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PATHOLOGY OF A NERVOUS DISORDER (PUSHING DISEASE OR "STOOTSIEKTE") IN CATTLE CAUSED BY THE PLANT *MATRICARIA NIGELLIFOLIA* DC. (ASTERACEAE)

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ABSTRACT

NEWSHOLME, S. J., KELLERMAN, T. S. & WELMAN, WILHELMINA G., 1984. Pathology of a nervous disorder (pushing disease or "stootsiekte") in cattle caused by the plant, *Matricaria nigellifolia* DC. (Asteraceae). *Onderstepoort Journal of Veterinary Research*, 51, 119–127 (1984).

Brains from 10 bovine field cases of pushing disease, a nervous disorder caused by the plant, *Matricaria nigellifolia*, were examined by light microscopy. Moderate to marked encephalitis, characterized by predominantly perivascular microgliosis, perivascular lymphocytic infiltrates and reactive changes in astrocytes, was present in all the brains. The lesion was concentrated in the white matter throughout the forebrain and midbrain.

Dried, milled *M. nigellifolia* was dosed to 6 steers. Clinical signs of pushing disease, which included docility, clumsiness and pushing against objects, appeared abruptly in 5 of the steers after a latent period that varied from 16–44 days. The lowest total dose of plant that proved toxic was 10 g/kg. The length of the latent period appeared to be related inversely to the total dose. Encephalitis, which was similar in nature and distribution to those in the field cases, was demonstrated in the 5 affected steers. The lesion was minimal in the brain of the steer that did not develop pushing disease.

The cerebral lesion is sufficiently consistent and distinctive to be useful in establishing a diagnosis of pushing disease. The perivascular distribution of microgliosis suggests that the site of the toxic insult is the cerebral vasculature. Botanical information is presented.

INTRODUCTION

Pushing disease (PD)(Afrikaans—stootsiekte) is a nervous disorder with distinctive clinical signs affecting cattle in parts of Southern Africa. Andrews (1923) described outbreaks of PD in the midlands of Natal and established that the disease is caused by ingestion of the plant, *Matricaria nigellifolia*. Affected cattle consistently become docile, wander aimlessly and often lean or push forcibly with the head against any fixed object. Severely affected cattle cannot eat or drink, and they become recumbent and die. There is a latent period of several weeks from the time of ingestion of the plant until clinical signs first appear. The toxic principle of the plant is not known. Although the clinical signs are well documented, there exists no detailed report on the pathology of PD.

Outbreaks of PD have recurred sporadically in recent years on certain farms in northern Natal. This report describes pathological findings in some of the cases from these farms and the results of a dosing trial with *M. nigellifolia*.

DESCRIPTION, DISTRIBUTION AND ECOLOGY OF THE PLANT

Family: Asteraceae (Compositae)

Name: Matricaria nigellifolia DC.

Common names: Rivierals, stootsiektebossie, waterkerwel, staggers weed.

Description: (Fig. 1–3) Soft, straggling, creeping or decumbent annual or perennial herb, glabrous or hispidulous, glaucous or grey-green, with many simple or sub-simple prostrate or weakly erect leafy stems from the base, up to 60 cm long, sometimes reddish purple, often rooting at the nodes, forming dense covers. *Leaves* alternate, sessile, up to 6 × 6 cm, ovate in outline, bi- or tripinnate, ultimate segments linear, acute, base half-clasping with several linear or filiform, acute, nerved lobes. *Capitula* solitary on nude penduncles up to 8 cm long in the upper leaf axils, globose, about 10 mm across. *Involucral bracts* elliptic to ovate, obtuse, 2 mm broad, biseriate, more or less equal, margins scarious. *Receptacle* conical, elongating in fruit, epaleate. *Ray florets* short and broad, entire, white. *Disc florets* 4-toothed, yellow. *Achenes* 1,5 mm long, oblong, mostly with 2 pale thickened lateral ribs, large sessile glands on each face, crowned with a callous thickened rim, pappus absent; ray achenes somewhat flattened, disc achenes somewhat 4-angled. *Flowering time* from September to May, but mostly from November to March (Harvey, 1865; Hilliard, 1977; Vahrmeijer, 1981).

Two varieties of *M. nigellifolia* occur in South Africa. The var. *tenuior* DC. differs from the typical variety described above in having heads about 5 mm across and involucral bracts 1 mm broad.

Distribution: (Fig. 4) The plant occurs from the south-eastern Cape, through Natal, to the Transvaal. It has been recorded in the following districts: Transvaal–Soutpansberg, Pilgrim's Rest, Marico, Krugersdorp, Vereeniging and Tshituni (Venda). Natal–Paulpietersburg, Vryheid, Hlabisa, Weenen, Nkandla, Umvoti, Lion's River, Pietermaritzburg, Camperdown, Inanda, Lower Tugela, Durban, Alfred, Port Shepstone. Cape Province, Ciskei and Transkei—Molteno, Queenstown, Glen Grey, Xalanga, Engcobo, Port St. John's, Mqanduli, Bedford, Fort Beaufort, Victoria East, Stutterheim, Komgha, King William's Town, East London, George, Alexandria, Albany. *M. nigellifolia* var. *tenuior* is known only from the eastern Cape and Natal.

Ecology: M. nigellifolia will grow on sand or clay from about sea-level to 1 500 m. It prefers the banks of rivers, streams, lagoons or irrigation furrows and is common in shady places, on damp or wet soil, in marshes or on the edges of dams and vleis. It will also be found in humid grassveld in forest clearings, and can even grow in river beds, stream gullies and in dried-up swamps. In addition, there are records from river flats and the borders of sugar cane fields. *M. nigellifolia* will also grow semi-submerged, sometimes with its stems floating in water up to 50 cm deep, and it can tolerate high mineral pollution in the water. Although it is an indigenous plant, *M. nigellifolia* can become a weed.

MATERIALS AND METHODS

Field Cases

Brains fixed in buffered 10 % formalin from 10 adult cattle were received from farms in the Vryheid and Glencoe districts of Natal. For each animal the clinical history

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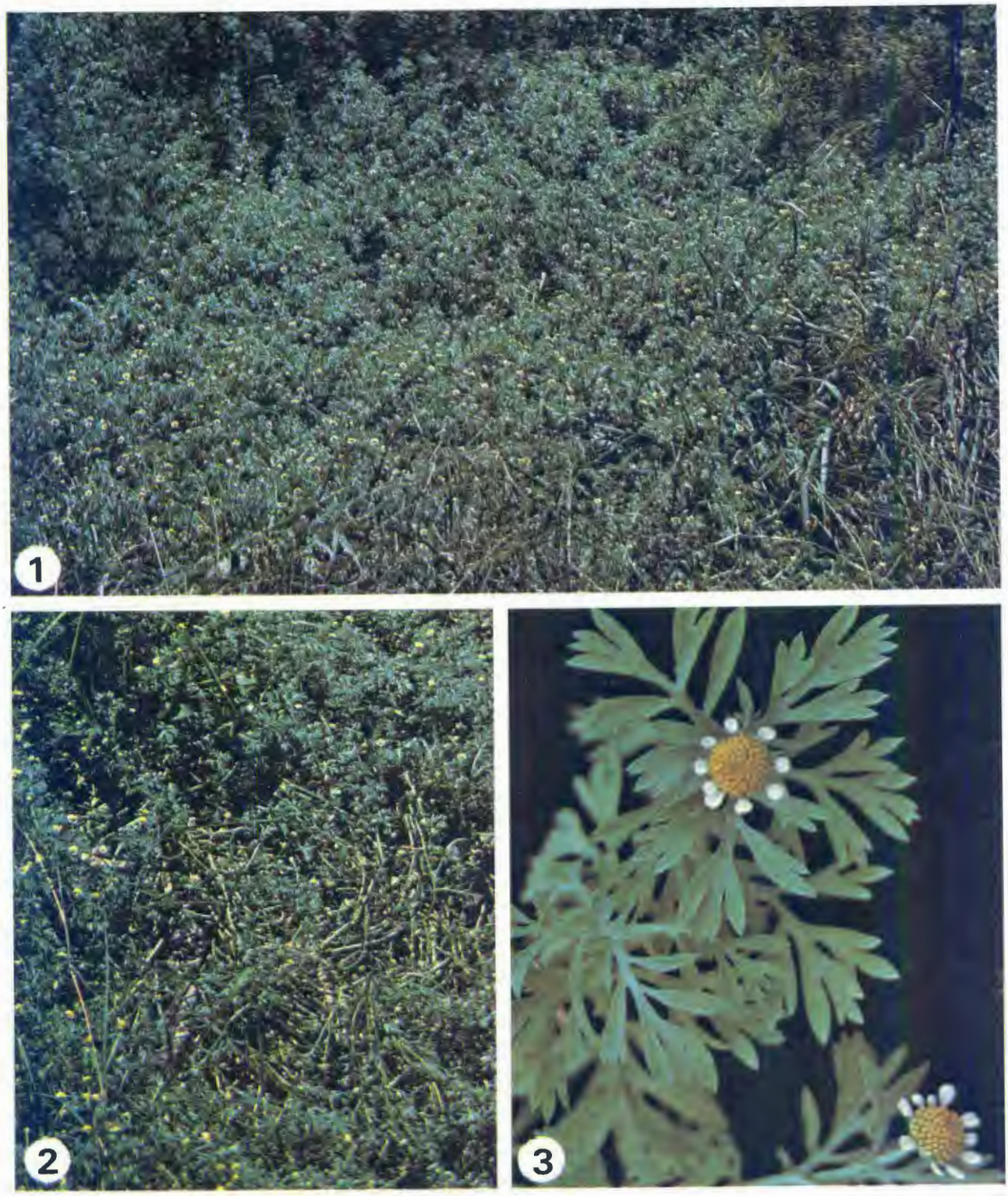


FIG. 1–3 *M. nigellifolia*

was compatible with PD, and there was known access to *M. nigellifolia*. For light microscopy tissue blocks from various parts of the brains were processed routinely and embedded in paraffin wax. Sections were cut at 5–6 μm thickness and stained with haematoxylin and eosin (HE). Selected sections were stained by the periodic acid-Schiff reaction (PAS) with and without previous diastase digestion (Cook, 1977), luxol fast blue (LFB) for myelin (Margolis & Pickett, 1956) and Schmorl's stain (Pearse, 1961). Frozen sections of selected fixed tissue blocks were cut at 15–20 μm thickness, using a Reichert-Jung Cryo-Cut II microtome, were post-fixed in formol ammonium bromide for 2–3 days and were impregnated by a modified Cajal's gold sublimate method for astrocytes (Luna, 1968) and by a silver carbonate method for neuroglia (Penfield, 1928). Sections of brains from cattle unaffected by nervous disease and which had not been exposed to *M. nigellifolia* were prepared and stained by the same methods for comparison, where required.

Experimental Cases

Plant: M. nigellifolia plants were collected in December 1982 from a farm in the Glencoe district of Natal

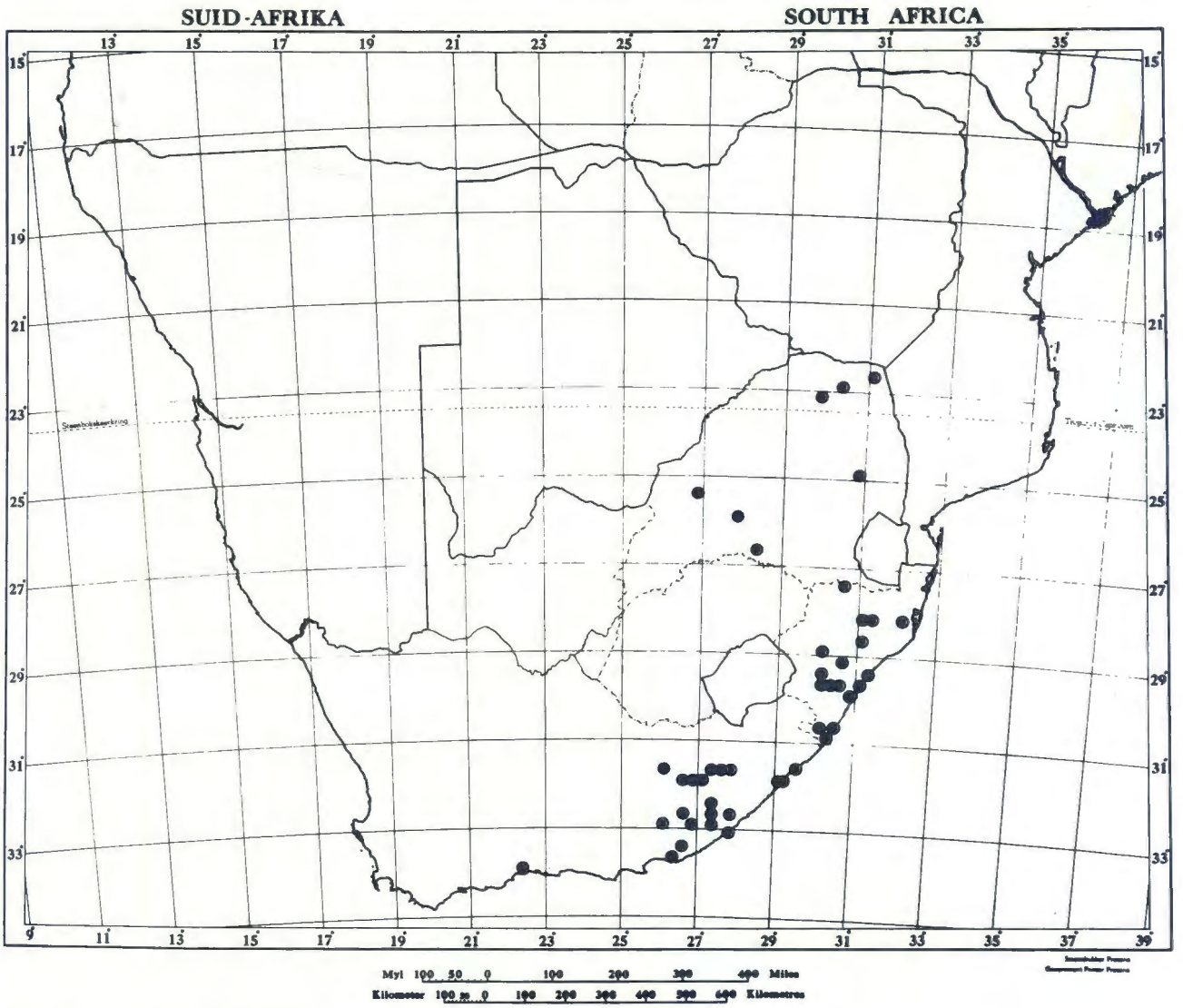


FIG. 4 The distribution of *M. nigellifolia*

upon which PD had occurred previously. The plants were transported to the Veterinary Research Institute, Onderstepoort, where they were stored at *c.* −5°C until required.

Animals and dosing procedure: After storage the plants were dried, milled and dosed by stomach tube to six 1-year-old Afrikander-type steers which had not been previously exposed to the plant (Table 1). Steers 1 and 2 regurgitated part of the dose on the 3rd day of dosing, and their rumens were subsequently enfistulated to provide a more satisfactory route of administration. Both of them, however, developed clinical signs of PD before more plant was given.

Clinical pathology: Venous blood was drawn from the steers both before dosing, during the latent period and after the onset of clinical signs. The following were measured by routine methods: packed cell volume, haemoglobin concentration, serum gamma glutamyl transpeptidase, serum aspartate aminotransferase and serum sodium, potassium, calcium and magnesium concentrations. Acid-base balance was evaluated from blood drawn from Steers 3 and 4 after the onset of clinical signs, according to the methods of Astrup, Jorgensen, Siggaard Andersen & Engel (1960).

Gross pathology: The steers were killed by intravenous injection of pentabarbitone sodium, and necropsies were done immediately.

Light microscopy: The brains, spinal cords, eyes and tissue specimens from livers, kidneys and brachial and sciatic nerves were collected and fixed in buffered 10 % formalin. Specimens were embedded and sections were prepared as for the field cases.

RESULTS

Light microscopy of field cases

Moderate to marked encephalitis, which was characterized by extensive, predominantly perivascular gliosis (Fig. 5 and 6) and by perivascular inflammatory cell infiltrates (Fig. 7), was present in all the brains. The lesion was concentrated in the white matter throughout the forebrain and midbrain, and extended into the cerebellar white matter in 2 of the brains. The grey matter was generally not affected, but mild perivascular gliosis occasionally extended from the affected white matter into closely adjacent zones of grey matter.

The perivascular glial cells were frequently arranged in a palisade pattern with their long axes lying perpendicular to the vessel walls (Fig. 6). They possessed an oval to rod-shaped nucleus with coarsely granular chromatin and lightly eosinophilic cytoplasm. Cell borders were indistinct with HE staining, but silver impregnation demonstrated occasional fine cytoplasmic processes (Fig. 11). These cells were interpreted to be microglia.

TABLE 1 The toxicity of dried, milled *M. nigellifolia* to steers

Steer		Dose (g/kg × n)	Dosing regimen			Latent period (days)	Duration of clinical signs	Duration of experiment (days)	Clinical signs
No.	Initial mass (kg)		Days on which dosed	Total dose (kg)	Total dose (g/kg)				
1	245	5 × 3 2,5 × 1*	0–3	4,20*	17,5*	21	8 h	21	Docility, clumsiness, pushing against objects, recumbency
2	238	5 × 3 2,5 × 1*	0–3	4,17*	17,5*	21	2 days	23	Docility, clumsiness, pushing against objects, recumbency
3	192	5 × 12	0–16	11,52	60	16	2 days	18	Docility, clumsiness, pushing against objects, recumbency
4	202	5 × 4	0–3	4,04	20	27	2 days	29	Docility, clumsiness, pushing against objects, recumbency
5	220	5 × 2	0–1	2,20	10	44	50 days	94	Inco-ordination
6	252	5 × 1	0	1,26	5	—	—	94	No clinical signs

g/kg × n = Dose × number of daily administrations
* : Values estimated since part of dose was regurgitated

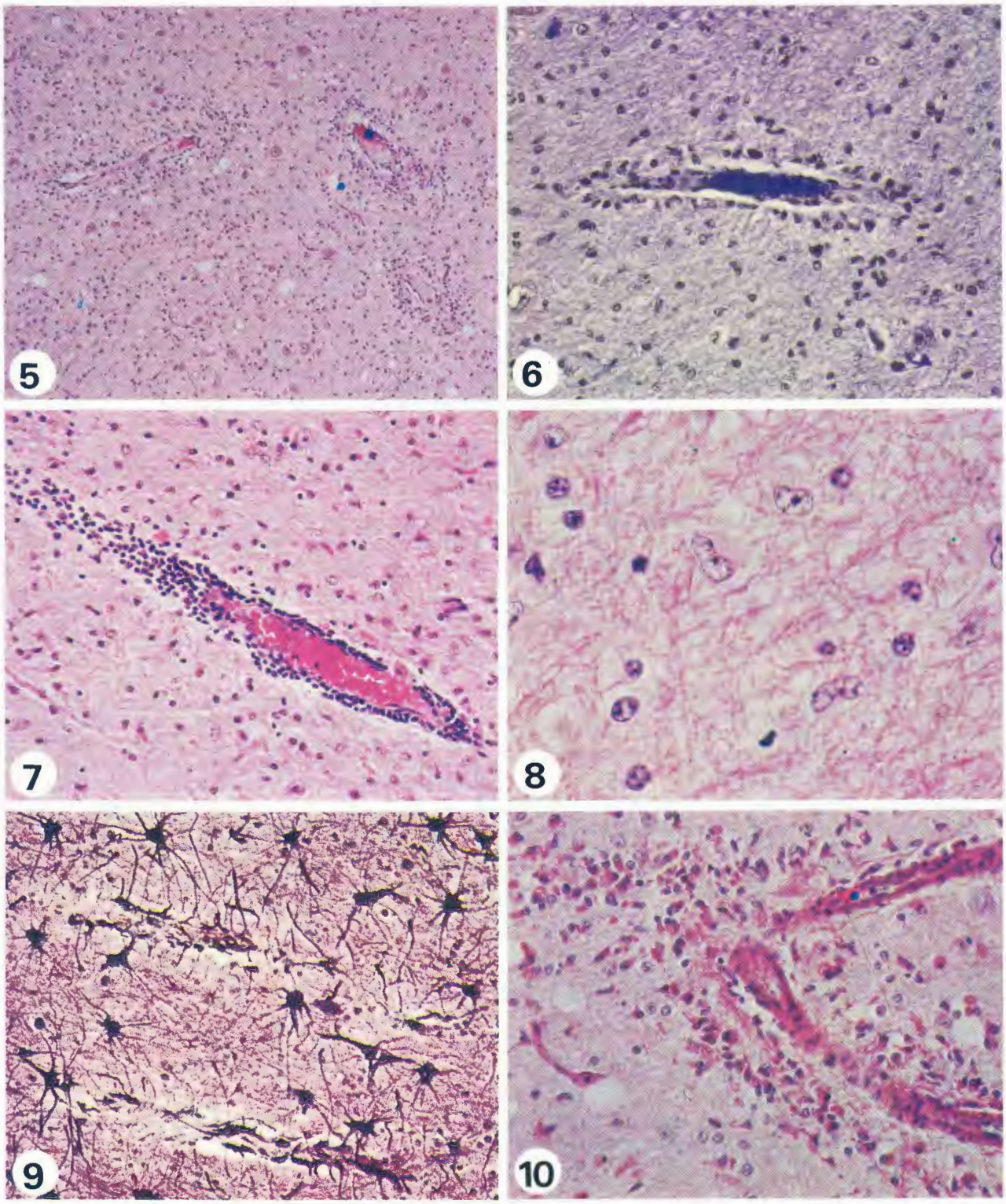


FIG. 5 Gliosis predominantly around blood vessels and mild *status spongiosus* in midbrain: HE × 100

FIG. 6 Perivascular gliosis with palisade pattern in white matter: HE × 250

FIG. 7 Perivascular lymphocytic infiltrate in white matter: HE × 200

FIG. 8 Astrocytes with abundant cytoplasm and large, eccentric, reniform nuclei in white matter: HE × 1 000

FIG. 9 Astrocytes with enlarged cytoplasm and thick processes in white matter: Gold sublimate method × 200

FIG. 10 Perivascular gliosis and abundant PAS-positive material within glial cytoplasm in midbrain: PAS × 400

The perivascular inflammatory cell infiltrates were restricted to the larger vessels, and consisted of lymphocytes (Fig. 7) and occasional plasma cells. Small groups of cells with a round nucleus and abundant cytoplasm filled with golden-brown pigment granules were seen around vessels in 4 of the brains. The pigment was PAS-positive, diastase-resistant and deep blue with Schmorl's stain. A few juxtavascular mitotic figures were seen in 3 brains, but the cell-type involved was not identified.

Changes were evident in many of the astrocytes throughout the white matter in the areas affected by encephalitis. Astrocyte nuclei were frequently eccentric

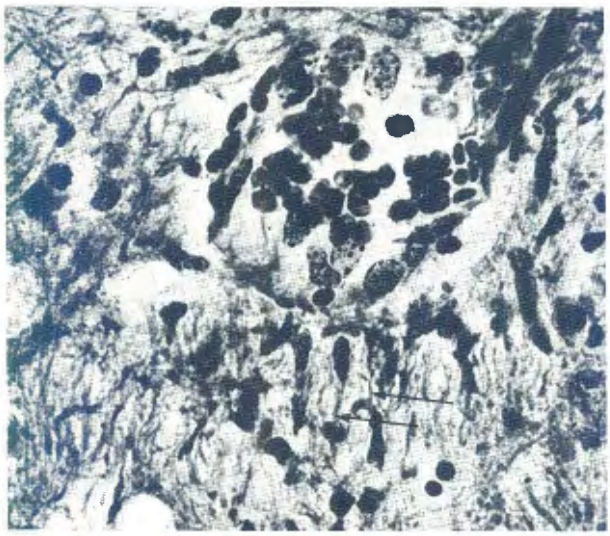


FIG. 11 Perivascular glial cells with oval to rod-shaped nuclei and fine cytoplasmic processes (arrowed): silver carbonate method × 1 000

and reniform or lobulated (Fig. 8) and were enlarged, measuring up to 14 μm through their greatest dimension (astrocyte nuclei in brains from unaffected cattle rarely measured more than 9 μm). The cytoplasm of these cells was abundant and lightly eosinophilic. In sections impregnated by Cajal's method astrocytes appeared to have more extensive cytoplasm and their processes seemed thickened (Fig. 9) compared with those in brains from unaffected cattle.

Mild *status spongiosus,* which was characterized by multifocal vacuolation of the white matter in the areas of gliosis (Fig. 5), was present in 5 of the brains. The structures affected by this vacuolation were not identified. Luxol fast blue staining failed to demonstrate loss of myelin.

Periodic acid-Schiff-positive, diastase-resistant cytoplasmic granules, which measured up to 3 μm diameter, were abundant in the perivascular microglia (Fig. 10), and a few similar granules were seen within astrocytes. Globules with similar histochemical characteristics but which measured up to 15 μm were present in the cytoplasm of pericytes throughout grey and white matter in all the brains. Such globules occurred with similar frequency and distribution in brains from unaffected cattle.

Experimental Cases

Clinical signs: (Table 1) The clinical signs appeared abruptly after a latent period between the administration of the 1st dose and the appearance of signs that varied from 16–44 days. The latent periods and total doses of plant are depicted in Fig. 12. The clinical condition of

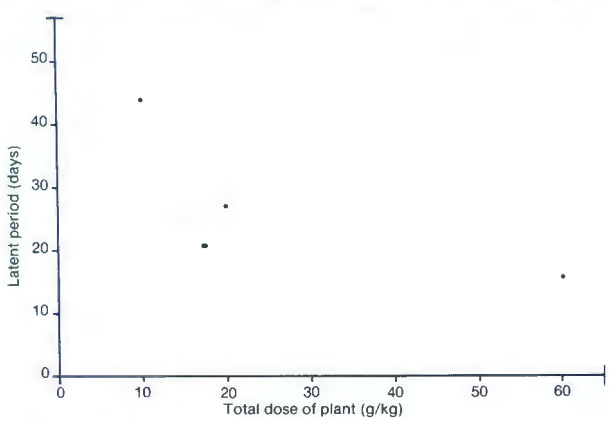


FIG. 12 Latent periods and total doses of *M. nigellifolia*

Steers 1–4 deteriorated rapidly over a period of 8–48 h. They became recumbent and were killed *in extremis*. Steer 5 showed pronounced clinical signs between Days 44–60, followed by progressive improvement. It was killed after recovery on Day 94 together with Steer 6, which had shown no signs.

The most striking and consistent signs were docility, apathy, clumsiness and pushing against objects. Docility was evident by the ease with which affected steers, which were unaccustomed to being handled, could be approached and led without resistance (Fig. 13–15). Responses to stimuli such as noise and sudden movement were lessened. Some of the steers, despite having a normal gait, tended to brush or bump clumsily against objects. Episodes of pushing with the head against fixed objects increased in frequency and duration during the course of the disease. Pushing varied from gentle (Fig. 16) to strenuous.

In Steer 5 signs of inco-ordination developed which included scuffing of the feet, knocking-on, brushing, occasional knuckling over at the phalangeal joints and apparent difficulty in walking backwards.

Clinical pathology: Values obtained from all the samples were within the normal ranges for this laboratory.

Gross pathology: Subcutaneous contusions were present over the head, neck and shoulders in Steers 1–3. The gyri of the cerebral cortex were swollen in Steer 4 (Fig. 17).

Light microscopy: The brains of Steers 1–5 showed gliosis, perivascular inflammatory cell infiltrates and changes in astrocytes which were similar in nature and distribution to those in the field cases. These changes extended to involve the cerebellar white matter in Steers 3 and 5. The degree of the changes was milder than in most of the field cases and was more uniform from case to case. In Steers 1 and 3 the perivascular inflammatory infiltrates were more mixed (Fig. 18), and included a few neutrophils. Several juxtavascular mitotic figures were seen in the cerebral cortex in Steers 2 and 3 (Fig. 19), but the cell-type involved was not identified. Mild *status spongiosus* was present in Steers 1–3. Changes seen in the brain of Steer 6 were limited to mild segmental perivascular lymphocytic infiltrates in the midbrain.

No changes were seen in the spinal cords, peripheral nerves or eyes. Mild to moderate centrizonal hepatocellular fatty change was present in Steers 1–5.

DISCUSSION

The clinical signs and latent periods in the experimental cases were compatible with those described for PD (Andrews, 1923).

The available evidence indicates that encephalitis is a consistent and reproducible feature of PD, since it occurred with similar distribution in all the field cases and in the experimental cases, but was minimal in the steer that did not develop PD: Perivascular gliosis in brains of cattle with PD has been noted elsewhere (Pienaar, 1977), but this is the first detailed description of the lesion in this disease.

Gliosis and perivascular lymphocytic infiltrates are features usually associated with infectious or allergic encephalitides. The association of these features with a plant toxicosis is unique to PD, as far as we are aware. Glial proliferation and inflammatory cell infiltrates have

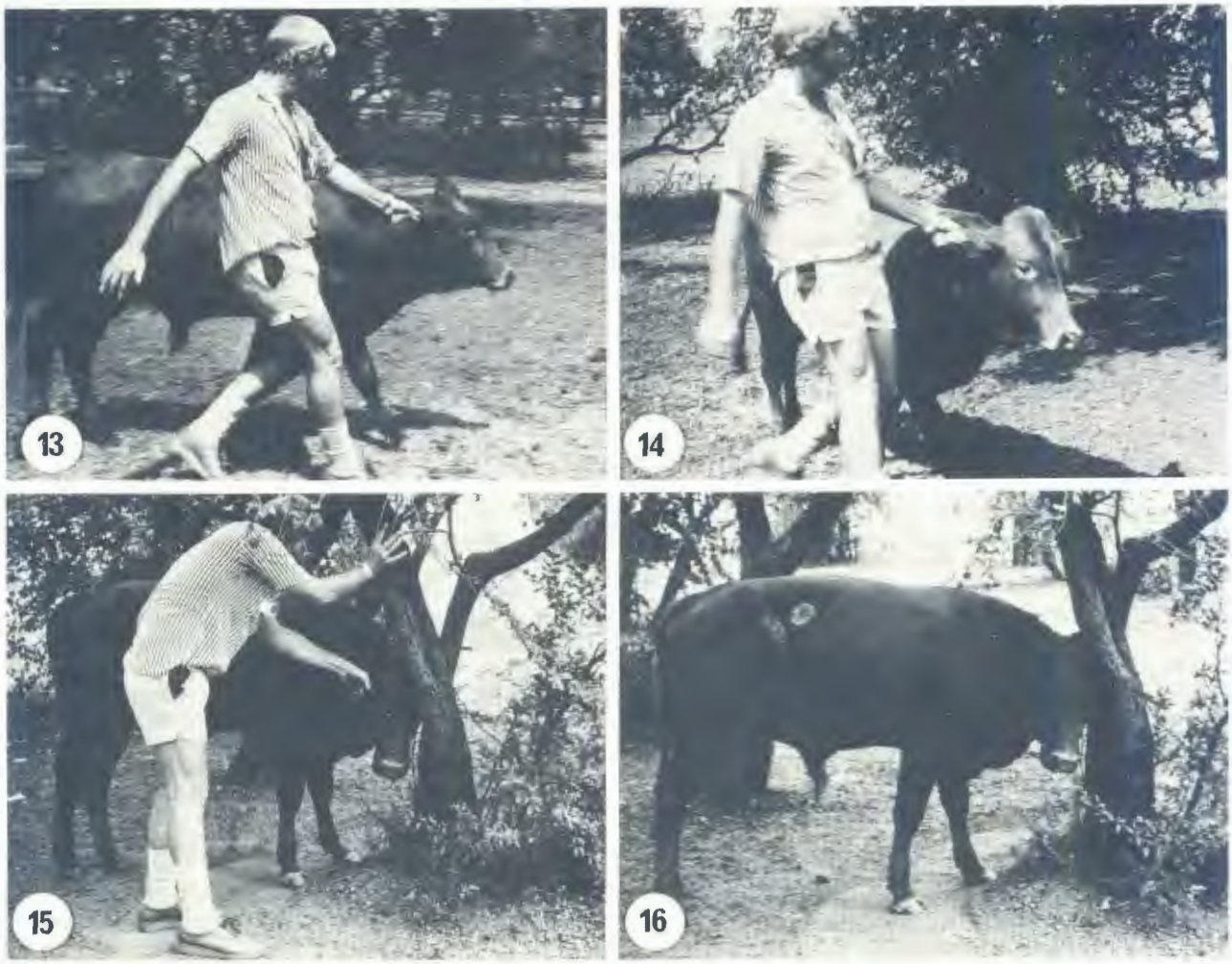


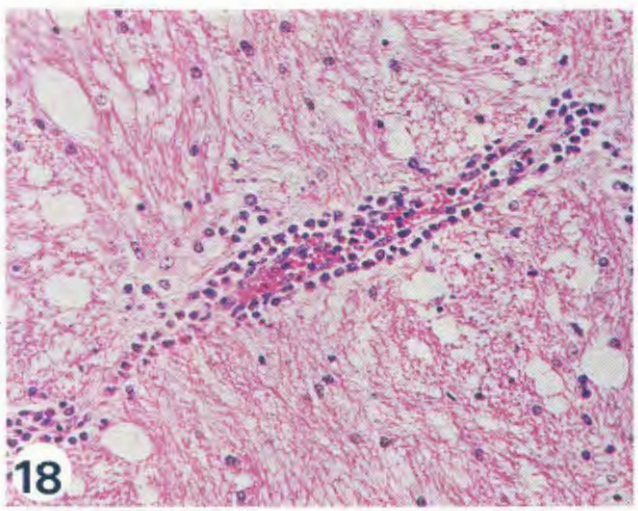
FIG. 13–16 Sequence to show docile, intoxicated steer being led to a tree against which it pushes

FIG. 17 Swollen cerebrocortical gyri in brain from Steer 4 (upper), compared with age-matched, unaffected brain.

been described in other plant or fungal toxicoses, such as equine nigropallidal encephalomalacia (Cordy, 1954) and equine leukoencephalomalacia (Marasas, Kellerman, Pienaar & Naudé, 1976), but, unlike PD, the cell infiltrates in these conditions are clearly associated with malacia. The lesion of PD might, indeed, be confused with viral encephalitis, but we believe that the predominantly perivascular pattern of gliosis and the concentration of the lesion in the white matter are sufficiently distinctive to substantiate a diagnosis of PD by light microscopy.

The predominantly perivascular distribution of gliosis with frequent palisade patterns suggests that the primary site of the toxic insult is the cerebral vasculature. Our interpretation of the perivascular glial cells as microglia is based on light microscopical evidence. More conclusive identification, however, would require electron microscopy, since the limitations of light microscopy alone in identifying microglia are well recognized (Greenfield & Meyer, 1963; Persson, 1976; Kitamura, Tsuchihashi & Fujita, 1978). Microglial proliferation is seen in many forms of encephalitis (Greenfield & Meyer, 1963). The proliferating cell-type represented by the juxtavascular mitotic figures could not be identified, but it is likely that these cells were either of microglial or of recent vascular origin.

Periodic acid-Schiff-positive, diastase-resistant material which was abundant in the cytoplasm of the microglia suggests that an episode of oedema may have occurred. Blakemore (1969), for example has found similar material within microglia and other cells in oedematous rat brains, and has presented evidence that the material represents plasma proteins which have leaked



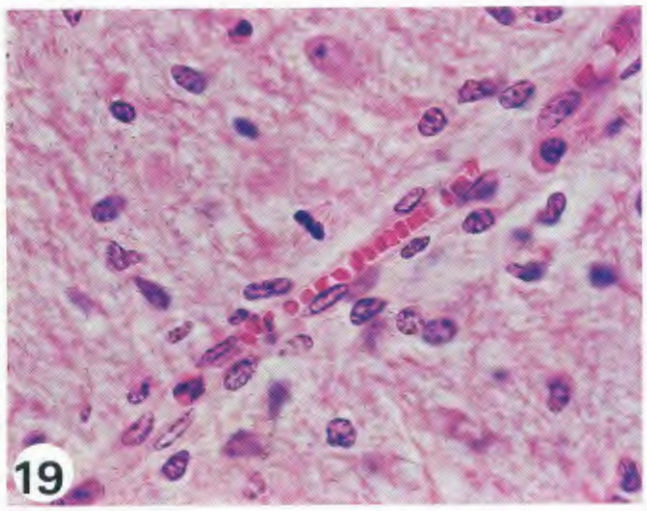


FIG. 18 Mixed perivascular, inflammatory cell infiltrate in white matter (Steer 1): HE × 200
FIG. 19 Juxtavascular cell in metaphase of mitosis (Steer 2): HE × 600

from vessels and have been taken up from the extracellular space. The larger, PAS-positive globules in the cytoplasm of pericytes, however, cannot be regarded as part of the lesion, since they were seen with similar frequency in the brains of cattle unaffected by PD. Jolly (1971) has reported the frequent presence of similar globules within pericytes of bovine brains. The staining properties of the golden-brown pigment which occurred within perivascular cells in some of the field cases were compatible with lipofuscin. The origin of the pigment was not clear.

The nuclear and cytoplasmic enlargement of astrocytes in the white matter was consistent with hypertrophy of these cells. Regressive astrocytic changes such as cytoplasmic vacuolation and nuclear pyknosis, were not seen, and in gold-impregnated sections there was no evidence of break-up of astrocytic processes (clasmatodendrosis). The astrocytes resembled the swollen-bodied or plump astrocytes (gemistocytes) which reportedly occur in moderate ischaemia, subacute encephalitis and oedema of white matter (Greenfield & Meyer, 1963). The origin of the PAS-positive, diastase-resistant material in the cytoplasm of some of the astrocytes is not clear. It may represent leaked plasma protein which has been taken up by these cells.

The selective involvement of white matter cannot readily be explained. There was no evidence of demyelination. Such a distribution, however, is consistent with that of oedema. Vasogenic cerebral oedema usually selects white matter (Klatzo, 1967; Manz, 1974). Hypoxia has also been reported to injure white matter selectively in some circumstances, but this apparently occurs when there is simultaneous oedema (Feigin, Budzilovich, Weinberg & Ogata, 1973).

The severity of the lesion varied among the field cases, but was more uniform among the experimental cases. No variations in the character or severity of the lesion were seen that could be related to differences in the dose of plant, the latent period or the chronicity of the disease. The stage at which the lesion develops is not known, but it probably begins before the onset of clinical signs, since gliosis and perivascular lymphocytes were present in Steer 1, which had a clinical course of only 8 h. Microglia take more than 30 h after injury before they begin to show evidence of proliferative activity, as was shown in a study of stab wounds in rabbit brains (Kitamura *et al.*, 1978), and perivascular lymphocytic infiltration is usually associated with lesions at only a subacute or chronic stage.

Andrews (1923) reported hepatic degeneration in cases of PD and implied that it might contribute to the pathogenesis of the disease. Hepatic alterations seen in our cases were limited to mild to moderate hepatocellular fatty change which we believe was not related to the pathogenesis of PD. Although clinical pathological parameters to assess hepatocerebral disease were not measured, there was no morphological evidence to suspect it. Alzheimer type II astrocytes, a characteristic feature of hepatic encephalopathy in humans (Adams & Foley, 1952), were not seen. Astrocytes in PD showed reactive changes, but the affected cells were confined to white matter and possessed abundant cytoplasm with thick processes. Such features are clearly distinct from those of Alzheimer type II astrocytes, as has been stressed by Norenberg & Lapham (1974). *Status spongiosus* of white matter in cattle has been associated with advanced hepatic lesions caused by a variety of agents (Hooper, 1972), but *status spongiousus* was mild and inconstant in our material.

The results of Andrews (1923) suggest that the length of the latent period is inversely related to the dose of plant, and our limited results support this suggestion. The shortest latent period that we recorded (16 days) was substantially less than that recorded by Andrews (1923), which was 23 days. The clinical severity appeared to be related to the total dose. Steer 5, which received a lower total dose than Steers 1–4, showed milder clinical signs.

That the lowest total dose of *M. nigellifolia* that proved toxic was small (10 g/kg) indicates that field outbreaks of PD could occur without an abundance of the plant. Although the clinical signs of PD are distinctive, light microscopical examination of the brain may prove useful in substantiating a diagnosis, particularly when access to *M. nigellifolia* is not suspected, either where the plant is scarce or if an animal has been moved during the latent period.

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