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PARALYSIS IN LAMBS CAUSED BY OVERDOSING WITH PARBENDAZOLE

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ABSTRACT

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An experiment was undertaken to determine whether an overdose of the anthelmintic parbendazole could cause paralysis in lambs when given to ewes during the early stages of pregnancy. Out of a total of 68 lambs, born from ewes treated at various stages of gestation with parbendazole at 180 mg/kg, 5 showed the paralysis syndrome, 5 showed skeletal deformities, 2 were ataxic, while 1 foetus was aborted. The ewes giving birth to paralysed lambs had been treated with parbendazole at 30, 32, 37 and 53 days of gestation. Cerebral hypoplasia was observed in 2 of these lambs, while 2 others showed internal hydrocephaly. Histopathological lesions observed in the lambs with cerebral hypoplasia included gliosis and areas of encephalomalacia in the cerebral white matter. Lesions present in the spinal cords of the 2 ataxic lambs included hydromyelia, syringomyelia, duplication of the spinal canal and an abnormal position of the canal. This is the first report describing brain lesions in lambs as a result of an overdose of parbendazole.

Résumé

PARALYSIE CHEZ L'AGNEAU CAUSÉE PAR UN SURDOSAGE DE PARBENDAZOLE

Une expérience a été entreprise pour déterminer si un surdosage de l'anthelmintique parbendazole, pouvait causer la paralysie chez les agneaux quand il était administré à des brebis aux stades précoces de leur gestation. D'un total de 68 agneaux, nés de brebis traitées à des stades variés de leur gestation, avec du parbendazole à 180 mg/kg, 5 montrèrent le syndrome de paralysie, 5 montrèrent des malformations squelettales, 2 furent ataxiques, tandis qu'un foetus avorta. Les brebis donnant naissance à des agneaux paralysés avaient été traitées avec du parbendazole à 30, 32, 37 et 53 jours de gestation. Une hypoplasie cérébrale fut observée chez deux de ces agneaux, tandis que deux autres montrèrent une hydrocéphalie interne. Des lésions histopathologiques observées chez les agneaux atteints d'une hypoplasie cérébrale comprirent une réaction gliale et des places d'encéphalomalacie dans la substance blanche des hémisphères cerebraux. Les lésions présentes dans la moelles épinière des deux agneaux ataxiques comprirent l'hydromyelie, la syringomyelie, le doublement du canal épinier et une position anormale du canal.

Ceci est le premier rapport décrivant les lésions du cerveau chez les agneaux à la suite d'un surdosage de parbendazole.

INTRODUCTION

Trials conducted with the anthelmintic parbendazole* in various countries have proved that under certain conditions teratogenic effects could be evoked in new-born lambs whose dams had been dosed during pregnancy (Saunders, Shone, Philip & Birkhead, 1974; Szabo, Miller & Scott, 1974; Shone, Philip & Fricker, 1974). Anomalies reported by these authors were primarily those of bones and joints. Skeletal lesions observed affected mainly the pelvis, long bones, joints and digits.

Various studies have proved that parbendazole exerts its teratogenic effect when given to pregnant ewes as a single dose of 60 mg/kg. The period of greatest embryonic susceptibility with respect to the skeletal system is between 12 and 24 days of pregnancy (Szabo *et al.*, 1974; Middleton, Plant, Walker, Dixon & Johns, 1974; Shone *et al.*, 1974).

Rubino (1976) reported congenital malformations in lambs related to the administration of parbendazole at approximately 40 mg/kg. Of the 81 lambs born from 140 ewes, 50 died, and of these 47 showed skeletal malformations. Hydrocephalus, characterized by excessive convexity of the cranium was observed in one of the lambs. In the same animal a duplication of the central canal of the spinal cord was reported.

The purpose of the present investigation was to report the macro- and microscopic lesions in the central nervous system (CNS) of lambs, the ewes of which had been dosed with parbendazole during the early stages of gestation.

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HISTORY

Since 1968 intermittent complaints of paralysis in lambs have been received from various parts of South Africa. An investigation of this phenomenon revealed that on some farms, in addition to paralysed lambs, lambs were born with various skeletal malformations. Some of the farmers admitted that they had been aware of the warning on the label, "Do not dose ewes during mating and for one month thereafter, as dosing during this period may lead to malformed lambs". Despite this warning the sequence was encountered normal lambs, paralysed lambs, teratogenic lambs and again normal lambs in at least 3 instances.

The paralysed lambs were unable to stand, although no skeletal malformations were present. As they were unable to suckle, affected lambs usually died of starvation; bottle-fed, however, they survived, but did not recover.

A careful study of the management on these farms revealed that parbendazole was used routinely at a normal dose of *c.*30 mg/kg during various stages of gestation. Although exact breeding data were not always available, it appeared that ewes giving birth to paralysed lambs had been treated with parbendazole between approximately 23 and 62 days of gestation (Prozesky & Joubert, 1979).

Because large numbers of sheep could not be used and the incidence of paralysed lambs encountered in the field was low, this experiment was undertaken to determine whether an overdose rather than the prescribed dose of parbendazole could cause paralysis in lambs.

MATERIALS AND METHODS

Ewes

A group of 250 two-tooth Merino ewes, 50 of which were used as controls, were treated at various stages of gestation with parbendazole at 180 mg/kg. Oestrus was synchronized with Estrumate* (chloprostenol) and pregnant mare serum (PMS), followed up by hand servicing. The animals were kept in a kraal under close observation and the mass of each animal was determined before treatment. Accurate records were maintained so that the ewe of each lamb born could be identified.

Lambs

Lambs were clinically examined on the day of birth. Those with the anomalies were mass-measured, photographed and X-rayed, while an electroencephalogram (EEG) was recorded on 5 animals. A complete autopsy was carried out on all affected lambs.

1. *EEG recordings*

EEG readings were recorded on 5 lambs. Two of the lambs were paralysed, 1 was ataxic, and 2 served as controls. After anaesthesia with pentobarbitone sodium** (Sagatal V), the head of each of the animals was shaved and subcutaneous scalp electrodes were inserted after electrode jelly had first been applied to the occipital and frontal regions. The electrode board was connected to a 8-channel recorder. Recordings were taken from both the frontal and occipital regions on each side (double frontal and occipital leads). All the recordings were taken at 200 microvolt, 30 Hertz, 0,3 seconds and 30 mm/second.

2. *Autopsies*

A total of 18 lambs, 6 of which served as controls, were autopsied. All the brains were mass-measured separately. The brains were fixed *in toto* in 10% formalin before coronal sectioning and the selection of blocks for histopathological examination. Specimens from various organs were collected in 10% buffered formalin. Tissue blocks were embedded in paraffin wax, sectioned 3–5 μm in thickness and stained with haemotoxylin and eosin (HE). The special staining techniques employed were Holzer's (Anon, 1960), Luxol fast blue- Holmes's (Margolis & Pickett, 1956) and Van Gieson's (Lillie, 1954). An ocular micrometer was used for counting different cell types in brain sections.

3. *MOP Kontron Messgeräte recordings*

A MOP 1 AMO3 Kontron Messgeräte was used to measure the form, area, perimeter and maximum diameter of the brains of 3 paralysed lambs, while 5 brains served as controls. Measurements were recorded by placing photographs of the brains, all of the same magnification, on a transparent evaluation tablet with a projection screen-plate. A cursor was used to make the measurements.

Serological tests

Approximately 1 month after the last lamb was born, a blood sample was collected in a vacuum tube without coagulant from each ewe which had given birth to a paralysed lamb, as well as from 17 control ewes. Sera collected were tested for the presence of antibodies against Rift Valley fever (RVF), Wesselsbron disease (WBD), bluetongue (BT), Akabana disease (AD) and mucosal disease viral-diarrhoea (MD-VD). The methods employed were as follows:

Rift Valley fever and Wesselsbron disease

A haemagglutination inhibition test was performed, as described by Clark & Casals (1958).

Bluetongue and Akabana disease

A complement fixation test was performed (McIntosh, 1956).

Mucosal disease viral diarrhoea

1. *Fluorescent-antibody technique*

Sheep sera were tested by the indirect fluorescent-antibody technique to demonstrate the presence of neutralizing antibodies (Cunningham, 1973). The test sera were allowed to absorb on tissue culture cells infected with MD-VD virus. A commercially available rabbit anti-sheep IgG conjugate* was applied.

2. *Serum-virus neutralization test*

Fivefold dilutions of sheep sera were mixed with an equal volume of virus suspension containing 100 $TCID_{50}$/0,1 mℓ. Secondary foetal calf lung cells were inoculated with the above-mentioned mixtures. Inhibition of virus growth at 1:5 dilutions and at higher serum dilutions was regarded as positive.

RESULTS

Ewes

A total of 68 lambs were born from the treated ewes. Five of the lambs were paralysed, although no musculoskeletal anomalies were observed. Two lambs were ataxic, 5 showed skeletal deformities and one foetus was aborted. No abnormalities were observed in 18 lambs born from ewes in the control group.

Serological results. The serological results of ewes giving birth to lambs with congenital anomalies are summarized in Table 1.

TABLE 1 Post-lambing serological titres of ewes giving birth to paralysed lambs, against Rift Valley fever (RVF), Wesselsbron disease (WBD), mucosal disease (MD), bluetongue (BT) and Akabana disease (AD)

Ewe No.	RVF	WBD	MD	BT	AD
1.....	—	1/40	—	1/4	1/4
2.....	—	—	—	1/4	1/4
3.....	—	—	—	1/4	1/4
4.....	—	—	—	1/16	1/4
5.....	—	—	—	1/4	1/4

Lambs

Clinical examination

1. *Paralysed lambs*

The paralysed lambs were unable to stand, although no skeletal malformations were present (Fig. 1). The animals were able to suckle if bottle-fed, could urinate and defaecate normally. A neurological examination revealed normal pupil and pain reflexes. Two of the lambs lay on their sides and showed galloping movements, while the other 3 made jerky horizontal movements with their heads. All 5 animals bleated

* "Chloprostenol" ICI
** "Sagatal" Maybaker

* Miles Laboratories, Cape Town

continuously. The ewes giving birth to paralysed lambs had been treated with parbendazole on Days 30, 32, 32, 37 and 53 of gestation (Table 2).

TABLE 2 Clinical and/or pathological anomalies in lambs born from ewes treated with parbendazole

Lamb No.	Stage of pregnancy when ewe was treated with parbendazole (days)	Clinical and/or pathological anomalies
1.....	17	Ataxia with syringomyelia
2.....	20	Ataxia with syrongomyelia
3.....	30	Paralysis
4.....	32	Paralysis
5.....	32	Paralysis and hydrocephalus
6.....	37	Paralysis and hydrocephalus
7.....	53	Paralysis

2. *Ataxic lambs*

Hopping movements were produced during locomotion when both limbs of the hindquarters of the 2 ataxic lambs were used simultaneously. The condition of both animals deteriorated rapidly. In one of the animals that survived for 30 days, the pasterns dropped progressively lower and almost touched the ground before death. An interesting phenomenon in both animals was the presence of a constriction at the base of the tail (Fig. 7).

The ewes giving birth to ataxic lambs had been treated with parbendazole on Days 17 and 20 of gestation (Table 2).

3. *Lambs with skeletal anomalies*

Skeletal anomalies observed in the 5 affected animals were mainly confined to the long bones. The main malformations observed were rotational and flexing deformities of the limbs, overflexion of the carpal joints and abnormalities of posture or gait (Fig. 2 & 8). Ewes giving birth to these lambs had been dosed with parbendazole during the first month of pregnancy.

EEG results

The EEG recordings of 2 paralysed lambs corresponded with those of the control animals.

MOP Kontron Messgeräte results

The results of the MOP Kontron Messgeräte recordings are summarized in Tables 3, 4 & 5.

TABLE 3 Parameters on the entire brains of lambs as recorded with the MOP Kontron Messgeräte apparatus

Control lambs

Lamb No.	Form	D. max.	Length	Area	Mass (g)
8.....	0,9	48,3	140,8	1 420,4	54,9
9.....	0,9	49,9	145,8	1 444,8	60,4
10.....	0,9	48,6	141,6	1 377,6	55,7
11.....	0,9	49,0	145,6	1 461,5	55,3
12.....	0,9	51,7	149,5	1 560,3	65,8
13.....	0,9	49,1	146,8	1 480,4	61,3
Average.....	0,9	49,43	145,0	1 457,5	58,9

*Paralysed lambs**

Lamb No.	Form	D. max.	Length	Area	Mass (g)
3.....	0,9	47,8	140,7	1 343,2	43,9
4.....	0,8	45,6	135,3	1 227,5	41,0
7.....	0,8	47,9	139,6	1 310,2	40,7
Average.....	0,833	47,1	138,5	1 293,6	41,86

Comparison of control and paralysed lambs

Lamb No.	Form	D. max.	Length	Area	Mass (g)
Control lambs	0,9	49,43	145,0	1 457,5	58,9
Paralysed lambs	0,833	47,1	138,5	1 293,6	41,86
Percentage difference....	9	4,7	4,48	11,24	28,93

* Paralysed lambs with hydrocephalus are not included

Form = Factor based on $F = \frac{4\ \pi\ \text{area}}{\text{perimeter}^2}$

D. max. = Largest diameter of circumscribed feature (mm)

Length = Length from point A to point B (A→B) (mm)

Area = Area in mm². Area determination based on perimeter trace

TABLE 4 Parameters on the cerebral hemispheres of lambs as recorded with the MOP Kontron Messgeräte apparatus

Control lambs

Lamb No.	Form	D. max.	Length	Area
8.....	0,8	39,6	135,8	1 203,4
9.....	0,8	39,5	135,5	1 195,4
10.....	0,8	38,9	135,1	1 166,5
11.....	0,8	43,3	143,6	1 313,1
12.....	0,8	44,6	145,8	1 398,0
13.....	0,8	42,0	141,2	1 318,4
Average.....	0,8	41,31	139,5	1 265,8

*Paralysed lambs**

Lamb No.	Form	D. max.	Length	Area
3.....	0,8	39,3	133,6	1 110,1
4.....	0,8	37,3	128,0	1 026,5
7.....	0,8	37,1	128,1	1 026,8
Average.....	0,8	37,9	129,9	1 054,46

Comparison of control and paralysed lambs

Lamb No.	Form	D. max	Length	Area
Control lambs.....	0,8	41,31	139,5	1 265,8
Paralysed lambs....	0,8	37,9	129,9	1 066,46
Percentage difference	—	8,25	6,88	15,74

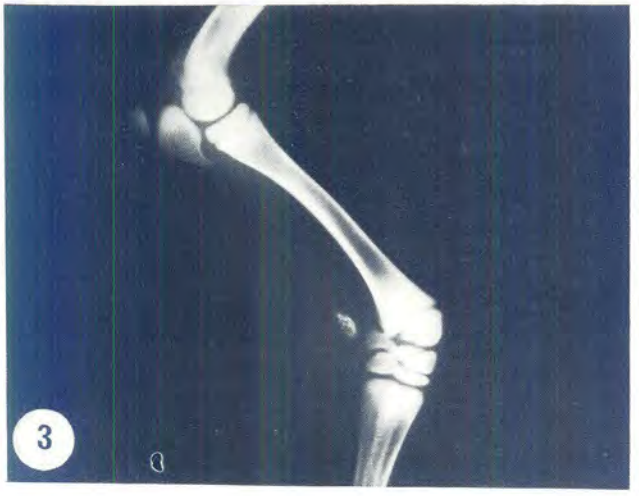
Form = Factor based on $F = \frac{4\ \pi\ \text{area}}{\text{perimeter}^2}$

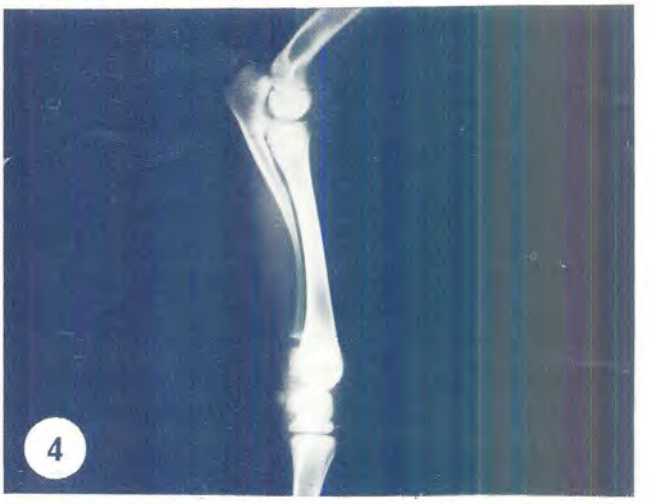
D. max. = Largest diameter of circumscribed feature (mm)

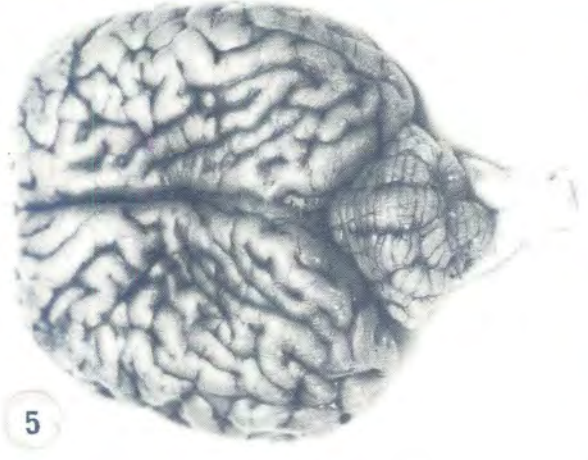
Length = Length from point A to point B (A→B) (mm)

Area = Area in mm². Area determination based on perimeter trace

* Paralysed lambs with hydrocephalus are not included







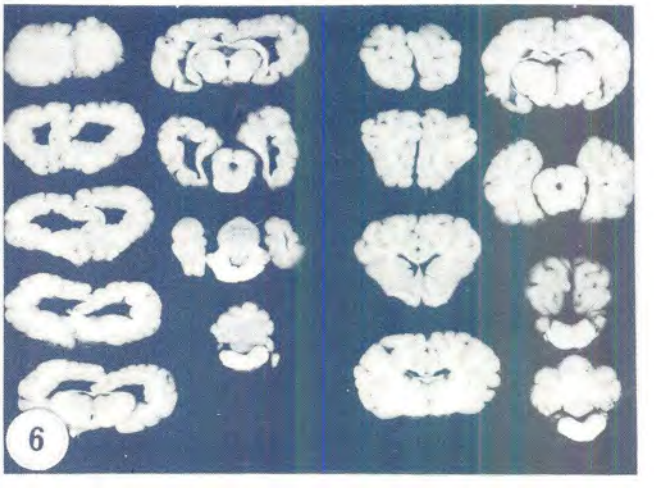


FIG. 1 A paralysed lamb born from a ewe treated with parbendazole at 32 days of gestation

FIG. 2 Lamb with a skeletal deformity born from a ewe treated with parbendazole at 26 days of gestation. Note the lateral rotation of the front limbs

FIG. 3 Radiograph of the radius and ulna from a lamb the ewe of which was treated with parbendazole during the first month of gestation. Note the absence of the shaft of the ulna

FIG. 4 Radiograph of the radius and ulna of a control lamb

FIG. 5 Brain from a paralysed lamb with hydrocephalus. Note the collapsed appearance of the cerebral hemispheres

FIG. 6 Note the hydrocephalus in brain sections cut at different levels on the left as compared with a control on the right

Analysis of variance between the brain masses of control, paralysed and teratologic lambs was done. A significant F value was determined. Using the Bonforonis multiple comparison procedure, a highly significant value ($P \leq 0,01$) in the difference of brain masses between control and paralysed lambs was obtained, while the difference between the control and teratologic lambs was not significant. A significant difference ($P \leq 0,05$) was found between paralysed and teratologic lambs.

TABLE 5 Parameters on the cerebellums of lambs as recorded with the MOP Kontron Messgeräte apparatus

Control lambs

Lamb No.	Form	D. max.	Length	Area
8	0,7	15,2	59,9	203,7
9	0,7	17,2	64,5	226,3
10	0,7	17,0	60,0	208,2
11	0,6	13,2	56,5	145,8
12	0,7	14,2	56,3	168,5
13	0,7	14,9	56,1	170,1
Average	0,683	15,28	58,88	187,1

*Paralysed lambs**

Lamb No.	Form	D. max.	Length	Area
3	0,7	16,6	60,4	215,1
4	0,8	15,8	58,2	201,8
7	0,8	16,0	62,0	241,1
Average	0,77	16,13	60,2	219,3

Comparison of control and paralysed lambs

Lamb No.	Form	D. max.	Length	Area
Control lambs	0,683	15,28	58,88	187,1
Paralysed lambs	0,77	16,13	60,2	219,3
Percentage difference	—11,2	—5,26	—2,19	—14,68

Form = Factor based on $F = \frac{4\pi \text{ area}}{\text{perimeter}^2}$

D. max. = Largest diameter of circumscribed feature (mm)

Length = Length from point A to point B (A→B) (mm)

Area = Area in mm². Area determination based on perimeter trace

* Lambs with hydrocephalus are not included

As no information regarding the surface area, length, and maximum diameter of brains of teratologic lambs was available, no comparisons on the above brain parameters between these lambs and paralysed or control lambs could be done. Student's T test was used to compare the average value of the surface area, length and maximum diameter of the entire brain, the cerebral hemispheres and the cerebellum of control and paralysed lambs. Highly significant differences ($P \leq 0,01$) were obtained when comparing the total surface area of the entire brain and cerebral hemispheres of control and paralysed lambs, whilst significant differences ($P \leq 0,05$) were determined for the lengths of the entire brain and cerebral hemispheres, as well as for the maximum diameter of the entire brain. There was no significant difference when the maximum diameter of the cerebral hemispheres of control and paralysed lambs were compared. No differences could be found when the surface area, length and maximum diameter of the cerebellum of control lambs were compared with those of paralysed lambs.

The coefficient of variance for all the above analyses varied between 2 and 12%, which is within acceptable normal limits.

Radiographic examination

No abnormalities were observed in the 5 paralysed and 2 ataxic lambs. Various skeletal malformations such as rotational and flexing defects of the limbs and overflexion of the carpal joints were present in the lambs with teratology (Fig. 3).

Macroscopic pathology

Paralysed lambs

The brains of 2 of the 5 paralysed lambs were smaller than those of the controls (Fig. 9). In the 3rd lamb the brain was normal in size. In the last 2 lambs the cerebral hemispheres had a flattened appearance (Fig. 5). Sagittal sectioning of the last 2 brains revealed dilatation of both the lateral ventricles (hydrocephalus) (Fig. 6). The normal finger-like pattern of the white matter in the cerebral hemispheres was not present but was replaced by a collapsed mass of white matter (Fig. 10). Structures affected by the dilatation of the lateral ventricles included the caudate nucleus, the thalamus and lateral geniculate bodies. The *corpora quadrigemina* appeared smaller than normal. In both animals the *corpus callosum* and *fornix* were either very thin or absent in some sections.

Ataxic lambs

The dorsal surface of the spinal cord in both animals revealed a space approximately 4 mm in diameter beneath the dura mater (Fig. 11). The latter extended from L2–5 in one animal and from L5–S2 in the other. A unilateral renal aplasia was present in one of these animals. The ewe of this lamb had been dosed with parbendazole at 20 days of gestation.

Lambs with skeletal anomalies

Apart from the lesions observed during the clinical and radiological examinations, no other lesions were observed.

Aborted foetus

Although the exact age of the aborted foetus was not known, it was clear from the size of the foetus that it had been aborted during the last third of gestation. Lesions observed included the following: anasarca, ascites, hydrothorax with pulmonary hypoplasia and bilateral cystic dilatation of the renal pelvis and ureters (Fig. 12 & 13).

Brain masses of lambs

The brain masses of affected and control lambs are summarized in Tables 3 & 6.

TABLE 6 Comparison of brain masses of control and paralysed lambs and lambs with teratology (average figures)

Lambs	Brain mass (g)	Percentage difference as compared with control lambs
Control lambs	58,9	—
Paralysed lambs	41,86	28,93%
Lambs with teratology	53,68	8,87%

Microscopic pathology

Paralysed lambs

1. *Paralysed lambs with hydrocephalus*

A prominent feature of the cerebral cortex in the 2 lambs was the thinning or complete absence of the molecular layer at the tips of the gyri. Oedema seen as a vacuolation of affected areas was occasionally observed. A disturbed architectural structure of the cerebral cortex and medulla was a prominent feature

in both animals. In one of the animals 3–4 spaces, lined by ependymal cells and 20–40 μm in diameter. were observed in the cerebral grey matter adjacent to the dilated lateral ventricles.

2. *Paralysed lambs without hydrocephalus*

In the cerebral white matter of all 3 lambs, an increased number of glia cells was observed in the vicinity of the *radiatio corporis callosi* and *radiatio optica*. In 2 of these lambs the gliosis was accompanied by areas of encephalomalacia (Fig. 14 & 15). A prominent Gitter cell reaction was noticed in the affected area in one of the lambs (Fig. 16).

Lambs with ataxia

An examination of the lumbo-sacral spinal cord of both lambs revealed the presence of 1 or 2 cavities in the dorsal *funiculi* (syringomyelia) (Fig. 17 & 18). The cavities varied in size in different sections and almost completely replaced the dorsal *funiculi* in the lumbar area. These cavities were surrounded by a homogeneous layer of degenerated neural elements. Sections stained with the Van Gieson's stain for collagen revealed a thin layer of connective tissue lining the inner side of these degenerated neural elements.

Some of the cavities contained an eosinophilic staining fluid, while in others a few dark staining glial nuclei were observed lining the inner surface of the cavities. Hydromyelia was observed in some sections (Fig. 19). In both lambs the central canal in the sacral region of the spinal cord was ectopic and appeared more ventral than normal. Portions of white matter appeared to be embedded within the grey matter above or around these central canals. A few swollen axis-cylinders, seen in Luxol fast blue-Holmes's stained sections, surrounded the cavities. Duplication of the central canal in the lumbar area was observed in one of the lambs.

Lambs with skeletal anomalies

In one of the lambs duplication and even triplication of the central canal of the lumbo-sacral area were observed (Fig. 20 & 21). Ectopic white matter was observed between these central canals.

Discussion

Data in the present study revealed a brain mass difference of 28,93% between control and paralysed lambs (Table 3). Statistical analysis (analysis of variance) showed that this difference was highly significant ($P \leq 0,01$). However, no statistically significant difference was observed when the brain masses of control lambs were compared with those of lambs exhibiting various skeletal malformations (Table 6).

Student's T test was used to compare the average value of the surface area, length and maximum diameter of the entire brain, the cerebral hemispheres and the cerebellum of control and paralysed lambs. Highly significant differences ($P \leq 0,01$) were obtained when comparing the total surface area of the entire brain and cerebral hemispheres of control and paralysed lambs, whilst only a significant difference ($P \leq 0,05$) was determined for the lengths of the entire brain and cerebral hemispheres as well as the maximum diameter of the entire brain. No difference could be found when the surface area, length and maximum diameter of the cerebellum of control lambs were compared with those of paralysed lambs (Tables 3 & 4). Two of the lambs born from ewes treated with parbendazole at 32 and 37 days of gestation exhibited internal hydrocephalus with prominent dilatation of the lateral ventricles (Table 2). These results together with the presence of encephalomalacia in the white matter of the cerebral hemispheres in 2 paralysed lambs indicate that the cerebral hemispheres are the part of the brain having the greatest susceptibility to parbendazole.

The period of greatest embryogenic susceptibility regarding the central nervous system was between 17 and 53 days of gestation (Table 2). Lesions in the cerebral hemispheres ranging from cerebral hypoplasia to internal hydrocephalus were observed in lambs, the ewes of which had been treated with parbendazole between 30 and 53 days of gestation (Table 2).

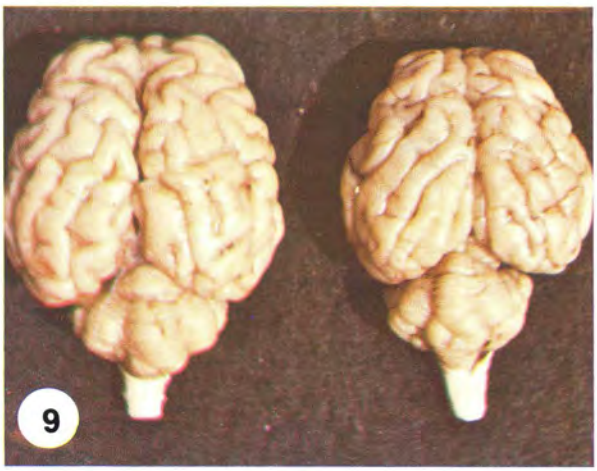
According to McIntosh, Baghurst, Potter & Hetzel (1979), the major increase in mass in the cerebral hemispheres in the ovine foetus occurs between 40 and 90 days of gestation, with a second increase after 95 days. The first phase represents neuroblast multiplication and the second phase glial multiplication. In the ovine foetus neuronal multiplication occurs mainly between 40 and 80 days of gestation and glial multiplication between 95 and 130 days. These 2 rapid growth phases represent the most vulnerable periods for developmental damage (McIntosh *et al.*, 1979).

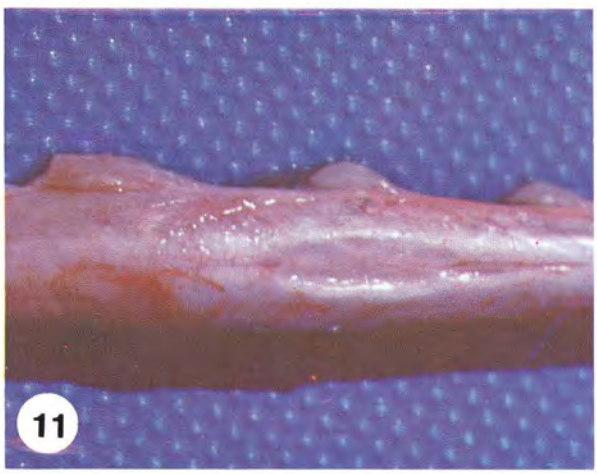
Lapras, Delatour, Labe, Panarin & Richard (1975) showed that parbendazole had an antimitotic effect when administered to rats with leucaemia, while the chronic administration of parbendazole inhibited spermatogenesis and haemopoiesis. Benzamidazoles inhibit DNA synthesis probably on account of their antimetabolic nature (Corbett, 1974).

According to Seiler (1975), benzamidazoles are excreted rapidly in mammals, over 90% being excreted within 48–72 hours. No accumulation of metabolites of these drugs has been observed in mammals. The metabolites are approximately equally distributed in various organs within the animal body, and are excreted mainly via the kidneys (Di Cuollo & Miller, 1974). Little information on the metabolism of parbendazole in the ovine foetus is available. The concentration of parbendazole in different foetal organs, if compared with equivalent values in the ewe, can vary significantly. Likewise organ concentration values of twin lambs can vary considerably (Eastwood, 1969).

We deduce from the above results that actively dividing cells are affected during an approximately 48-hour period after administration of parbendazole. As regards the developing CNS of the ovine foetus, it is to be expected that neuroblasts of the cerebral hemispheres are the cell type which would be most severely affected, and this would inevitably result in cerebral hypoplasia and even internal hydrocephalus in severely affected animals, as the present study shows.

In an attempt to prove a reduction in the number of neurons and neuroblasts in the cerebral hemispheres, direct ocular counting methods were employed. However, because of serious problems encountered (including defining criteria for counting, the young age of the animals and the very wide variation in cell numbers in different areas of the brain) comparisons with control animals were difficult, and thus this method was abandoned.





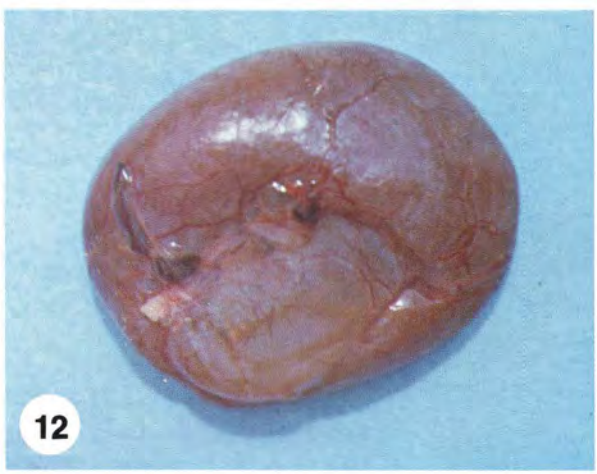


FIG. 7 A constriction of the base of the tail of an ataxic lamb. The ewe of this lamb was treated with parbendazole at 17 days of gestation

FIG. 8 Lamb with skeletal deformities born from a ewe treated with parbendazole during the first month of gestation. Note the flexion of the carpal joints

FIG. 9 Cerebral hypoplasia in a paralysed lamb (right) compared with that of a control (left)

FIG. 10 A collapsed mass of white matter in the cerebral hemisphere of a paralysed lamb with hydrocephalus (right) compared with the same area of a control (left)

FIG. 11 Syringomyelia in a lamb with ataxia

FIG. 12 Cystic dilatation of the ureter of an aborted foetus, the ewe of which was treated with parbendazole

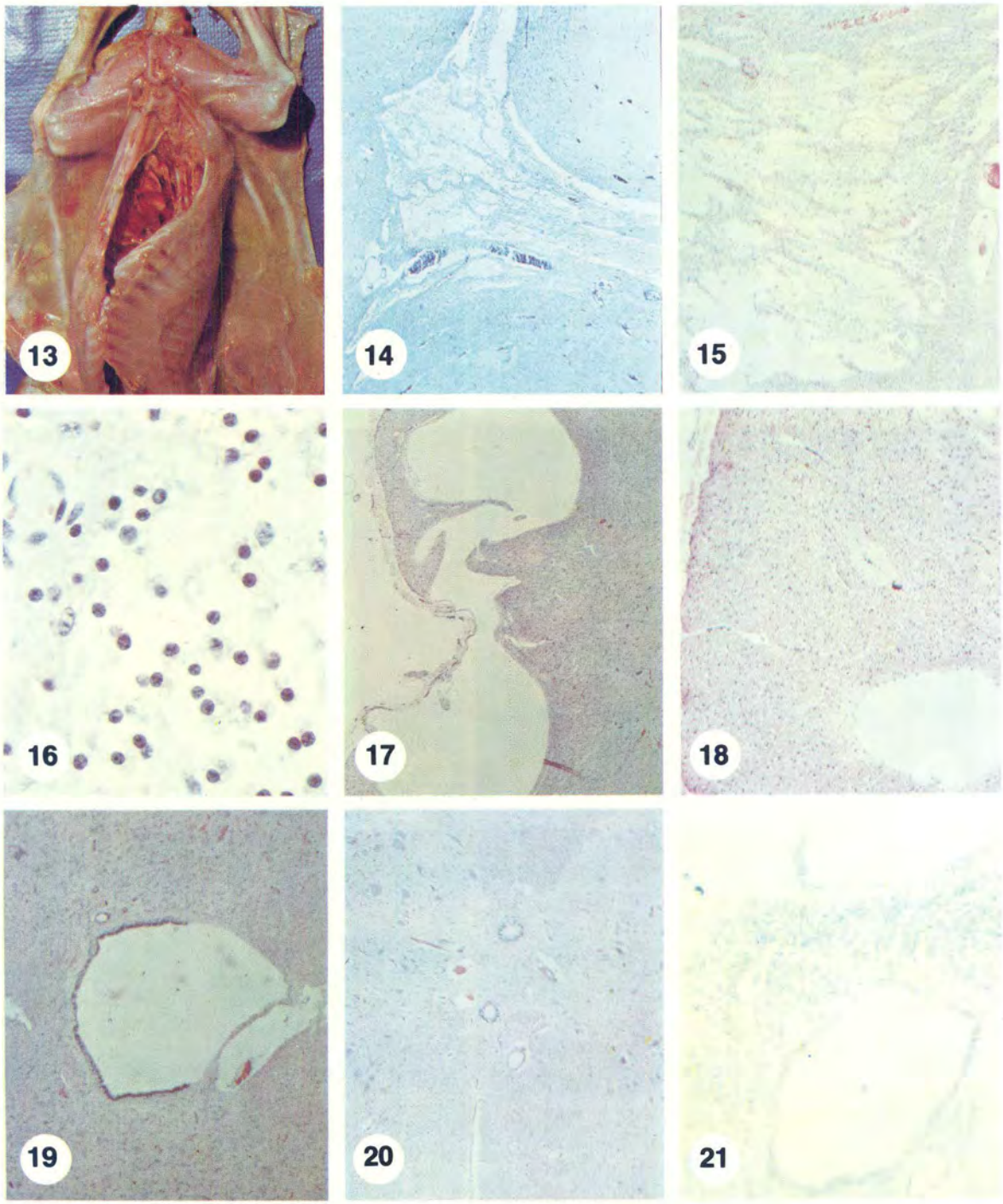


FIG. 13 Anasarca in an aborted foetus, the ewe of which was treated with parbendazole
FIG. 14 An area of encephalomalacia in the cerebral hemispheres of paralysed lamb with cerebral hypoplasia: Holzer's ×100
FIG. 15 An area of encephalomalacia in the cerebral hemispheres of a paralysed lamb with cerebral hypoplasia: HE ×150
FIG. 16 A higher magnification of the area of encephalomalacia. Note the Gitter cells: HE ×1 200
FIG. 17 Syringomyelia in an ataxic lamb: HE ×100
FIG. 18 Syringomyelia in an ataxic lamb. Note the homogeneous layer of degenerated neural elements surrounding the cavity: HE ×100
FIG. 19 Hydromyelia in an ataxic lamb. Note the partial absence of the epithelium lining the ependymal canal: HE ×100
FIG. 20 Duplication of the central canal in a lamb with skeletal deformities: HE ×100
FIG. 21 Duplication of the central canal in a lamb with skeletal deformities: Luxol fast blue—Holmes's ×500

An interesting feature in the present study was the presence of focal areas of encephalomalacia in the cerebral white matter of 2 paralysed lambs, both of which exhibited cerebral hypoplasia. Seeing that the 2 lambs were exposed to parbendazole at 32 and 53 days of development respectively, 2 possible explanations for the lesions are proposed. The normal development of tissue depends on the correct balance of proliferation, migration and differentiation of cells. Interference, by any means, in any one of these systems might initiate a mechanism of malformation leading to disturbances in one or more of these systems and cause developmental abnormalities at both cellular and morphological levels (Poswillo, 1976). One possible explanation, therefore, would be that the administration of parbendazole to ewes, the lambs of which experience a vulnerable period regarding the development of the cerebral hemispheres, resulted in a disturbed balance of one or more of the above factors (proliferation, migration or differentiation of cells). Depending on the degree of interference, the outcome of the insult ranged from internal hydrocephalus to cerebral hypoplasia with or without areas of encephalomalacia. Therefore, the areas of encephalomalacia observed appear to be the remnants of a necrotic lesion inflicted during the early developmental stages.

Patterson, Sweasey & Herbert (1971) reported that myelination in developing sheep occurs mainly during 2 peak periods, namely, at 20 days prior to and 10–20 days after birth. Since benzamidazoles are excreted rapidly, it is unlikely that the administration of the latter to ewes during the early stages of gestation would interfere directly with myelination. Thus, a possible explanation for the encephalomalacia would be that the administration of parbendazole during the early stages of gestation interferes with the proliferation, migration and differentiation of oligodendroglia and that this results in a metabolic failure of the latter and the formation of unstable myelin with subsequent degeneration.

There are 2 major categories of hydrocephalus—compensatory and obstructive. With compensatory hydrocephalus, cerebrospinal fluid (CSF) accumulates in spaces in the cranial cavity not occupied by brain parenchyma. Examples include destruction of tissue associated with ischaemia, inflammation or injury. Obstructive hydrocephalus results from obstruction to flow or absorption of CSF (De Lahunta, 1977). The presence of areas of encephalomalacia in 2 lambs with cerebral hypoplasia suggests that the hydrocephalus observed in 2 of the paralysed lambs is compensatory in form. It is suggested that the accumulation of fluid in the dilated lateral ventricles resulted in the disturbed organization of the cerebral cortex and medulla observed both micro- and macroscopically.

Apart from the macroscopic lesions observed in the cerebral hemispheres of the 2 lambs with hydrocephalus, i.e. dilatation of the lateral ventricles and a disturbed architectural structure of the white matter, microscopic lesions were confined to the cerebral cortex. A thinning or total absence of the molecular layer could be ascribed to compression caused by the dilatation of the lateral ventricles.

Considering the extent of the compensatory hydrocephalus observed, one would expect to see remnants of tissue debris in affected areas. As this was not the case, the question is raised as to how the embryo is capable of removing large numbers of necrotic cells. Langman & Cardell (1977) suggested that most of the cellular debris is decomposed *in situ* without being phagocytized and they considered this to be a form of autolysis. Since ewes giving birth to paralysed lambs were exposed to parbendazole during the early stages of gestation (Table 2), it is likely that lesions inflicted on the cerebral hemispheres of the lambs with hydrocephalus would have resolved by the time of birth. This would explain the absence of a cellular response in these lambs.

Various viruses, including those responsible for Rift Valley fever, Wesselsbron disease, bluetongue, Akabana disease and mucosal disease viral diarrhoea, can damage the foetal CNS should the ewe be infected during certain stages of gestation (Coetzer & Barnard, 1977; Hartley, De Saram, Dellaporta, Snowdon & Shepherd, 1977; Jubb & Kennedy, 1970). The post-lambing serological results (from ewes which gave birth to paralysed lambs for the above-mentioned teratogenic viruses) are summarized in Table 1. As the latter sera were collected at least 1 month after the ewes had lambed, and as there were no significant titres to these teratogenic viruses, it can be concluded that they played no role in the cause of the paralysed lamb syndrome. Furthermore, as all the lambs born from control ewes appeared normal, parbendazole would appear to be the cause of the lesions observed.

The period of greatest embryogenic susceptibility to parbendazole with respect to the musculoskeletal system is between 12 and 24 days of gestation (Szabo *et al.*, 1974; Saunders *et al.*, 1974), and innervation is necessary for survival, growth and differentiation of myofibres (Zelena, 1962; Shafiq, Asiedu & Milhorat, 1972). Should congenital articular rigidity (CAR) occur in animals in association with CNS defects such as hydrocephalus, the CNS lesion may be exerting a direct effect on muscle either transneuronally or by modifying prenatal muscle activity (Swatland, 1974). In the present study no anomalies developed in the musculoskeletal system in the 5 paralysed lambs. Brain masses of lambs with CAR compared favourably with those of control animals (Table 6). Although it is realized that the results may be confusing because of the limited number of animals with congenital anomalies, it appears that with parbendazole toxicity there is no relation between the CNS and musculoskeletal lesions observed.

The EEG waves recorded from the scalp surface are a direct result of changes on the membranes of pyramidal neurones (Remond, 1974). The latter receive impulses from sensory receptors throughout the body to reach the reticular activating system, the thalamic nuclei and finally the cortical neurones (Brazier, 1968; Remond, 1974). Although an EEG was recorded from only a limited number of lambs, the recordings from both the control lambs and the 2 paralysed lambs were similar. This merely indicates that the neurones within the cerebral cortex of these 2 paralysed lambs exhibited normal depolarization and hyperpolarization.

Syringomyelia can have various causes, either primary (congenital) or that related to secondary acquired factors (Cohrs & Saunders, 1965). It is described as slowly progressive, with a variation in course, and is rarely the cause of death (Benda, 1959, cited by Cohrs & Saunders, 1965). Gardner (1959) stressed the relationship between hydrocephalus, hydromyelia and syringomyelia. Anomalies of the central canal associated with syringomyelia include hydromyelia, syringomyelia, duplication of

the central canal, absence of the central canal and a central canal represented by an ependymal microrossette, the last having an abnormal location in the grey matter (Cohrs & Saunders, 1965).

The present study leads to the conclusion that the spinal dysraphism resulting from the administration of parbendazole stems from a disturbed flow of the CSF, with consequent hydromyelia, rupture of the ependymal lining and subsequent syringomyelia. This would explain why the walls of the cavities consisted of degenerated neuronal and neuroglial tissue.

The administration of parbendazole to pregnant ewes during the first month of gestation has been known to result in various anomalies of the kidneys (Saunders *et al.*, 1974), among which are the following: unilateral renal aplasia or hypoplasia accompanied by hyperplasia of the opposite kidney; dilatation of the ureter and pelvis draining the hyperplastic kidney, the results of the accumulation of fluid and bilateral polycystic kidneys (Prozesky, unpublished observations, 1979). In the present study, the bilateral dilatation of the ureters and renal pelvis in the aborted foetus most probably was the result of a partial or complete obstruction of the ureters. This would explain the ascites, hydrothorax and anasarca seen in the foetus.

The data reported here emphasize that slight differences in the timing of administration of parbendazole to pregnant ewes may lead to significant differences in the congenital anomalies observed in lambs.

Middleton *et al.*, (1974) reported a significant reduction in the number of ewes, which had been treated with parbendazole on the 12th day of pregnancy at a dosage rate of 30–60 mg/kg, that lambed, after the initial service. The dramatic reduction in the lambing percentage observed in this study can be attributed to the antimitotic activity of parbendazole (Lapras *et al.*, 1975), the dosage rate used and the frequent handling of the animals during the course of the experiment.

As the dosage rate of parbendazole used in the present study was 4 times that recommended in the RSA, the authors consider it of importance to compare the experimental findings with those which occur under field conditions.

The paralytic syndrome observed in the lambs under field conditions was indistinguishable from that seen in the experimental cases. Macroscopic examination revealed hydrocephalus in both natural and experimental cases. The number of lambs studied is too low, however, for any significant statistical conclusions to be arrived at as regards the incidence of hydrocephalus. The histological examination of 10 natural cases of paralysed lambs revealed gliosis in the cerebral hemispheres of 1 lamb. In another 2 lambs, lesions comparable to those observed in experimental cases were noted in the spinal cord (L. Prozesky, unpublished data, 1981). It is evident that the symptoms and pathological lesions of lambs exhibiting the paralysed lamb syndrome are similar in natural and experimental cases, although the extent of the lesions under experimental conditions are exaggerated, as higher dosage rates were used.

According to Saunders *et al.*, (1974) the danger period of parbendazole for the ovine foetus occurs during the third week of gestation. It now appears that even up to 53 days of gestation there is still danger of resultant brain damage. Considering the data of McIntosh *et al.*, 1979), even 80 days should be considered risky.

As far as could be ascertained, these are the first reported cases describing brain lesions in lambs which can be attributed to the administration of parbendazole to pregnant ewes.

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