

## FIRST REPORT OF ANNUAL RYEGRASS TOXICITY IN THE REPUBLIC OF SOUTH AFRICA

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### ABSTRACT

SCHNEIDER, D. J., 1981. First report of annual ryegrass toxicity in the Republic of South Africa. *Onderstepoort Journal of Veterinary Research*, 48, 251-255 (1981).

The occurrence of annual ryegrass toxicity (ARGT) in sheep and cattle is reported for the first time in South Africa. To date it has been diagnosed conclusively in South African Mutton Merino sheep on a farm in the Caledon district and in cattle on 3 farms, 2 of which are situated in the Bredasdorp district and 1 in the Ceres district. It is a neurological disease characterized by symptoms of tremor, ataxia, intermittent epileptiform seizures, nystagmus, opisthotonus, abortions and high mortality.

The history, clinical signs and experimental reproduction of the disease as well as the pathology of 4 experimental and 10 natural cases in sheep and 2 in cattle are described.

### Résumé

#### PREMIER RAPPORT SUR LA TOXICITÉ DU RYEGRASS EN REPUBLIQUE D'AFRIQUE DU SUD

L'occurrence de la toxicité annuelle du ryegrass (ARGT) chez le mouton et le bétail est rapportée pour la première fois en Afrique du Sud. A ce jour, un diagnostic certain a été fait chez le mouton South African Mutton Merino dans une ferme du District de Caledon et dans le bétail de 3 fermes, deux desquelles sont situées dans le District de Bredasdorp et une dans le District de Ceres. C'est une encéphalose caractérisée par des symptômes de frémissement, ataxie, crise épileptiforme intermittente, nystagmus, opisthotonos, avortements et une mortalité élevée.

L'anamnèse, les signes cliniques et la reproduction expérimentale de la maladie ainsi que la pathologie de quatre cas expérimentaux et de 10 cas naturels chez le mouton et 2 chez le bétail sont décrits.

### INTRODUCTION

Annual ryegrass toxicity (ARGT), a neurological disease affecting sheep and cattle ingesting annual ryegrass species parasitized by an *Anguina* sp. and a *Corynebacterium* sp., was first described in South Australia in 1967 (McIntosh & Thomas, 1967) and in Western Australia in 1971 (Gwynn & Hadlow, 1971). Since these first reports of ARGT, when the disease was confined to only a few farms, the syndrome had been diagnosed on several hundred farms covering extensive areas of the 2 states of Australia. The evidence suggests that it takes 10-15 years after introduction of the infection into a ryegrass paddock for it to reach toxic levels for stock (Michelmores & Mackie, 1977).

In contrast to ryegrass staggers, which has a low mortality rate, ARGT can kill large numbers of stock quickly, losses of 70-90% being not uncommon (Michelmores & Mackie, 1977; Michelmores, McKay & Mackie, 1980). The toxic component has been found to be concentrated in the wall of the capsule filled with masses of yellow *Corynebacterium*, whereas the nematode galls and other parts of the plant are non-toxic (Lanigan, Payne & Frahn, 1976; Stynes, Petterson, Lloyd, Payne & Lanigan, 1979; Stynes, 1980). It is accepted that annual ryegrass cannot become toxic in the absence of the *Anguina*, because the nematode is essential for carrying the bacteria into the plant (Price, 1973; Fisher, 1977; Price, Fisher & Kerr, 1979a). Apart from the characteristic neurological signs and high mortality, mention is also made of many abortions (McIntosh & Thomas, 1967; Berry & Wise, 1975; Trotman, 1978). The toxic component is not affected when dried for 3 h at 100 °C and ryegrass remains toxic for years when stored (Culvenor, Frahn, Jago & Lanigan, 1978).

The object of this paper is to report outbreaks of a clinically identical syndrome in sheep and in cattle grazing annual ryegrass (*Lolium* hybrid) parasitized by

a nematode, *Anguina agrostis*, and a bacterium, *Corynebacterium* sp. The syndrome was experimentally reproduced by feeding to 4 sheep annual ryegrass hay and seed harvested from the toxic camp in which the ovine outbreak had occurred. In the case of the cattle experimental reproduction was not attempted.

### HISTORY

#### Occurrence in sheep

Between December 1979 and April 1980 a disease in South African Mutton Merino sheep, characterized by intermittent epileptiform convulsions and other signs of neurological disturbance of sudden onset, high mortality and abortion, was investigated on a farm in the Caledon district.

This is a winter rainfall area in which dietary supplementation during late summer and autumn is usually necessary. Wheat and sheep farming are the 2 main types of farming in this area and the average annual rainfall over the preceding 8-year period was 377,6 mm. Hay is used by this farmer as a method of providing supplementary food for the stock when necessary. All cases of mortality described here were associated with one particular camp of about 24 hectares. This camp was sown during May 1979 with home-grown Heros oats and 150 kg purchased *Vicia villosa* seed for hay production. Annual ryegrass had never been sown on this farm, but it had been present for many years and had become such a weed in grain lands during recent years that it had to be controlled by strategic burning, ploughing and by selective herbicide spraying. As this particular crop was meant for hay production, the ryegrass was not eradicated. On stubble and fallow lands in this area the increase of annual ryegrass is welcomed because of its value as winter grazing.

Because of unfavourable prevailing weather conditions, haymaking was delayed, and as a result the final product contained a high percentage of ripe seed of oats and ryegrass and some *Vicia*. Different



bales contained varying mixtures of these 3 plants, but the approximate proportions were 60% oats, 37% ryegrass and 3% *Vicia* by mass.

Seventy ewes and 62 8-week-old lambs were allowed to graze this stubble land for a period of 4 days after the bales of hay had been harvested. No abnormal symptoms were noticed amongst these sheep. Five days after these sheep had been removed from the camp, 120 7-month-old lambs were put into this same camp on the 2nd December 1979, until a permit for their slaughter had been received. Three days later a few dead and sick sheep were noticed by the owner, veterinary assistance was sought and all the sheep were taken out of this camp. During the next 4 days 95 of these lambs died, after which mortality ceased.

No problems were encountered for the next 6 weeks. Between the end of January 1980 and the end of April 1980, when the diagnosis was made, the remaining mortalities (226) occurred and approximately 200 ewes aborted. Mortalities and abortion, which always started 8–16 days after commencement of the feeding of bales of hay originating from this particular camp, affected various groups of sheep grazing different camps on the farm. Symptoms of disease and mortality ceased 4–6 days after this hay had been withheld.

#### *Occurrence in cattle*

Between 8th and 13th of October 1980, 13 out of a group of 90 one-year-old crossbred beef cattle died on a farm in the Bredasdorp district after showing neurological signs. These signs were noticed for the first time 10 days after introduction onto a pasture established in 1973 with lucerne, clover, Italian (*Lolium multiflora*) and Wimmera (*Lolium rigidum*) ryegrass. At this stage, however, the 2 *Lolium* spp. had overgrown the lucerne and clover completely and were in full seed, as the camp had not been grazed for 3 months pending hay production. One affected animal was brought to the laboratory at Stellenbosch for autopsy.

Between the 3rd and the 10th of November 1980, 4 out of 15 young stud Simmenthaler cattle died on a farm near Prince Alfred Hamlet. The 4 that died were all in the 18-month age group and all showed pronounced neurological symptoms. These were first observed approximately 14 days after the cattle were introduced into a camp in which *Medicago* sp., subterranean clover and barley were dominated by annual ryegrass in full seed. This pasture was established in 1978 from *Lolium rigidum* seed harvested from ryegrass grown from seed which had originally been bought and sown on this farm 8 years before. The pasture had not been grazed for 2 months and was in full seed.

Between the 13th and 18th of November 1980, 4 out of a group of 19 stud Hereford cows died on a farm in the Bredasdorp district after showing neurological symptoms. For a period of 60 days, 19 cows with their calves and 350 sheep grazed oats and annual ryegrass on an old oat stubble land.

When the grazing had been all eaten, the animals were moved to a new oat stubble land in an adjacent camp. While in transit, 1 cow developed neurological symptoms and after 12 days on the stubble land another 6 cows contracted ARGV.

One dead cow, in excellent condition, was brought to the Regional Veterinary Laboratory for autopsy. None of the sheep on this farm contracted the disease.

#### *Morbidity and mortality*

In the outbreak of ARGV in sheep, virtually all the sick sheep eventually died. In cattle, however, 1 or 2 clinically affected animals survived on each of the 3 farms investigated. None of the many tranquilizers or sedatives used proved to be of definite therapeutic value.

#### *Animals affected*

Cattle and sheep have been observed to contract the disease under natural conditions and no breed, age or sex predisposition to the disease has been found. In a feeding experiment white mice were found to be highly susceptible.

#### *Clinical signs*

The clinical symptoms of ARGV are due to a malfunction of the central nervous system (CNS) and are characterized by a sudden onset and marked neurological symptoms.

In severe cases animals will be found lying on their sides with legs extended, showing opisthotonus, nystagmus, shivering, twitching, foaming from the mouth and, in some cases, epileptiform seizures. Usually, these animals died within 4–12 hours of the onset of the signs (Fig. 1).

In less acute cases, which were more often found in cattle than in sheep, the animals appear normal from a distance, but when disturbed they will usually move a few metres, lower their heads, and fall on their sides, with legs extended and head held back, in a severe epileptiform seizure lasting 1–3 minutes. After recovery from the seizure, animals will usually stand again and some may even start to graze. In most cases, however, the animals appear to be unaware of their surroundings. Some show muscular twitching, appear unsteady on their legs and lift their legs high when walking. Especially in the case of cattle, they sometimes appear bewildered, excitable and run through fences when disturbed.

In the outbreak in sheep numerous abortions coincided with the typical neurological symptoms when ewes in late pregnancy were affected. The owner estimated that approximately 200 out of 557 ewes aborted.

## MATERIALS AND METHODS

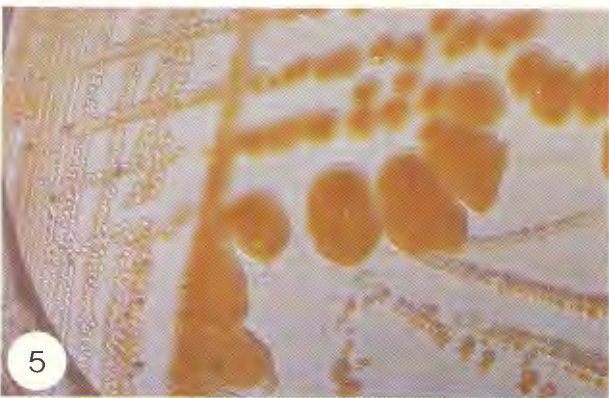
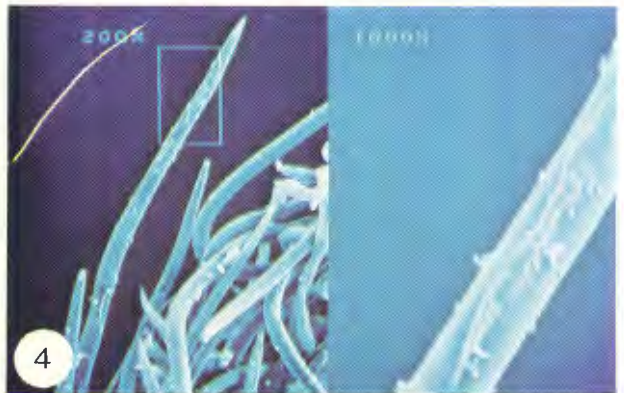
#### *Feeding trials with sheep*

The 3 plant components of hay from the toxic pasture at Caledon, i.e. oats, ryegrass and *Vicia*, were meticulously separated by hand and fed to crossbred Dorper sheep varying in age from 6 months to 6 years. In addition, the following fractions of the hay were fed to the sheep; a mixture consisting of equal parts *Lolium* sp. and oat hay; a mixture of chaff containing numerous seed heads obtained by passing the hay through a combine harvester; pure *Lolium* sp. seed and *Lolium* sp. straw without seed (Table 1).

The sheep were examined daily. Because no significant clinical pathological changes in blood values could be found in the case of the natural outbreaks, routine blood analyses were not done on the experimental sheep.

Necropsies were performed on all the sheep in which the disease could be reproduced, as well as on 10 sheep and 2 cattle that were naturally affected. Specimens were collected from various organs, fixed in 10% buffered formalin, processed according to standard procedures, and stained with haematoxylin and eosin (HE).





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FIG. 1 Affected sheep  
FIG. 2 Parasitized *Lolium* sp. plants  
FIG. 3 Normal *Lolium* sp. seed (left); nematode galls (centre); bacterial galls (right)  
FIG. 4 *Anguina* larvae  
FIG. 5 *Corynebacterium* cultures



### Feeding trials with white mice

Fifteen groups of 5 white mice in each were fed on finely ground *Lolium* sp. seed which was obtained from the toxic hay mixture and made into pellets with the addition of 10% pure molasses. One control group of 5 mice was fed on commercial mouse ration. Histopathological specimens of organs from 4 of the mice that died were prepared as previously described.

### Identification of the ryegrass

Specimens of annual ryegrass from all the affected camps were tentatively identified as hybrids of *Lolium temulentum* L. at the Bolus Herbarium, University of Cape Town, and the Botanical Research Institute, Pretoria. It should be mentioned that *Lolium* spp. hybridize very readily.

### Bacteriological examination

The galls were examined for bacteria as described by Stynes *et al.* (1979) at the Stellenbosch Regional Veterinary Laboratory (RVL).

### Examination for nematodes

*Lolium* sp. seed from each of the 4 outbreaks were examined for nematodes at the Stellenbosch RVL according to the method of Berry & Stynes (personal communication, 1980).

Specimens of *Lolium* sp. seed from the outbreak in sheep were also submitted for nematological examination to Dr B. Meyer of the University of Stellenbosch, and seed, hay and green *Lolium* sp. plants from the outbreak in sheep and green *Lolium* sp. plants from 1 outbreak in cattle to Dr E. van den Berg of the Plant Protection Research Institute, Pretoria.

## RESULTS

### Feeding trials with sheep

The results are summarized in Table 1.

*Sheep 1*, which was fed pure *Vicia vilosa* hay *ad lib.* for 19 days, consumed 13,1 kg of hay without ill effect.

*Sheep 2*, which received only oat hay until the experiment was ended after 19 days, consumed 17,0 kg and also showed no abnormal clinical signs.

*Sheep 3*, which ate only *Lolium* sp. hay, consumed 8,2 kg in total and was found on the morning of the 10th day lying on its side in the crate and unable to stand, even when lifted onto its legs. Twitching of the muscles, nystagmus and frothing from the mouth,

and occasional epileptiform seizures occurred. To prevent post-mortem changes developing overnight in case of death, this sheep was slaughtered on the same afternoon.

*Sheep 4* was found on the morning of the 10th day also with severe neurological symptoms after it had eaten 3,4 kg of a 50:50 mixture of oat and *Lolium* sp. hay. The sheep was unable to stand, had opisthotonus, nystagmus, twitching and severe muscular spasms, with extended legs. The symptoms intensified and the sheep died during the afternoon.

*Sheep 5* consumed 2,5 kg of the chaff mixture until it was found on the morning of the 10th day lying on its side with extended legs and muscles twitching. This sheep could stand initially, but developed epileptiform seizures when handled and would then fall on its side. These neurological signs intensified and the sheep died later on the same day.

*Sheep 6* consumed 2 kg of *Lolium* sp. seed originating from the toxic hay before it was found on the morning of the 6th day showing intermittent neurological signs. The sheep appeared perfectly normal when standing on its own in the crate. When touched lightly by hand, however, the sheep lowered its head slowly, the eyes became glazed, the head began to shake and an epileptiform seizure developed. These seizures lasted only a few seconds in the morning, but intensified progressively until the sheep died in the afternoon.

*Sheep 7*, which consumed 4,0 kg *Lolium* sp. straw without seed heads over a period of 7 days, showed no symptoms. The experiment was terminated because of lack of material.

*Sheep 1, 2 and 7* were observed for a further 2 months, and remained healthy. None of the affected sheep gave any indication of illness prior to the onset of neurological symptoms.

The neurological symptoms of these 4 experimentally produced cases, as well as all the sheep and cattle examined from field cases, could be dramatically intensified when chased, handled or disturbed.

In both naturally and experimentally intoxicated sheep it was noticed that the venous blood had a bright red colour almost resembling arterial blood.

### Feeding trials with white mice

All the mice fed on the *Lolium* sp. seed died between the 5th and 12th day. None of the 5 control mice showed any clinical symptoms.

TABLE 1 Feeding trials with different components of toxic hay

Sheep No.	Age	Initial live mass (kg)	Material	Total mass ingested (kg)	Interval between commencement of trial and death (days)	Duration of experiment (days)	Fate
1.....	4 years	44	<i>Vicia</i> hay	13,1	—	19	Discharged
2.....	6 years	49	Oat hay	17,0	—	19	Discharged
3.....	5 years	52	Ryegrass hay	8,2	9	—	Killed <i>in ex-</i> <i>tremis</i>
4.....	8 months	32	50% ryegrass and 50% oat hay	3,4	9	—	Died
5.....	1 year	40	Mixed chaff*	2,5	9	—	Died
6.....	6 months	26	<i>Lolium</i> sp. seed	2,0	5	—	Died
7.....	1 year	37	<i>Lolium</i> sp. straw	4,0	—	7	Discharged

\* This chaff mixture was obtained after the seed was removed by a combine from the toxic bales of hay



Clinical signs in affected mice developed suddenly. They became listless, showed signs of being cold, huddled together and died within 24 hours of showing the first signs. No neurological symptoms were noticed in any of these mice.

#### Chemical pathological findings

No conspicuous chemical-pathological changes could be found in the blood of 6 sheep and 4 cattle naturally intoxicated.

#### Pathological findings

Since the pathological findings in the natural cases and the 4 experimental sheep were identical, no distinction will be made in describing the lesions of the 2 groups. No lesion of important diagnostic value was found.

**Gross pathology:** Post-mortem examinations were performed on 14 sheep (10 naturally and 4 experimentally affected) and 2 cattle from field outbreaks. Organs from a 3rd bovine animal were received and examined histopathologically.

The following lesions were found in most animals examined: rapid onset of rigor mortis, severe lung oedema accompanied by various degrees of congestion and froth in the trachea and bronchi. Haemorrhages, varying in size from petechial to echymoses, were constantly found on the endocardium and often also on the epicardium. The liver was always pale in colour and had signs of diffuse degeneration. The kidneys were pale brown in colour with diffuse degeneration of the cortex. In most cases the cranial cavity contained more fluid than normal. The lymph nodes were oedematous, enlarged, and congested, especially those in the anterior half of the body. The ruminal contents appeared somewhat smaller than normal and the small intestines were usually mildly congested.

**Histopathology:** Histopathological sections from various organs from 13 sheep and 3 cattle were examined, but nothing of significant diagnostic value was found.

Moderate congestion and oedema of the brain were constantly present, most animals showing small foci of acute hyaline degeneration and necrosis of the myocard but no cellular reaction. Various degrees of fatty degeneration and cloudy swelling of the hepatocytes were consistently seen. The kidneys usually showed mild tubular degeneration, the lymph nodes mild lymphoid proliferation with oedema, and the lungs invariably congestion with oedema.

The 4 mice all showed some oedema and congestion of the brain, congestion and fatty metamorphosis of the liver, focal haemorrhages in the myocard and congestion of the kidneys.

#### Examination of *Lolium* sp. plant and seed material

**Macroscopic examination:** In the case of the sheep outbreak no macroscopic abnormality could be detected in the hay or seed.

In the 3 outbreaks in cattle, which occurred on *Lolium* sp. dominant pastures, the pastures were inspected at the time of mortality. The *Lolium* sp. plants were in the seed-bearing, ripening stage. When carefully examined, some seed heads appeared glistening and were slightly sticky when touched. These charac-

teristics disappeared rapidly after rain or drying of the seed heads. Occasionally, some seed heads were either totally or partially undeveloped, had bent or kinky stems and distorted seed heads with yellow and swollen florets (Fig. 2).

**Examination for nematodes:** Parasitized seed or "galls" could be identified from all of these outbreaks and, after being soaked in water for 24 hours, numerous living nematode larvae could be seen when these "galls" were opened. Most infected "galls" contained hundreds of 2nd stage *Anguina* sp. larvae (Fig. 3 & 4).

Meyer (personal communication, 1980) reported finding the 2nd stage larvae of an *Anguina* sp. in the *Lolium* sp. seed from Caledon. Dr E. van den Berg (personal communication, 1981) examined *Lolium* sp. seed and hay from the toxic camp at Caledon as well as green *Lolium* sp. plants from this camp and from a camp in Bredasdorp, where the 2nd outbreak occurred during September and October 1980. She found adult parasites on *Lolium* sp. plants from both farms and could identify these as *Anguina agrostis*.

**Bacteriological examination:** After the dry *Lolium* sp. seed had been soaked in water for 12 hours or more, the bacterial infected "galls" became pale yellow and could easily be identified with the naked eye, but more accurately under a stereoscopic microscope. When smears were made of this yellow material, masses of short gram positive rods were seen. They were slightly pleomorphic, had rounded ends and measured  $0,75-1,0 \times 0,5 \mu\text{m}$  in size.

These bacteria could readily be cultured, as described by Stynes *et al.* (1979). After 72 hours incubation at 28 °C, numerous pale yellow, convex colonies could be seen. These colonies were 1-3 mm in size, glistening, slimy and slightly sticky (Fig. 5). The bacteria from these colonies corresponded with those seen on direct smears from the infected galls and are non-motile, oxidase negative, nitrate reduction negative, urease negative, catalase positive, indol negative, methyl red negative and voges-proskauer negative.

These characteristics correspond with those described for *Corynebacterium* sp. (Price, 1973; Berry & Wise, 1975; Bird & Stynes, 1977; Price, Fisher & Kerr, 1979b).

#### DISCUSSION

The *Anguina*- and *Corynebacterium*-infected galls were found in *Lolium* sp. seed collected from each of the 4 camps where ARGT was diagnosed, but none in ryegrass samples collected (Schneider, 1980, unpublished data) from 190 farms situated in 24 districts in the winter rainfall area of the Republic of South Africa.

The clinical symptoms produced in the experimental sheep were indistinguishable from those present in the natural cases and were identical with ARGT as described in Australia. These findings, the abortions and the fact that an *Anguina* sp. and *Corynebacterium* sp. could only be isolated from toxic *Lolium* sp. plant material prove conclusively that these mortalities were caused by ARGT.

As 2 of the 4 farmers had never sown annual ryegrass on their farms, and Australian scientists (Michellmore & Mackie, 1977) have found evidence which suggests that it takes 10-15 years after introduction of infection for a land to reach toxic levels, it is most



likely that his infection had been introduced into the Republic of South Africa many years before. If this is the case, then it is logical to expect that this infection is prevalent, at low levels, on many other farms. The fact that no seed infected with *Anguina* sp. or *Corynebacterium* sp. could be found in samples from 190 farms must not be taken as evidence that this infection is only confined to the originally infected farms, as sample collection was not always representative. Many samples were too small, others were collected before they could have been parasitized and it is accepted that tests of this nature can only identify an infection if the number of infected seed per hectare has reached a particular level (Fisher, Dubé & Watson, 1979).

It is significant that on these 4 farms the ryegrass was not grazed by stock during the flowering and early stages of seed development, thereby allowing undisturbed multiplication of the *Anguina* sp. and *Corynebacterium* sp. in the seed heads. These conditions exist when *Lolium* sp. plants are allowed to grow amongst grain crops or when *Lolium* sp. pastures are preserved during spring for hay production or merely as reserve grazing.

The most likely explanation for the flock of 70 ewes and 62 lambs grazing the toxic stubble land for 4 days without suffering ill effects would be that these sheep, on introduction into this camp, selectively ingested mainly oats and *Vicia* since these plants are more palatable. When the flock of 120 older lambs were introduced 5 days later, they were forced to eat more of the *Lolium* sp. plant material.

No scientific explanation can be given why, in the 1 outbreak in cattle at Bredasdorp, none of the sheep died while sharing the camps with the cattle. The most likely explanation, however, would be that the cattle, owing to their high grazing habit, ingested proportionately more of the toxic ryegrass seed heads.

There remains little doubt that the *Lolium* sp. seed heads, infected with *Corynebacterium* sp. which passed through the combine with the chaff, caused the death of Sheep 5.

Considering the potency and stability of this toxin, as was experienced in cattle, sheep and mice, it is to be expected that all farm animals eating ryegrass might potentially be affected by this toxin.

The nature of the toxin has not been fully established, but Stynes *et al.* (1979) found indications that the toxin might be a phytoalexin produced by the plant in response to the presence of the invading *Corynebacterium* sp.

In Australian pen trials, the tranquillizer Chlor-diazepoxide gave promising results (Richards, Peterson & Purcell, 1979). The cost of the drug and secondary complications after treatment, however, render this drug impracticable for large scale use in field outbreaks.

## ACKNOWLEDGEMENTS

I am indebted to Mr W. J. van Rensburg for bacteriological work; to Mr E. Heine for toxicological and biochemical examinations; to Drs N. P. J. Kriek, J. A. W. Coetzer, M. G. Collett and J. M. Pletcher for histopathological examination; to Miss L. Hugo for botanical assistance; to Mrs Ina Boshof and Philip van der Merwe from F.F.T.R.I. for the photographs; to Drs R. Wilson, I. Herbst, F. Freeman, J. Adam and P. le Roux for referring the outbreaks; to Dr P. M. S. Masters for encouragement and preparation of the manuscript and to Mr J. S. Maree for willing assistance on the farm.

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