Onderstepoort J. vet. Res., 52, 255–259 (1985)

PARALYSIS AND LIPOFUSCIN-LIKE PIGMENTATION OF FARM STOCK CAUSED BY THE PLANT, *TRACHYANDRA LAXA* VAR. *LAXA*

RINA C. GRANT[1], P. A. BASSON[2] and A. B. KIDD[3]

ABSTRACT

GRANT, RINA, C., BASSON, P. A. & KIDD, A. B., 1985. Paralysis and lipofuscin-like pigmentation of farm stock caused by the plant, *Trachyandra laxa* var. *laxa*. *Onderstepoort Journal of Veterinary Research*, 52, 255–259 (1985)

A paralytic condition of farm stock in South West Africa, characterized by prominent neuronal and some mild extraneuronal pigmentation, is described. The distribution of the pigment, which was mainly located in the larger neurones of the brain and spinal cord, is given. Experimental evidence, obtained by feeding the plant, is presented that the condition is caused by *Trachyandra laxa* var. *laxa*. The histochemical features of the pigment proved to be compatible with a lipofuscin.

INTRODUCTION

History of outbreaks

A syndrome of progressive, ascending paralysis has occurred in cattle, sheep, horses and pigs in the Kalahari Sandveld of South West Africa (SWA)/Namibia since 1954. The first outbreak was reported in that year in a small area of the Gobabis district (Basson, unpublished reports, 1955; 1956). The affected farms, Marie, Panda, Lora, Rosendal, Becker and Naunas, had been overgrazed, and the state of the vegetation was much poorer on these farms than on the unaffected neighbouring farms. In an investigation by Basson, Adelaar & Schulz in 1956 (unpublished report) macroscopical pigmentation of the brains of the affected aninmals was observed, and 2 plants, which were readily eaten, were mentioned as possible aetiological agents, viz., *Anthericum erratic-um (=Trachyandra laxa)* and *Sarcostemma viminale*. A suspected fungal rustiness was described on the former. The outbreak continued until 1957, and the disease became known as "Marie paralysis", a connotation derived from one of the original farms which had been affected. No further cases were reported until October 1976, when another outbreak occurred in the same district. The number of outbreaks escalated and reached a peak during 1979, when 478 sheep, 54 cattle and 61 horses on 22 farms were thought to have died from this disease.

Only a few cases were reported during 1980, and none in 1981. All the cases reported were in the central and south-eastern parts of SWA/Namibia, in the districts of Gobabis and Mariental (Aranos area). These districts are mainly in the red Kalahari Sandveld area (Fig. 1).

During the first outbreak in 1954–1957, only cattle, pigs and sheep were reported to be affected. In the second outbreak (1976–1980), goats and horses were also affected, but not pigs. Pigs, however, were then properly housed and did not have access to the plant. The outbreaks occurred mainly in the hot, dry months from August to December, and ceased soon after the onset of good rains and improvement of the pastures. All the species were not equally affected on the different farms, and the species affected appeared to depend on the availability of various palatable plants. For instance, where the vegetation also included edible bush cover, such as *Acacia mellifera* that is eaten by cattle, the cattle were seldom affected, while on the same farms outbreaks occurred in sheep and horses that do not utilize these plants. Lactating and young animals and active rams appeared to be more severely affected than other animals.

Clinical signs

The clinical signs were similar for all the species affected, and the condition was usually characterized by a progressive paresis and paralysis which frequently appeared to be ascending. The animals showed signs of pain, various degrees of hypersensitivity and muscle twitching. They moved with difficulty and tended to knuckle over at the hind or front fetlock joints (Fig. 2). Some animals were aggressive, probably because of weakness. At this early stage, horses improved clinically with exercise, but they tired very easily and respiration became laboured. Hind leg spasticity was present in some cases. In rare cases, spasticity was seen in both hind and forelegs. Knuckling over and overflexion of the hock and carpal joints were more commonly present. Paresis and paralysis usually progressed until the animals could no longer support themselves and preferred sternal recumbency. Nystagmus was noticeable in some.

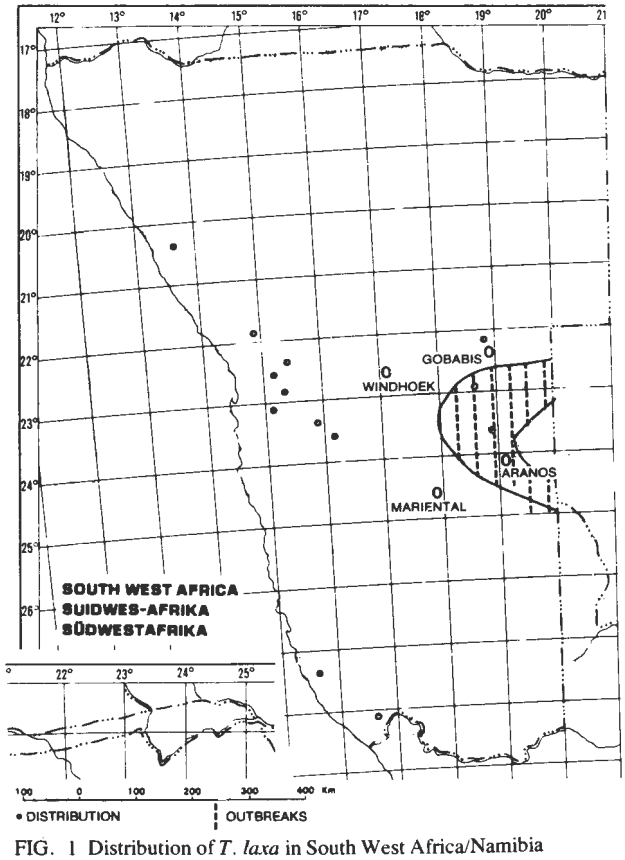

FIG. 1 Distribution of *T. laxa* in South West Africa/Namibia

[1] Central Veterinary Laboratory, Private Bag 13187, Windhoek, South West Africa/Namibia, 9000

[2] State Veterinarian, P.O. Box 81, Grootfontein, South West Africa/Namibia, 9000

[3] State Veterinarian, P.O. Box 27, Gobabis, South West Africa/Namibia, 9000

Received 9 July 1985—Editor

FIG. 2 Knuckling over at carpus and front fetlocks of an affected sheep

FIG. 3 *Trachyandra laxa* var. *laxa*

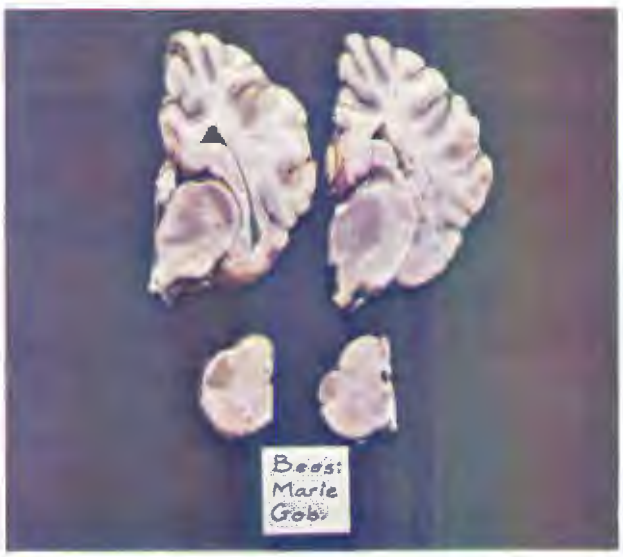

FIG. 4 Bovine: Pigmentation of the grey matter

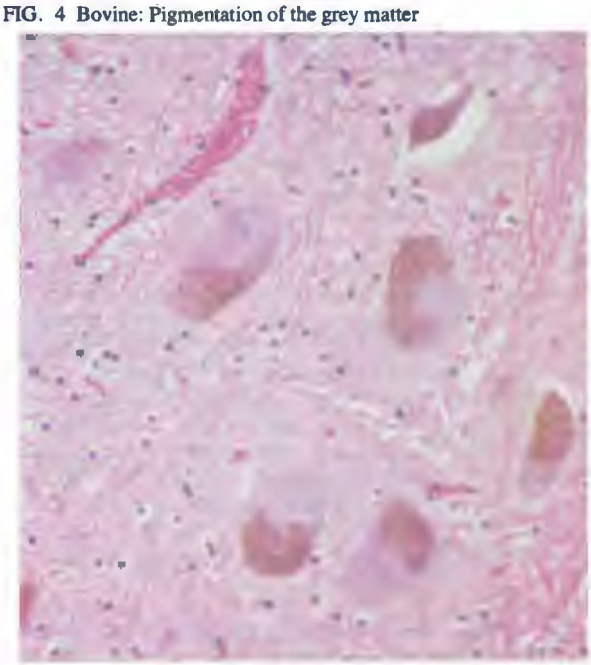

FIG. 5 Sheep: Intracytoplasmic neuronal pigment: HE × 400

Initially, affected animals could still use their front legs to crawl to shade, food or water, but many died from exposure, inanition or lack of water. During the early stages, animals could recover with proper stall feeding or with improvement of the grazing, but recovery sometimes took as long as 6 months. Certain animals recovered partially, but one or other limb remained paretic. Once the forelegs became completely paralysed there appeared to be no hope of recovery. At no stage was any decrease in tail or tongue tonus detectable. Some sheep, however, became constipated. Recumbent sheep often succumbed to secondary *Pasteurella* pneumonia. Affected animals usually died 1–3 months after the onset of clinical signs, the shortest period in a few cases being about 2 weeks.

We report in this paper the findings of a general survey, the pathological features of field cases and the results of dosing trials, which provide evidence that the condition is caused by *T. laxa* var. *laxa*.

DESCRIPTION AND DISTRIBUTION OF *TRACHYANDRA LAXA* (N.E. BR.) OBERM. (LILIACEAE) (Fig. 3)

Description: Plants glabrous, up to 60 cm high. *Roots* many, somewhat fleshy or thin, often whith a lanate pubescence of root hairs. *Rhizome* small, irregular, woody. *Squamae* narrow tubular, membranous, brown, several surrounding each leaf- and scape-base separately but none surrounding a shoot. Leaves many, terete, up to 40 cm long, 30 mm in diameter, erect or drooping, often glutinous from secretions of sub-epidermal glands situated in longitudinal lines. Inflorescence a divaricate much-branched raceme, branches either alternate or the first 3 arising trichotomously from a clavate apex, the following branches alternate, laxly flowered; bracts ovate, *c.* 3 mm long, membranous, white with a brown keel; pedicels up to 6 mm long, erect in bud, pendulous during anthesis, erect in fruit. *Flowers* pendulous with perianth 1 cm in diameter recurved from near the base, forming a "ball" around pedicel, white, dark-keeled, with 2 yellow spots near the base, scentless, producing much nectar; 3 outer stamens scabrid, 3 inner forming a tube, curving outwards above ovary and there yellow and densely retrorsely scabrid; the basal area touching the ovary, smooth with lateral, and dorsal fringes; ovary with *c.* 10 ovules per cell.

Capsule c. 6 mm long, globose, dry. *Seed* dark brown, angled. Common name: tumbleweed, rolbossie, sand-ui.

There are 2 varieties which are distinguished as follows:

Branches of inflorescence alternate, scape not clavate at theapex; plants rather slender..... var. *laxa*

Three lowest branches of inflorescence trichotomous; scape clavate at the apex; sturdy plants ..... var. *ridiga*.

Flowering times: var. *laxa* appears to flower throughout the year; var. *rigida* flowers mostly in the late summer months, January to March.

Distribution: Both varieties occur in South West Africa/Namibia, Botswana, western Transvaal, northern Orange Free State, northern Cape Province and Namaqualand. On Kalahari sands and other sandy soils (Obermeyer, 1962).

MATERIALS AND METHODS

General survey

Brain samples were initially collected for the exclusion of rabies by using the fluorescent antibody technique. Serum samples from affected horses were tested for dourine by a complement-fixation test, and water samples from affected farms were collected and forwarded to the Department of Water Affairs, Windhoek, for analysis. Liver samples of animals showing the typical syndrome were collected in 10 % formalin and analysed for copper, iron, zinc and manganese by atomic absorption spectrophotometry.

A thorough examination of the vegetation was made on several of the affected farms (Langpan 429, Marie 500, Rusgevonden 610, Wolwepan 632, Saffier 638 and Oupembamewa 79). As many farmers suspected that "rust" occurring on the plant *T. laxa* was responsible for the syndrome, complete specimens of these plants were collected, air-dried and submitted to Onderstepoort for fungal isolation. Questionnaires were sent out to all the farmers, and rainfall data were obtained from three rainfall stations in the area.

Pathology of field cases

Necropsies were performed on 9 sheep, 4 cattle and one horse. Brains, spinal cords and specimens from a wide range of tissues were collected and fixed in buffered 10 % formalin. Paraffin sections were prepared and stained with haematoxylin and eosin (HE) according to routine procedures for light microscopy. Selected sections were stained by the periodic acid-Schiff Reaction (PAS) (Pearse, 1961), Perl's Berlin blue method for iron (Pearse, 1961), Schmorl's method for lipofuscin (Pearse, 1961), Lillie's ferrous iron uptake method for melanin (Lillie, 1957), a long Ziehl Neelsen method for acid-fast lipofuscins (Pearse, 1961) and oil-red-O (ORO) (Pearse, 1961).

Feeding trials

The following trials were done with *T. laxa*, the plant suspected of causing the syndrome. Tissue specimens from the majority of animals *(vide infra)* were collected in 10 % formalin, and sections were prepared for light microscopy as for the field cases.

Experiment 1: Three karakul sheep were fed the plant *ad libitum* on the farm Rusgevonden within the endemic area. All 3 animals originated from the farm. The animals were fed for 4 months. Clinical signs were recorded and the sheep were slaughtered at the end of the trial. Specimens in formalin were collected from only the one animal that showed clinical signs.

Experiment 2: One young horse was obtained from the Otjiwarongo district, where the plant does not occur. It was taken to one of the affected farms (Uitkomst) and fed on fresh plants for 6 weeks. The horse died 2 days after the onset of clinical signs. Tissue specimens for light microscopy were collected at necropsy.

Experiment 3: Ten 18-month-old Karakul sheep with no previous exposure to *T. laxa* were introduced from the Neudamm Research Farm onto the farm Uitkomst *(vide supra)*. Five of these sheep were fed twice daily with freshly cut *T. laxa* leaves as well as with the entire plants in order to ensure that all relatively palatable parts of the plant were ingested. The plants were fed *ad libitum* and no other food was given. The other 5 sheep were kept as controls and were fed on locally obtained hay which was supplemented with lucerne when necessary. The trials commenced early in August 1980 when the plants were still young, and continued until January 1981 when the plants became lignified at the end of their growing season. Consequently the intake of the plants varied and decreased towards the end of the period. All the animals were slaughtered after 6 months, and tissue specimens for light microscopy were collected from both groups.

RESULTS

General survey

The vegetation and area survey revealed the following: The soil type was classified as soft red sand (Leistner, 1967). The vegetation consisted mainly of *Acacia erioloba* and *A. mellifera* trees, and ground cover being mainly unpalatable grasses, such as *Aristida meridionalis, Cassia italica, Indigofera* spp., *Trachyandra laxa*, and, at times, in confined areas, variable stands of *Sarcostemma viminale, Crotalaria* spp. and *Geigeria* spp. *T. laxa* was the only plant that flourished and it had been well grazed on all the affected farms, except on Oupembamewa 79, where no *T. laxa* was found. *T. laxa* is a typical geophyte (Leistner, 1967) with soft, palatable, green leaves early in the growing season, which begins in August.

Although *Geigeria* can cause paralysis in ruminants (Du Toit, 1928), these plants did not occur in large numbers, and there was no evidence that they had been heavily grazed. The same was true of *Crotalaria* spp. and *S. viminale*. Plants of the *Indigofera* spp., which have been reported to cause lameness and overgrowth of hooves (Watt & Breyer-Brandwijk, 1962), were found on most farms but did not appear to have been heavily grazed.

The mean mineral concentrations in the livers of affected sheep were all well within the normal ranges for SWA/Namibia (Grant, unpublished data, 1980.)

The following fungi were isolated from *T. laxa: Alternaria alternata, Cladosporium* sp., *Curvularia* sp., *Drechslera rostrata, Fusarium* sp., *Penicillium* sp., *Pithomyces chartarum, P. cynodontis, P. sacchari* and *P. karoo*.

Botulism was excluded as a possible cause, because regular vaccination against the disease did not protect the farm stock from the paralytic syndrome. The tests for rabies and dourine were negative.

The majority of the questionnaires were not returned, but the information obtainable from the rest confirmed that the outbreaks occurred during 2 periods (1954–1957 and 1976–1980) of above-average rainfall. They occurred mainly during hot, dry months from August to December. Cattle and sheep were the animals mainly affected, but cases were also reported in horses. The water analyses indicated that the water was suitable for human and animal consumption.

Pathology of field cases

Macroscopical findings: Necropsies were often completely negative, but one or more of the following changes were sometimes present:

Khaki-brownish pigmentation of the grey matter of the brain and/or spinal cord, especially of the larger nuclear areas (Fig. 4), pulmonary emphysema and oedema, secondary pneumonia, mild hepatic pigmentation and degeneration and very mild renal pigmentation. Overt pigmentation of the latter 2 organs, however, was usually absent. Petechial haemorrhages were seen in the urinary bladder of a few animals.

Light microscopical findings: The most striking and consistent feature observed was undoubtedly the presence of neuronal pigment. Yellowish brown to greyish brown intracytoplasmic granules were present within the neurones of both brain and spinal cord (Fig. 5) as well as in the ganglionic neurones that were studied. The pigment was more conspicuous and abundant in larger neurones, but was absent from the cerebellar Purkinje's cells.

The most frequently and severely pigmented sites included the thalamus (lateral geniculate, external reticular, ventro-caudal and ventro-lateral nuclei), hypothalamus, putamen, amygdaloid, midbrain (anterior and posterior colliculi, substantia nigra and oculomotor nucleus) and the medulla oblongata (especially the olives, solitary, facial, dorsal vagal, hypoglossal and trigeminal nuclei). The roof nuclear area of the cerebellum, the corpus striatum and cerebral cortex were usually either less intensely involved or unpigmented. In the spinal cord, pigmentation was more pronounced within the dorsal horns, particularly at the level of the cervical and lumbar enlargements.

Aggregates of similar pigment were frequently, but not invariably, found in the Kupffer's cells of the liver, within the renal tubular cells of the cortico-medullary zone, in the medullary cells of the lymph nodes and in the spleen. In some cases, macrophages containing pigment were seen in the myocardium and within the walls of cerebral, myocardial and hepatic blood vessels. In these extraneural tissues, however, the pigment was usually indistinguishable from the lipofuscin pigment which so frequently occurs in ageing tissues. The tinctorial features of the pigment are recorded in Table 1.

Other lesions encountered less consistently included mild spongiosity of the cerebral white matter in 4 out of 9 sheep and very mild scattered axonal swelling in one ox. Mild centrilobular fatty changes were present in the livers of 3 sheep. Hypostatic pneumonia was present in one sheep. A sheep and one ox showed prominent degenerative arterial changes in the myocardium. The same ox had mild hepatic portal fibrosis, thrombosis in the spleen as well as suspected degenerative changes of a few cerebral neurones.

TABLE 1 Histochemistry of the neuronal pigment of field and experimental cases

Method	Result	Interpretation
Schmorl's method for lipofuscin	Usually greenish: seldom bluish	+ (mild)
Modified Ziehl Neelsen	Brown	—
PAS	Brownish red	+ (mild to moderate)
Berliner Blue	Brown	—
Lillie's Melanin Stain	Light brown	—
ORO	Brown	—

Feeding trails

Clinical observations: One of the 3 sheep in Experiment 1 lost control of its balance and became paretic after 4 months. It went down in lateral recumbency and was slaughtered shortly afterwards.

The horse in Experiment 2 became paralytic after 6 weeks and died within 2 days. None of the experimental animals in Experiment 3 showed any distinct clinical signs.

Macroscopical findings: Pigmentation of the brain was noticeable in the one sheep that showed clinical signs in Experiment 1, and in 3 in Experiment 3. Mild pigmentation of both liver and kidneys occurred in 2 sheep in the latter experiment.

The horse was cachectic and had congestion and oedema of the lungs and subepicardial petechiae and periaortic haemorrhages.

Light microscopy

Experiment 1: Specimens were collected only from the sheep in which pigmentation of the brain was seen macroscopically. The pigmentation proved to be very prominent in the brain and mild to moderate in the spinal cord. It corresponded morphologically, tinctorially and in distribution to the pigment of natural cases.

Pigmentation and mild fatty changes were seen in the liver. A prominent but varying degree of *status spongiosus* of the white matter was present in various areas of the brain, such as the cerebrum, thalamus, corpus striatum, midbrain, cerebellum and *medulla oblongata*. The cerebrocortical junction between the white and grey matter, including the inner laminar layer, was also affected. Scattered axons were swollen. One small area of haemorrhage and malacia was present in the cerebellar white matter.

Experiment 2: The intraneuronal pigmentation was present, but was mild, and its recognition required examination with the oil immersion objective. Its colour differed from that in the field cases. With HE it consisted of light-reddish to light-yellowish brown granules. These stained positive with both Schmorl's (blue) and PAS (deep red). These 2 special staining methods revealed prominent neuronal pigmentation and mild hepatic pigmentation.

A few swollen axis cylinders were also found in the brain.

Experiment 3: Specimens from all the experimental and control animals were studied.

Sheep in feeding trials: Moderate to severe neuronal pigmentation of both brain and spinal cord was present in all 5 animals, but the pigmentation of the cord was less intense in 2 cases. The pigment corresponded in all respects to that of the field cases. Similar, mild to moderate pigmentation was noticeable in the liver and kidneys of the majority of sheep. The intestinal villi of all the sheep contained many macrophages with abundant pigment granules which resembled the neuronal pigment. Pigmentation of the spleen seemed to be due mainly to haemosiderin. Mild spongiosity of the white matter of the brain was present in 3 sheep. Additional changes were seen in the recumbent, paralytic sheep. These included muscular atrophy and the presence of eosinophilic, intracytoplasmic granules and globules in the adrenal cortex and medulla respectively.

Control sheep: Neuronal pigment was absent in all 5 sheep, but very mild to mild pigmentation was noticeable in the liver, kidney and spleen of 3 animals. Some of the pigment, particularly in the spleen, stained positive by Perl's method, which indicated that it was haemosiderin. Other pigment granules, however, could not

be differentiated with certainty from the pigment in the experimental and natural cases. Much of the pigment was blue and some green with Schmorl's stain, in contrast to more abundant greenish pigment in the experimental cases. In general, with HE, the controls had less hepatic and renal pigment. No pigmentation of the intestinal villi was observed. A very mild *status spongiosus* of the cerebral white matter was present in one sheep. A few animals in both control and experimental groups revealed mild focal disseminated myocarditis.

Discussion

The results of the feeding trials and other available evidence indicate that *T. laxa* was the cause of the syndrome.

Clinical evidence was produced in one horse and one sheep that prolonged intake to *T. laxa* over a period of 3–6 months can result in a paralytic syndrome indistinguishable from that thus far known as "Marie paralysis". The typical intraneuronal pigmentation seen in natural cases was reproduced in these 2 animals as well as in 5 subclinical cases.

The histochemical features of the pigment are compatible with a lipofuscin. Initially, as we saw in the horse in Experiment 2, it is very light reddish brown and stains positive with Schmorl's (blue) and PAS (red). With ageing, as with lipofuscins, it becomes progressively yellowish brown to greyish brown and loses its strong positive reaction with Schmorl's and eventually also with PAS. In the majority of clinical cases, however, it stains greenish with the former method, and brownish red with PAS.

The pigment present in the macrophages within the intestinal villi, lymphoid tissues and Kupffer's cells could not be distinguished from lipofuscins associated with normal ageing, but it is clearly more abundant than in normal animals of the same age. It follows that the feature which distinguished the condition from the normal aging process is not the presence of the pigment, but its abundance.

The cause of the mild to severe *status spongiosus* of the brain in some clinical and experimental cases is not clear. This change may be due to *T. laxa* but it was also present in some of the controls. The plant, *Helichrysum argaerosphaerum*, which causes such lesions (Basson, Kellerman, Albl, Von Maltitz, Miller & Welman, 1975) occurs in the area. *Strongyloides papillosus* is a common parasite that can also produce similar lesions (Pienaar & Basson, unpublished data, 1970). The possibility of other causes, including hepatotoxins, cannot be excluded.

The myocardial vascular lesions and foci of myocarditis did not occur consistently and were also seen in the controls, which suggests that they were not related to *T. laxa* intoxication.

The fact that the disease occurred shortly after 2 wet cycles and mainly on overgrazed farms could be explained by an increase of the plant during these wet cycles.

The fact that lactating animals, young animals and working animals were those mainly affected could be explained by the higher nutrient requirements of these animals which could force them to utilize less palatable species.

The syndrome and pigmentation resemble those described for *Phalaris* staggers in certain respects (Hartley, 1978), but the pigments in these 2 conditions differ histochemically and in their distribution.

We suggest the term, *Trachyandra* paralysis, for this condition.

Acknowledgements

We are grateful to Dr P. Andreae and Dr J. van der V. Basch for their assistance, as well as to Mr M. E. J. Bezuidenhout of the farm Uitkomst, other farmers in the area and various Stock Inspectors for their co-operation. Dr T. S. Kellerman, Section of Toxicology, Veterinary Research Institute, Onderstepoort, is thanked for the fungal survey; Dr S. J. Newsholme, Section of Pathology at Onderstepoort for his valuable assistance in the histopathological studies, the same section at Onderstepoort for the preparation of the histopathological sections and Miss Clare Reid, Botanical Research Institute, Pretoria, for the description of the plant. The assistance of Mr M. A. N. Muller of the Herbarium in Windhoek in preparing the distribution map for SWA/Namibia is gratefully acknowledged.

References

BASSON, P. A., KELLERMAN, T. S., ALBL, P., VON MALTITZ, L. J. F., MILLER, E. S. & WELMAN, WILHELMINA G., 1975. Blindness and encephalopathy caused by *Helichrysum argyrosphaerum* DC. (Compositae) in sheep and cattle. *Onderstepoort Journal of Veterinary Research*, 42, 135–148.

DU TOIT, P. J., 1928. Investigations into the cause of vermeersiekte in sheep. *Report of Veterinary Research, Union of South Africa*, 13/14, 109–153.

HARTLEY, W. F., 1978. Chronic phalaris poisoning or phalaris staggers. *In:* KEELER, R. F., VAN KAMPEN, K. R. & JAMES, L. F. (eds). Effects of poisonous plants on livestock, 391–393. New York, San Francisco & London: Academic Press.

LEISTNER, O. A., 1967. The plant ecology of the southern Kalahari. *Botanical Survey Memoirs*, No. 38, 118. Pretoria: Government Printer.

LILLIE, R. D., 1957. Ferrous iron uptake. *Archives of Pathology*, 64, 100–103.

OBERMEYER, A. A., 1962. A revision of the South African species of *Anthericum*, *Chlorophytum* and *Trachyandra*. *Bothalia*, 7, 669–767.

PEARSE, A. G. E., 1961. *Histochemistry, theoretical and applied*. 2nd ed. London: Churchill.

WATT, J. M. & BREYER-BRANDWIJK, M. G., 1962. The medicinal and poisonous plants of Southern and Eastern Africa. 2nd ed. Edinburgh: E. & S. Livingstone Ltd.