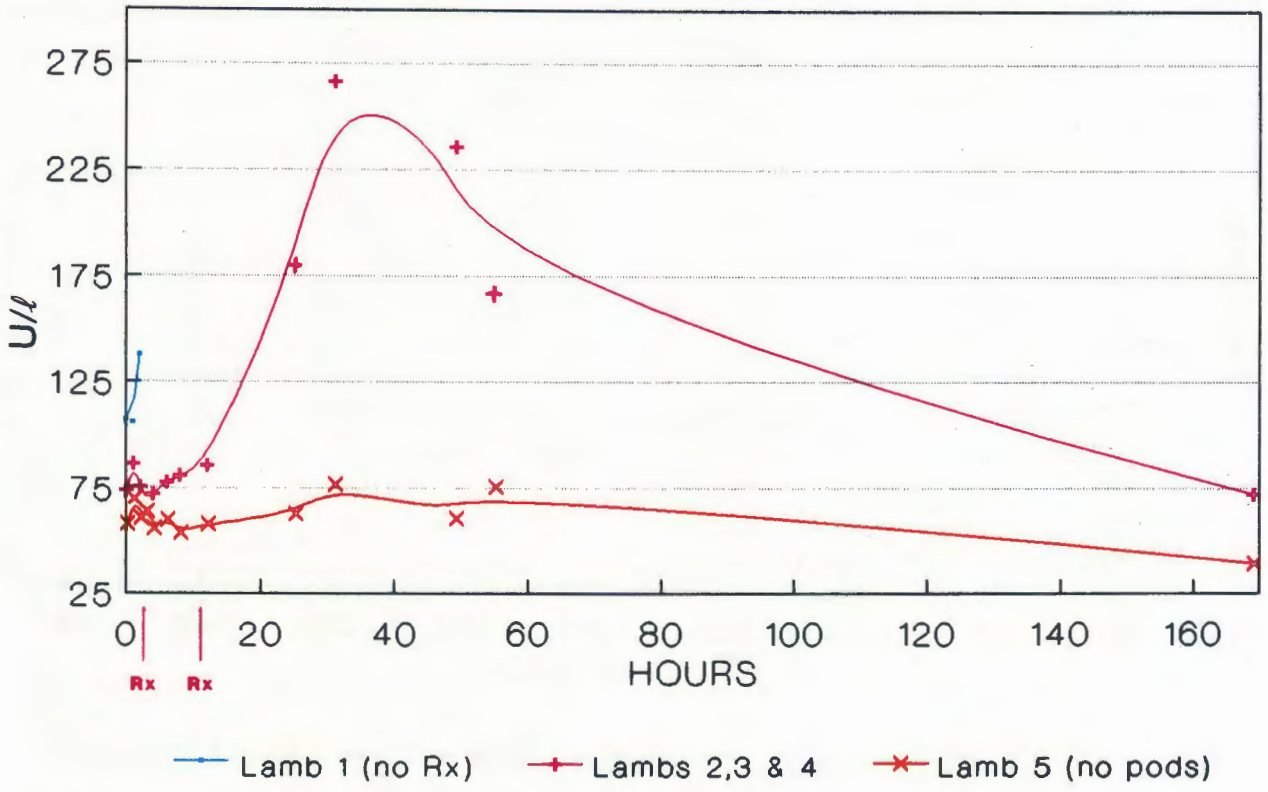

FIG. 6. Serum AST levels in lambs dosed with *A. versicolor* pods over time

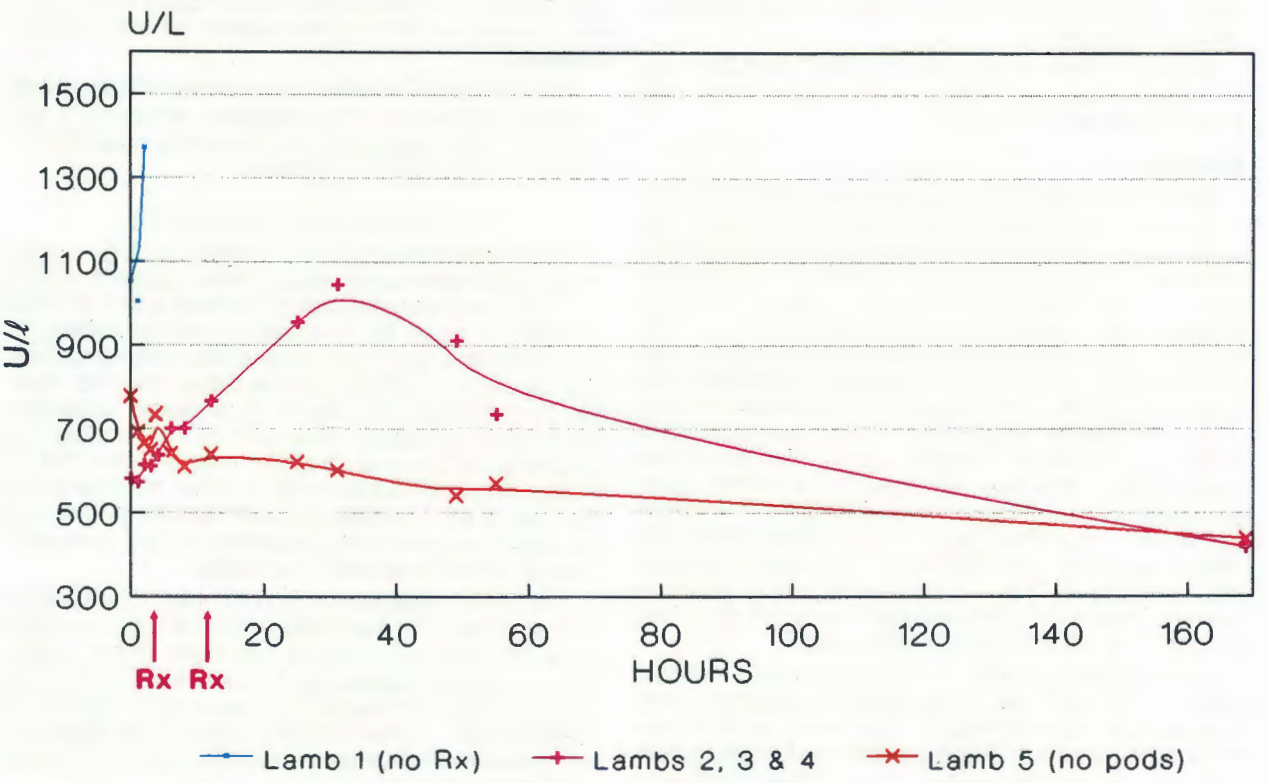

FIG. 7. Serum LDH levels in lambs dosed with *A. versicolor* pods over time

was attributed to muscle spasms and the rise in CK, AST and LDH enzyme activities to muscle damage secondary to the muscle spasms. The fact that the enzymes originated from skeletal muscle was further supported by the fact that the main CK isoenzyme involved was the muscle bound MM fraction. The MB fraction was virtually non-detectable which supported the histopathological findings of an absence of myocardial lesions. This differs from *A. tanganyicensis* intoxication where areas of myocardial degeneration were seen (Basson, Adelaar, Naude & Minne, 1970).

The rise in glucose levels, seen in all the animals except the one that died, was attributed partially to the unavoidable stressing of the animals that occurs during the course of such experiments (Fig. 4). The fact that the animals showing symptoms had slightly higher glucose levels than the treated control was probably due to increased stress in these cases caused by their symptoms. These findings confirm those of Needham & Lawrence (1966) who in addition noted a glycosuria in intoxicated sheep which they ascribed to adrenal cortical activation that was secondary to the acute convulsive activity. The hypoglycaemic state of the animal that died was probably due to physical exhaustion brought about by the muscle tremors and convulsions.

The haemoglobin and haematocrit levels remained normal throughout the course of the experiment.

Evidence of cyanosis in the carcase of the fatally poisoned sheep indicated that the animal was in a hypoxic state at the time of death. This may be due to an antagonistic effect by the neurotoxin of *A. versicolor* on vitamin B_6 which is required, by virtue of its action as an essential co-enzyme in transamination reactions (Bateman, 1985), for normal cellular respiration.

A convincing explanation for the nervous symptoms has yet to be put forward. Initially it was thought that a shortage of glutamate due to inhibition of transaminase, inhibition of glycogen phosphorylation and a deficiency in GABA synthesis may explain the nervous symptoms but work in guinea-pigs has shown pyridoxal, the active form of the vitamin, to be an ineffective treatment (Gummow *et al.*, 1990). This suggests that the main pathways where Vit B_6 is currently known to act are not being affected and that the structure of pyridoxine HCl and not other isomers of Vit B_6 is the key to the solution. In addition pilot trials using GABA as a possible treatment for albiziosis met with no success (Gummow, unpublished data, 1990). It seems therefore that a lot more research needs to be done before the mechanism of action of the toxin can be determined.

At autopsy Needham & Lawrence (1966) and Basson *et al.* (1970), noted cyanosis, generalised congestion and widespread haemorrhages in experimental and natural cases of *A. versicolor* and *A. tanganyicensis* intoxication. In this report similar lesions were noted in the lamb that died. Basson *et al.* (1970) reported the presence of petechial haemorrhages in the diaphragm and muscles of the forelimbs, hindlimbs and back. They did not however note any suggillations or haematomas in the muscles in the region of the cisterna magna and lumbosacral cistern. One can therefore conclude that in this report the latter haemorrhages as well as the haemorrhagic appearance of the CSF probably resulted from manipulation during the clinical procedures for collection of CSF from the above 2 cisterns.

The cerebral and spinal cord lesions included focal haemorrhages, generalised congestion and a mild diffuse oedema of both the gray and white matter but in particular the latter. These lesions are not specific but have been reported previously in cases of *A. tanganyicensis* toxicity (Basson *et al.*, 1970).

The renal and hepatic lesions were very mild and again non-specific in nature. They could have arisen as a result of hypoxia and/or hyperthermia. Basson *et al.* (1970) also noted a mild nephrosis and hepatosis. They too regarded these lesions to be non-specific and attributed them to the effects of hyperthermia as a result of the tetanic muscle spasms.

The intestinal lesions could be ascribed to a coccidial infection and were not associated with the dosing of *A. versicolor*.

The symptomatology and pathology were virtually identical to that described for *A. tanganyicensis* poisoning in sheep (Basson *et al.*, 1970) providing circumstantial evidence that *A. versicolor* and *A. tanganyicensis* probably contain identical toxins. It is therefore highly likely that treatment of *A. tanganyicensis* poisoning with pyridoxine HCl will also be successful.

In conclusion, this trial proved that pyridoxine HCl is an effective treatment for *A. versicolor* toxicity in sheep. The most effective treatment regimen for sheep is probably 20–25 mg/kg pyridoxine given i.v. when symptoms are severe, followed by further treatment as required. Due to the possibility of relapses occuring some 8 h after the initial treatment it is recommended that affected animals be kept under observation for a few days after ingesting the pods. Prophylactic treatment is possible, provided one bears in mind that the therapeutic effect of the drug appears to be approximately 8 h in sheep. With this treatment a once fatal plant intoxication can now have a very good prognosis.

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