Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin. II. Macropathology and histopathology

STELLA S. BASTIANELLO[1], N. FOURIE[2], L. PROZESKY[1], P.W. NEL[3] and T.S. KELLERMANN[2]

ABSTRACT

BASTIANELLO, S.S., FOURIE, N., PROZESKY, L., NEL, P.W. & KELLERMAN, T.S., 1991. Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin. II. Macropathology and histopathology. *Onderstepoort Journal of Veterinary Research*, 62:5–18

This report contains an account of the gross and histopathological lesions of 20 cattle and four sheep in 15 field outbreaks of poultry litter toxicity, one steer fed *ad lib.* and six sheep dosed with toxic poultry litter, and ten sheep fed experimental rations containing *c* 2,5 ppm and 5 ppm maduramicin.

The principle macroscopic lesions in most cattle that died in field outbreaks were indicative of congestive heart failure. The lesions in sheep were similar, but generally milder. Cardiac dilatation was observed in both sheep and cattle. Microscopically, the cardiac lesions were more pronounced in cattle and comprised varying degrees of atrophy, hypertrophy, degeneration, necrosis of myocardial fibres, and interstitial fibrosis. Skeletal muscle lesions were usually more severe in sheep, particularly in the muscles of the hindquarters which appeared pale, oedematous and mottled.

One of the sheep in the poultry litter dosing trial developed signs of congestive heart failure and the hearts of two others were dilated. Extensive hypertrophy and atrophy of myocardial fibres were evident in the steer fed *ad lib.* with this material. As in field cases, the myocardial lesions of the sheep were less severe than those of the steer.

Mild cardiac dilatation was present in four of the seven sheep in the maduramicin feeding trial. Diffuse hypertrophy of myocardial nuclei was present in all seven cases, myocardial fibre atrophy in six, multifocal fibrosis and necrosis in six and two cases, respectively, and focal endocardial thickening in two. The skeletal muscles revealed granular degeneration and foci of necrosis and regeneration.

The cardiac and skeletal lesions in the field outbreaks, poultry litter feeding trials and maduramicin feeding trials, were highly comparable. This suggests that this form of poultry litter intoxication is a chronic form of ionophore toxicity, the pathology of which is characterized by a dilated cardiomyopathy with congestive heart failure and mild (cattle) to severe (sheep) skeletal muscle lesions.

Keywords: Cardiomyopathy, ruminant, poultry litter, ionophore, maduramicin

[1] Section of Pathology, Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort 0110

[2] Section of Toxicology, Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort 0110

[3] Private practitioner, P.O. Box 440, Christiana 2680

Accepted for publication 2 February 1995—Editor

INTRODUCTION

Poultry litter is a cheap source of protein for ruminants. In South Africa it is commonly fed to cattle and sheep, especially during the winter or in periods of drought (Nel, Kellerman, Prozesky & Van der Pypekamp 1987).

Feeding of poultry litter has been associated with mortalities due to botulism, copper toxicity and salmonellosis (Fourie, Bastianello, Prozesky, Nel & Kellerman 1991). Between 1986 and 1990, mortalities which could not be ascribed to any of the above causes were encountered in cattle and sheep fed poultry litter. The epidemiological, clinical and clinicopathological findings in 15 field outbreaks of this condition and the results of feeding trials with toxic poultry litter and maduramicin have been documented in the first part of this report (Fourie, Bastianello, Prozesky, Nel & Kellerman 1991). An ionophore antibiotic, *viz.* maduramicin, was shown to be the toxic component in the litter from some of the field outbreaks.

This report deals with the pathological findings in cattle and sheep in the above field outbreaks and poultry litter and maduramicin feeding trials.

MATERIALS AND METHODS

Field Outbreaks

Necropsies were performed on 20 cattle and four sheep from 15 outbreaks (Table 1). Fourteen cattle and one sheep were necropsied in the field and the remaining nine animals at the Division of Pathology of the Onderstepoort Veterinary Institute (OVI).

Representative specimens of the heart were collected in 10% buffered formalin in all 24 cases, the liver in 19 cases (16 cattle, three sheep), the lungs in 19 cases (15 cattle, four sheep), the skeletal muscles of the neck, back and hind limbs in nine cases (six cattle, three sheep). Various other tissues including the kidney and brain, were also collected.

In eight cases, specific regions of the heart were collected, namely the left and right free ventricular walls, septum, apex and left and right atria. The tissues were routinely processed and stained with haematoxylin and eosin (HE). Myocardial sections from selected cases were stained with the Masson's trichrome (MT) stain for collagen (Anonymous 1968) and the Verhoeff's stain for elastic tissue (Bradbury & Gordon 1977).

The myocardial lesions have been tabulated according to seven histologic features, *viz.* myocardial fibre degeneration; myofibre hypertrophy; myofibre atrophy; myofibre loss and fibrosis; hyalinization of fibres; necrosis with an attendant cellular infiltration; and focal endocardial thickening (Table 1).

Hypertrophy was assessed qualitatively based on criteria outlined by Davies (1975) and included an apparent increase in the number of myocardial fibre nuclei; changes in size, shape and staining characteristics of the nuclei; an apparent increase in the diameter of fibres and, occasionally, focal disarray of fibres.

Myocardial fibres were qualitatively judged to be atrophic if they met with three criteria: an apparent decrease in diameter; prominence and clustering of myocardial and interstitial cell nuclei; and a wavy course on longitudinal section (De Girolami & Smith 1982; Prozesky, Fourie, Neser & Nel 1988).

The skeletal muscle lesions were categorized and tabulated on the basis of five histologic criteria: granular degeneration and swelling of fibres; hyalinization of fibres; foci of necrosis with cellular inflammation and/or fibroplasia; regeneration of fibres; and vacuolation of fibres (Table 2).

Poultry litter feeding trials

The details of the dosing regimen for one steer and six sheep have been reported in Part 1 of this investigation (Fourie *et al.* 1991). Briefly, poultry litter from field outbreaks where mortalities had occurred was fed *ad lib.* to the steer and dosed per rumen fistula to the sheep at daily doses ranging from 5–15 g/kg for 23–114 d. One of the sheep and the steer died suddenly 35 and 43 d into the trial. The remaining five sheep were euthanased *in extremis* at 34, 107, 107, 109 and 114 d, respectively, following the development of one or more of the following symptoms: tachycardia; cardiac arrythmia; gallop rhythm; weakness; or lethargy. The animals were necropsied on the days noted above. Representative specimens of the heart, liver and lung from all seven cases, skeletal muscles from four sheep and a wide range of other tissues were collected in 10% buffered formalin. In four sheep, specific regions of the heart, namely the apex, interventricular septum and left and right free ventricular walls, were collected separately. The tissues were routinely processed and stained with HE and the lesions in the cardiac and skeletal muscle categorized and tabulated as for the field outbreaks (Tables 3 and 4).

Maduramicin feeding trials

The dosing data for ten sheep (three control and seven experimental cases) were reported in Part 1 of this report (Fourie *et al.* 1991).

In summary, one ewe in a pilot trial (sheep 35) was fed a commercial poultry broiler ration containing *c* 5 ppm maduramicin (ration A). Following the mortality of the above sheep, a trial was devised in which nine wethers were randomly divided into three groups of three each. One group (sheep 32–34) received a complete balanced pelleted ration (control ration). The second group (sheep 36–38), received ration B, equivalent to

TABLE 1 Histopathological lesions in the myocardium of 20 cattle and four sheep from 15 field outbreaks of poultry litter toxicity (cases 1–24)

Histopathological lesions	Distribution of lesions	Number of animals			Percentage of animals affected
		Cattle	Sheep	Total	
Myocardial fibre degeneration	Diffuse	20	4	24	100
Myocardial fibre hypertrophy*	Focal	2	2	24	100
	Diffuse	18	2		
Myocardial fibre atrophy	Focal	9	4	19	79
	Diffuse	6	0		
Foci of fibre loss and fibrosis	Isolated	2	1	15	63
	Scattered	12	0		
Foci of hyalinization	Isolated	4	1	11	46
	Scattered	5	1		
Foci of necrosis and cellular inflammation	Isolated	2	0	8	33
	Scattered	6	0		
Endocardial thickening	Focal	2	0	2	8

* Nuclear hypertrophy: 24 cases
Increase in fibre diameter and disorganization: 20 cases

TABLE 2 Histopathological lesions in the skeletal muscles of six cattle and three sheep from 15 field outbreaks of poultry litter toxicity

Histopathological lesions	Distribution of lesions	Number of animals affected			Percentage of animals affected
		Cattle	Sheep	Total	
Granular degeneration and swelling of fibres	Focal	1	1	8	89
	Diffuse	5	1		
Foci of hyalinization	Isolated	3	0	5	56
	Scattered	2	0		
Necrosis of fibres with cellular infiltration/ fibroplasia	Numerous	0	1	1	11
Regeneration of fibres	Scattered	0	1	1	11
Vacuolation of fibres	–	0	0	0	0

the control ration, with *c* 5 ppm maduramicin, and the third group (sheep 39–41), ration C which contained *c* 2,5 ppm maduramicin. The sheep received 2 kg of the pellets per day.

Case 35 died suddenly, following the development of dyspnoea and a stiff gait 28 d after commencement of feeding. The remaining nine cases (three controls and six sheep on rations B and C) were euthanased via

TABLE 3 Histopathological lesions in the myocardium of one steer and six sheep in poultry litter feeding trials (cases 25–31)

Histopathological lesions	Distribution of lesions	Number of animals affected			Percentage of animals affected
		Cattle (case 25)	Sheep (cases 26–31)	Total	
Myocardial fibre degeneration	Diffuse	1	6	7	100
Myocardial fibre hypertrophy*	–	1	6	7	100
Foci of hyalinization	Isolated	0	2	6	86
	Scattered	0	4		
Myocardial fibre atrophy	Focal	0	1	2	28
	Diffuse	1	0		
Foci of necrosis and cellular inflammation	Isolated	0	1	1	14
	Scattered	0	0		
Foci of fibre loss and fibrosis	Isolated	0	0	1	14
	Scattered	1	0		
Focal endocardial thickening	Focal	0	0	0	0

* Nuclear hypertrophy: 7 cases
Increase in fibre diameter and disorganization: 4 cases (57 %)

TABLE 4 Histopathological lesions in skeletal muscles of four sheep (cases 26, 27, 28 and 31) in poultry litter feeding trials

Histopathological lesions	Case number	Distribution of lesions	Number of animals affected	Percentage of animals affected
Foci of hyalinization	27, 28	Isolated	4	100
	26, 31	Scattered		
Granular degeneration and swelling of fibres	26, 27, 31	Diffuse	3	75
Necrosis of fibres with cellular infiltration/fibroplasia	26	Isolated	2	50
	31	Scattered		
Regeneration of fibres	–	–	0	0
Vacuolation of fibres	–	–	0	0

intravenous administration of an overdose of pentobarbital sodium or by the use of a captive bolt 57 d (cases 36, 37 and 38), 78 d (cases 32 and 39), 81 d (cases 33 and 40), and 92 d (case 41) into the trial. No clinical signs were noted in the control wethers. During the course of the feeding trial the remaining six sheep developed clinical signs characterized by rapid tiring, stiff gaits, reluctance to move, muscle tremors and collapse following forced exertion. No symptoms were noted when the animals were at rest. Necropsies were performed on all the sheep on the days noted above.

TABLE 5 Histopathological lesions in the myocardium of ten sheep in the maduramicin feeding trials

Histopathological lesions	Distribution of lesions	Case number and ration type										Number of affected cases in control group (n = 3)	Number of affected cases in experimental group (n = 7)	Percentage of affected animals in experimental group (n = 7)
		Control ration			Ration A	Ration B			Ration C					
		32	33	34	35	36	37	38	39	40	41			
Myofibre degeneration	Diffuse	–	–	–	+	+	+	+	+	+	+	0	7	100
Myofibre hypertrophy*	–	–	–	–	+	+	+	+	+	+	+	0	7	100
Myofibre atrophy	Diffuse	–	–	–	+	+	+	+	+	+	–	0	7	100
	Focal	–	–	–	–	–	–	–	–	–	+			
Foci of fibre loss and fibrosis	Isolated	–	–	–	+	–	+	+	+	+	+	0	6	86
Foci of hyalinization	Scattered	+	+	+	–	–	+	+	+	+	+	3	5	72
Foci of necrosis and cell inflammation	Isolated	+	–	–	–	–	–	–	–	+	+	1	2	28
Endocardial thickening	Focal	–	–	–	–	–	+	–	–	+	–	0	2	28

* Nuclear hypertrophy: 7 cases
Increase in fibre diameter and disorganization: 4 cases
\+ Present
– Absent

TABLE 6 Histopathological lesions in the skeletal muscles of 10 sheep in the maduramicin feeding trials

Histopathological lesions	Distribution of lesions	Case number and ration type										Number of affected cases in control group (n = 3)	Number of affected cases in experimental group (n = 7)	Percentage of affected animals in experimental group (n = 7)
		Control ration			Ration A	Ration B			Ration C					
		32	33	34	35	36	37	38	39	40	41			
Foci of hyalinization	Isolated	–	+	+	+	–	–	+	–	–	–	2	7	100
	Scattered	–	–	–	–	+	+	–	+	+	+			
Granular degeneration and swelling of fibres	Focal	+	–	–	–	–	–	+	–	–	–	1	7	100
	Diffuse	–	–	–	+	+	+	–	+	+	+			
Necrosis of fibres with cellular infiltration/fibroplasia	Isolated	–	+	–	–	–	–	+	–	–	–	1	7	100
	Scattered	–	–	–	+	+	+	–	+	+	+			
Regeneration of fibres	Isolated	–	–	–	–	–	–	+	–	–	–	0	7	100
	Scattered	–	–	–	–	–	–	–	+	+	+			
	Numerous	–	–	–	+	+	+	–	–	–	–			
Vacuolation of fibres	Isolated	–	–	–	–	–	–	–	+	–	–	0	3	43
	Scattered	–	–	–	–	+	+	–	–	–	–			

\+ Present
– Absent

At necropsy, tissues were collected in 10% buffered formalin. These included four separate specimens of the heart, namely the apex, interventricular septum and left and right free ventricular walls; skeletal muscles from the neck, chest, abdomen, diaphragm, back, fore- and hind limbs; as well as a wide range of other tissues. The specimens were routinely processed and stained with HE for histopathological examination. Selected sections from the heart and skeletal muscles were stained with the MT stain for collagen. Lung sections from sheep 34 (control ration) and the lung and liver from sheep 41 (ration C) were stained with the MT and Berlin blue (Perl's method) and Schmorls' stains for haemosiderin and lipofuscin (Pearse 1985).

The cardiac and skeletal muscle lesions were categorized as for the field outbreaks and tabulated in Tables 5 and 6, respectively.

RESULTS

Field outbreaks

Gross pathology

CATTLE

Of the six cattle necropsied at the OVI, five had lesions attributable to congestive heart failure. These varied in intensity from mild to moderate and included some or all of the following lesions: generalized congestion; hydrothorax, hydropericardium or ascites; pulmonary oedema; anasarca, particularly of the intermandibular region, neck and brisket; widespread intermuscular oedema and haemorrhages, especially in the muscles of the back and hindquarters; mediastinal, pharyngeal, peritracheal, perirectal and perirenal oedema; intestinal congestion; and accentuated hepatic lobulation, characterized by centrilobular pallor or haemorrhage.

One or more of the lesions listed above were also evident in the cattle necropsied in the field.

Mild cardiac dilatation and thinning of the right free ventricular wall was observed in one animal. Large, white chalky areas of mineralization, ascribable to pressure from prolonged sternal recumbency, were evident in the brisket muscles of case 17.

SHEEP

Mild to moderate signs of congestive heart failure were apparent in three sheep. These included generalized congestion, hydrothorax, intermuscular oedema and centrilobular hepatic pallor. In one animal the heart was mildly dilated and flabby, the left and right free ventricular walls were thinned, and the papillary muscles in both ventricles were flattened.

Prominent skeletal muscle lesions characterized by oedema, pallor and disseminated whitish areas of necrosis, were noted in the hindquarters of one sheep.

Histopathology

HEART (Table 1)

Degeneration. Degeneration of myocardial fibres, characterized by a fine granularity of the sarcoplasm and occasionally sarcoplasmic vacuolation was present in all the animals (Fig. 1).

Hypertrophy. Hypertrophy and hyperplasia of the myocardial fibre nuclei (evidenced by large vesicular, round, oval or elongated nuclei, many having indented or wavy outlines) were noted in all the animals. Two to three nuclei, and occasionally more, were frequently arranged in rows (Fig. 2 and 3).

Increase in diameter and disarray of myofibres were noted in all the sheep and 16 (89%) cattle. This lesion was much more striking in cattle, two of which showed a diffuse increase in myofibre diameter (Fig. 2 and 3).

Atrophy. The distribution of atrophic fibres varied from small multifocal areas below the endo- or epicardium or throughout the myocardium (four sheep and nine cattle) to diffuse atrophy (six cattle) (Fig. 3).

Myofibre loss and fibrosis. Isolated to scattered foci of attenuation, or loss of fibres with accompanying replacement fibrosis was present in 14 cattle and one sheep (Fig. 2 and 3). This change was most pronounced in the cattle with severe diffuse atrophy and/or hypertrophy.

Foci of hyalinization. Hyalinization of single, or small to large groups of fibres was noted in 11 animals (Fig.1). Affected fibres had a highly eosinophilic sarcoplasm, striations were indistinct or absent, and the nuclei were either unaffected or pyknotic.

Foci of necrosis and cellular inflammation. In eight cattle, small scattered foci of muscle fibre necrosis, associated with an infiltration of round cells (mainly lymphocytes as well as macrophages and some plasma cells) and occasionally neutrophils, were noted. These changes were most pronounced in the animals with severe fibre hypertrophy and/or atrophy.

Focal endocardial thickening. In the atria and ventricles of two cattle, the endocardium showed an increased density and cellularity of the deeper layer and oedema of the superficial layer. The MT and Verhoeff's stains revealed deposition, disorganization and disruption of collagen and elastic fibres (Fig. 4).

SKELETAL MUSCLES (Table 2)

One sheep exhibited severe skeletal muscle damage, characterized by extensive foci of hyaline or lytic necrosis accompanied by fragmentation and mineralization. Necrotic fibres were infiltrated by macrophages, fibroblasts and isolated neutrophils. Individual or small groups of regenerating fibres were evident throughout the affected muscles (Fig. 5 and 6). Granular degeneration and swelling of fibres were evident in eight cases

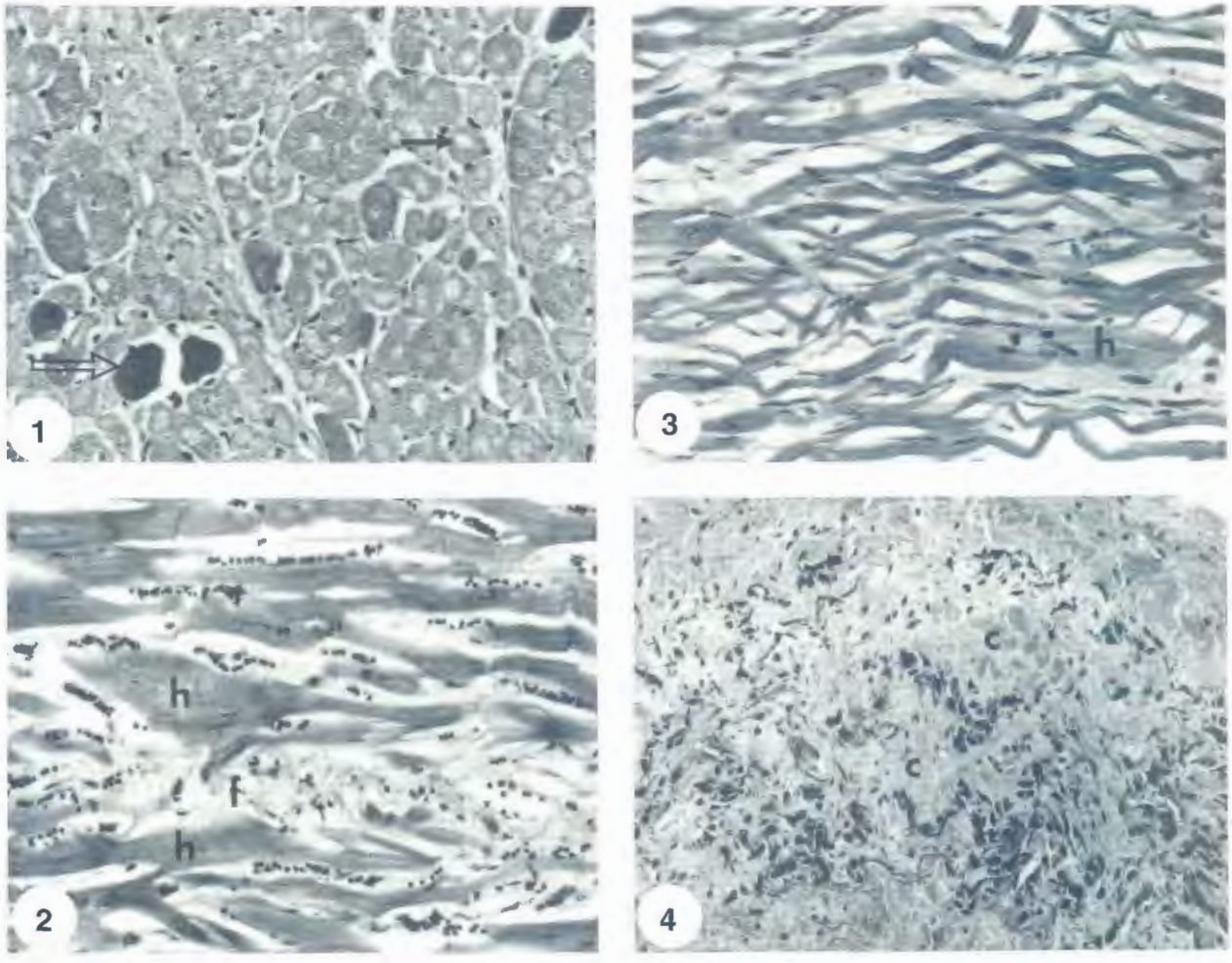


FIG. 1 Heart from a bovine field case. Central vacuolation of fibres (closed arrow) with hyalinization of scattered fibres (open arrow). HE X 220
FIG. 2 Heart from a bovine field case. Disarray of hypertrophic fibres (h) and focal fibrosis (f). HE X 220
FIG. 3 Heart from a bovine field case. Note atrophic fibres with a wavy course and interspersed hypertrophic fibres (h). HE X 220
FIG. 4 Heart from a bovine field case with endocardial thickening. Note disorganization, disruption and deposition of elastic fibres (black-staining) and collagen fibres (c). MT X 220

and contraction bands, hyalinization and/or lysis/fragmentation of single or small groups of fibres, in five animals.

LIVER

One or more of the following lesions consistent with heart failure were seen in 15 cattle and three sheep. In the cattle there was moderate to severe centrilobular necrosis in 12, centrilobular haemorrhage in seven, centrilobular to midzonal fatty changes in six and congestion and dilatation of the central veins and centrilobular sinusoids in three cases. One animal also manifested centrilobular fibrosis, haemosiderosis, sinusoidal distortion and hepatocellular loss.

Hepatic lesions in the sheep were mild, and included congestion and dilatation of the central veins and centrilobular sinusoids in three animals and centrilobular fatty changes in one.

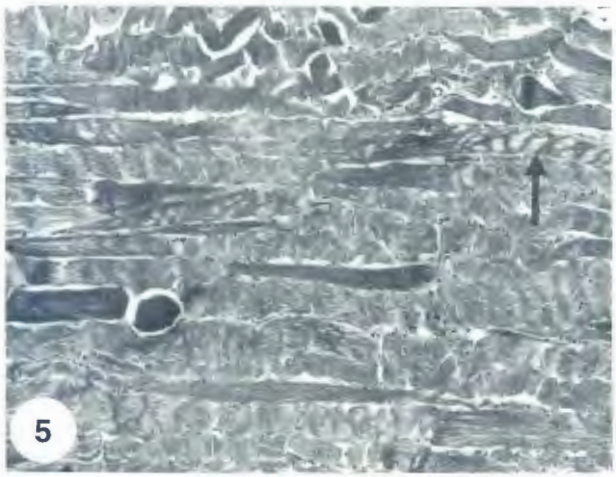
LUNGS

Lesions suggestive of congestive heart failure were noted in four sheep and in 14 of the 15 cattle examined. These consisted of congestion, alveolar oedema, thickening and collapse of the alveolar walls, intra-alveolar accumulation of macrophages and interlobular oedema. A mild focal fibrinopurulent pneumonia was present in three cattle.

Poultry litter feeding trials

Gross pathology

The steer displayed multiple subcutaneous and intermuscular haemorrhages, pulmonary congestion and



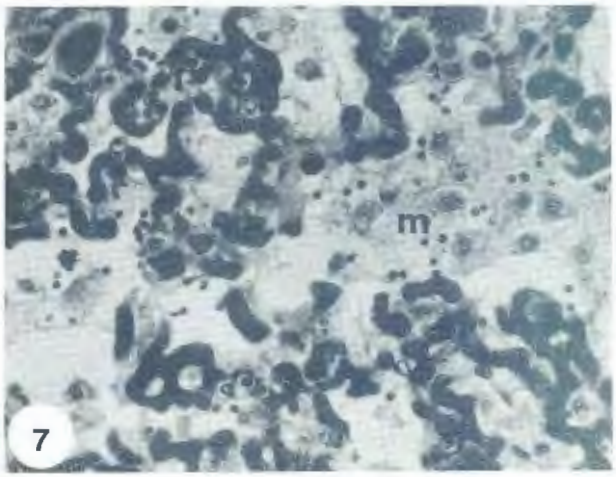


FIG. 5 Skeletal muscle from an ovine field case. Several fibres are hyalinized (dark staining). Others reveal partial lysis (arrow). HE X 90

FIG. 6 Skeletal muscle from an ovine field case. Extensive necrosis of muscle fibres and mild cellular infiltration. Scattered muscle fragments, though swollen, still reveal striations. HE X 220

FIG. 7 Lung from a bovine field case. Diffuse pulmonary congestion and oedema and focal intra-alveolar haemorrhage and aggregation of macrophages (m). HE x 270

oedema, oedema of the abomasal folds and gall-bladder and hepatic congestion with centrilobular pallor.

Signs of congestive heart failure, particularly hydropericardium and oedema of the subcutis of the ventral neck region were recorded in one sheep. In two animals, the hearts appeared rounded and dilated, and the free ventricular walls were thin. No significant gross lesions were seen in the remaining three cases.

Histopathology

HEART

The myocardial lesions are summarized in Table 3. Striking changes in the steer included diffuse hypertrophy and hyperplasia of the myofibre nuclei; diffuse atrophy of myofibres; multifocal areas of increase in myofibre diameter and disorganization of fibres; granular degeneration of fibres and scattered foci of replacement fibrosis.

Cardiac lesions in the sheep were less severe than those in the cattle. These included diffuse myofibre degeneration, hypertrophy of the myofibre nuclei, scattered foci of hyalinization and isolated foci of necrosis with an associated inflammatory reaction. Focal atrophy of myocardial fibres was noted in two sheep and foci of myofibre necrosis with cellular infiltration, in one.

SKELETAL MUSCLES

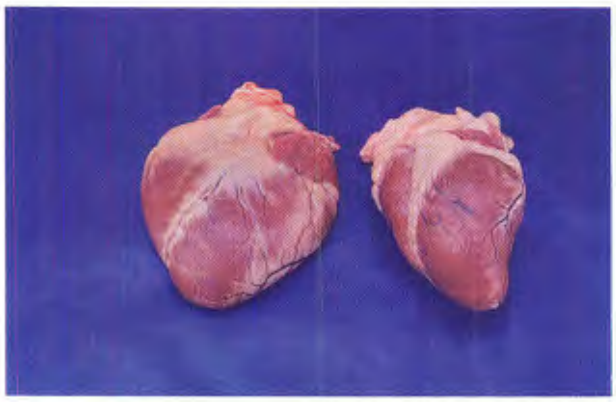
Lesions were mild and included isolated foci of hyalinization of fibres in four sheep, granular degeneration and swelling of the fibres in three cases and scattered small foci of necrosis with celular infiltration or fibrosis in two animals (Table 4).

LIVER

The hepatic lesions were similar to those encountered in the field cases, namely centrilobular necrosis and haemorrhage with fatty changes of the midzonal hepatocytes in the steer, and congestion with dilatation of the central veins and centrilobular sinusoids in the sheep.

LUNGS

Lesions in the steer included alveolar and septal oedema and congestion, with focal aggregates of intra-alveolar macrophages (Fig. 7). Pulmonary lesions in the



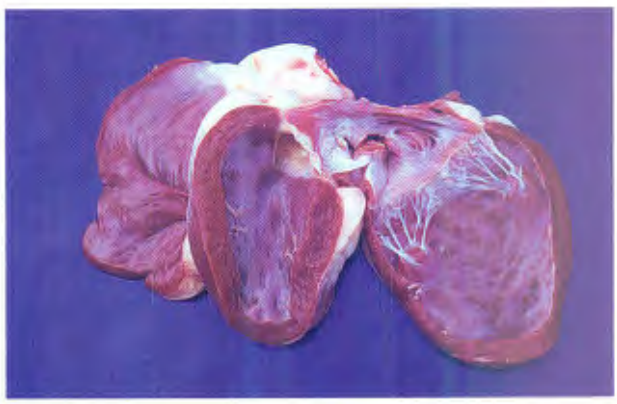


FIG. 8 Dilated heart with a rounded outline and apex, left, from a sheep on ration B. Normal-shaped heart, right, from a sheep on the control ration

FIG. 9 Heart from a sheep on ration B. Note the thin septum and ventricular walls and flattened and widened papillary muscles in the left ventricle

sheep were mild and consisted of focal thickening and collapse of the alveolar walls with a few aggregates of intra-alveolar macrophages.

Maduramicin feeding trials

Gross pathology

Mild ascites and/or hydropericardium were apparent in two of the sheep. In one animal the heart was pale, and in four, the heart was moderately dilated, with a rounded apex and outline (Fig. 8). The free ventricular walls and interventricular septa in these sheep were thin and flabby and the papillary muscles flattened (Fig. 9).

The skeletal muscles of three sheep were pale and oedematous. In five cases several fine yellow-white streaks were evident in the muscles of the fore- and hind limbs, neck, back, chest and abdomen.

Multifocal lobular atelectasis and occasional areas of adjacent emphysema were present in five animals. In most sheep, hepatic lobules were accentuated and the kidneys were slightly swollen. The urine of one animal was dark brown, suggesting myoglobinuria.

Histopathology

HEART

The lesions are summarized in Table 5. There were no meaningful differences as regards the type or distribution of lesions in the left and right free ventricular walls, apex and interventricular septum.

Examination of all treated sheep revealed diffuse, fine granularity of the sarcoplasm with, in some cases, focal areas of sarcoplasmic vacuolation (Fig. 10). Diffuse hypertrophy of the myocardial fibre nuclei was evident in all seven sheep (Fig. 10 and 11). Four animals displayed focal areas of increase in myofibre diameter and an accompanying disarray and interweaving of fibres (Fig. 11). Diffuse atrophy of myofibres occurred in six animals (Fig. 10) and focal atrophy in one.

Foci of myofibre loss with fibrosis were apparent in six sheep (Fig. 11) and foci of hyalinization in five. Foci of myofibre necrosis with cellular infiltration and focal endocardial thickening were present in each of two cases.

SKELETAL MUSCLES

The fibres of all the treated animals were finely granular, and isolated to scattered foci of hyalinization or lysis with cellular infiltration were apparent. Focal areas of fibre regeneration, evidenced by hypervascularization, hypernuclearity, basophilia and the central positioning of nuclei, were a distinctive feature in all the sheep. These foci were most prominent in the three sheep fed ration B (5 ppm maduramicin). In two sheep on ration B and one on ration C (2,5 ppm maduramicin), several centrally situated, large, rounded or irregularly shaped vacuoles, probably representing areas of myocytolysis or sarcoplasmic dilatation, were evident (Fig.12 and 13) (Table 6).

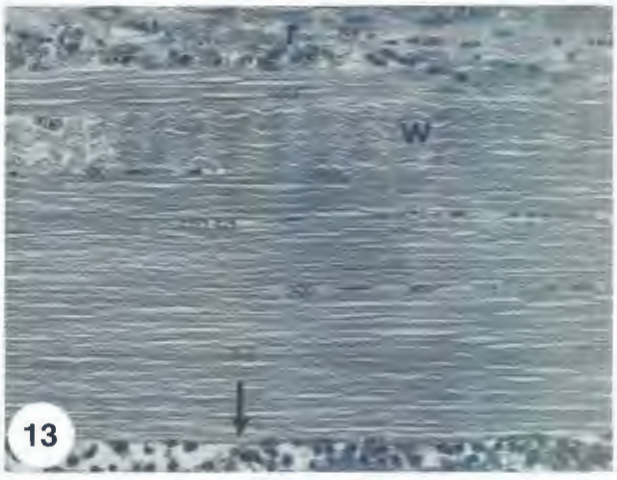
LIVER

There was mild cloudy swelling of the hepatocytes and dilatation of the central veins and centrilobular sinusoids in three cases.

LUNGS

The lungs in all the sheep fed rations A, B or C revealed diffuse thickening of the alveolar walls, leucocytosis (mainly monocytes and lymphocytes), multifocal alveolar collapse and the presence of haemosiderin-laden (Prussian-blue-positive) macrophages in the alveolar lumens (Fig. 14).

No specific lesions were evident in any of the other tissues examined.



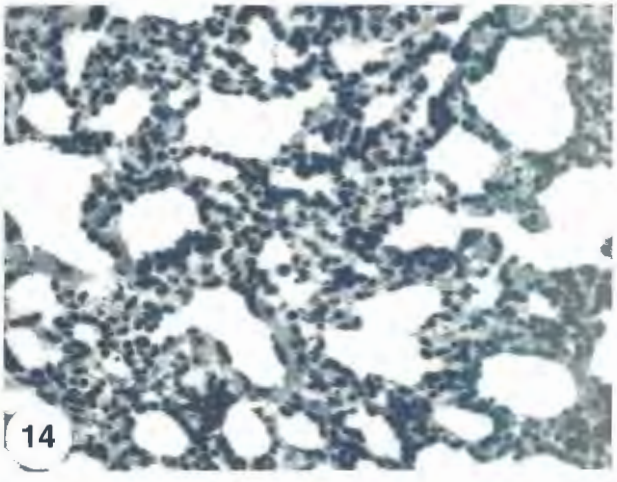


FIG. 10 Heart from a sheep on ration C. Moderately atrophic fibres with hypertrophic nuclei and scattered foci of myofibre disintegration and vacuolation. HE X 270

FIG. 11 Heart from a sheep on ration C. Disorganized hypertrophic (h) and atrophic (a) fibres with focal fibrosis (f). HE X 90

FIG. 12 Skeletal muscle from a sheep on ration C. Note hyaline necrosis (n) and myofibre vacuolation (arrow) and waviness (w). HE X 90

FIG. 13 Skeletal muscle from a sheep on ration B. The majority of fibres appear relatively normal. Note the myofibrillar waviness (w), hypercellular regenerating fibre (r) and lysed fibre infiltrated by cells (arrow). HE X 220

FIG. 14 Lung from a sheep on ration C. Chronic interstitial pneumonia characterized by congestion, thickening, hypercellularity and focal collapse of alveolar walls. HE X 270

DISCUSSION

The clinical signs, clinical pathological findings (Fourie *et al.* 1991) and gross and light microscopic lesions point to heart failure as the cause of death in both the field and experimental cases of poultry litter toxicity. Macroscopic lesions similar to those observed in the field cases and poultry litter trial, have been reported in cattle fed dried poultry litter in Israel (Perl, Schlosberg, Hoida, Davidson, Yakobson & Orgad 1991) and in cattle and sheep poisoned with monensin (Wardrope, Macleod & Sloan 1983; Newsholme, Howerth, Bastianello, Prozesky & Minné 1983).

Mottling of the heart resulting from myocardial necrosis and fibrosis occurs commonly in cases of ovine and bovine monensin and salinomycin poisoning (Newsholme *et al.* 1983; Van Vleet, Amstutz, Weirich, Rebar & Ferrans 1983; Bastianello 1986), but was absent in animals in this study. Instead, cardiac dilatation evidenced by rounding of the outline of the heart and thinning of the ventricular walls was noted in both field and experimental cases of poultry litter intoxication and in sheep in the maduramicin trial. Cardiac dilatation has been reported in field cases of gousiekte, a plant-induced cardiomyopathy of ruminants (Theiler, Du Toit & Mitchell 1923; Prozesky *et al.* 1988), but has not been recorded in other toxic myocardial conditions of sheep and cattle in southern Africa (Kellerman, Coetzer & Naude 1988). The higher prevalence of cardiac dilatation in the sheep fed rations incorporating maduramicin than in field cases, suggests that this feature may have been missed in the initial cases of poultry litter toxicity necropsied in the field. In subsequent outbreaks of poultry litter toxicity, not reported in this paper, cardiac dilatation was noted more commonly.

Histologically, the myocardial lesions in cases of poultry litter and maduramicin toxicity were similar, differing only in degree and extent. The most consistent lesions included diffuse granular degeneration, hypertrophy and atrophy of myocardial fibres with multifocal small areas of fibre loss and fibroplasia. Other less consistent lesions included necrosis of fibres with or without an attendant cellular inflammatory reaction and focal endocardial thickening.

Although no morphometric studies to evaluate hypertrophy and atrophy of myocardial fibres were done in this series, ample qualitative evidence of these changes were found. Hypertrophy and hyperplasia of the myocardial nuclei were noted in all the field and experimental cases of poultry litter toxicity and in all the animals in the maduramicin trial. Indications of more advanced fibre hypertrophy, such as an apparent increase in the diameter of fibres and focal disarray of fibres, were evident in 83 % of the cattle and sheep in the field outbreaks, and 57 % of the animals in the poultry litter and maduramicin trials. Diffuse atrophy of myocardial fibres occurred commonly in the sheep in the maduramicin trial, and in the field outbreaks 30% of the cattle manifested severe diffuse atrophy.

Meerson (1969) cited by Ferrans (1978) proposed that cardiac muscle fibres evolve through three distinct functional stages during the course of hypertrophy: firstly, a phase of increased energy production and protein synthesis; secondly, a stable state of hyperfunction; and, thirdly, a stage of exhaustion with inability of the heart to synthesize proteins, and consequent myofibrillar damage with fibre degeneration or atrophy. In this report, individual atrophic fibres or groups of atrophic fibres were often intermingled with hypertrophic fibres. This suggests that affected fibres initially became hypertrophic with eventual exhaustion and atrophy.

In this study, multifocal myocardial necrosis with an attendant inflammatory reaction or small areas of fibre loss and fibroplasia, were often encountered in animals with severe hypertrophy and/or atrophy. These foci therefore probably represent fibres in the final or exhausted stage of hypertrophy, which became atrophic, died off and were replaced by collagen. On the other hand, these lesions may be the result of the necrotizing effect of an ionophore, in particular maduramicin, in the ration as myocardial necrosis with consequent cellular infiltration or fibroplasia has consistently been observed in cases of acute ionophore toxicity in ruminants (Van Vleet & Ferrans 1986; Bastianello 1986).

Granularity of myocardial fibres, attributable to mitochondrial swelling, was consistently present in natural and experimental cases of poultry litter poisoning and in animals in the maduramicin trial. Sarcoplasmic vacuolation was occasionally also present in these fibres. Granularity and vacuolarity of fibres have regularly been reported in cases of monensin toxicity in cattle and sheep (Van Vleet *et al.* 1983; Newsholme *et al.* 1983). Vacuolation of myocardial fibres is also considered to be a prominent feature of dilated cardiomyopathy in humans (Unverferth 1985).

Focal endocardial thickening was observed in two cattle in field outbreaks of poultry litter toxicity and two sheep in the maduramicin trial. According to Davies (1975), this lesion represents a response of the endocardium to chronic myocardial damage or ventricular dilatation.

Hyalinization of individual myocardial fibres, or groups of them, was observed in natural and experimental cases of poultry litter poisoning and in animals in the maduramicin trial. Hyalinized foci have been observed in a variety of conditions and are apparently associated with the release of endogenous catecholamines (Newsholme 1982).

Cattle in the field outbreaks and the steer fed toxic poultry litter had minimal skeletal muscle lesions. This correlated with the occasional mild locomotory abnormalities encountered in cattle, *viz.* a stiff gait and fine tremors of the back and hindquarter muscles (Fourie *et al.* 1991). In sheep, however, severe locomotory disturbances, characterized by reluctance to move, a stiff gait, and eventually posterior ataxia and recumbency were encountered in one of the three field outbreaks involving sheep, in two sheep fed toxic poultry litter and in the seven sheep of the maduramicin trial (Fourie *et al.* 1991).

Skeletal muscle lesions in the sheep fed the maduramicin-containing rations were less pronounced and more chronic than the severe degeneration, necrosis and fibroplasia seen in the sheep from the field outbreak.

Fourie *et al.* (1991) recorded high levels of aspartate transaminase (AST) activity in the sera of sheep within 4–12 d of feeding the maduramicin ration. The AST activity peaked at *c* 8 d and tapered down to normal 50–90 d later when the sheep were slaughtered. The initial high AST levels probably correspond with the stage of extensive muscle damage, whilst the diminished levels at the time of necropsy can be attributed to the chronic reparative nature of the lesions at this stage.

Muscle lesions similar to those described in the field outbreaks in sheep have also been reported in acute monensin intoxication (Confer, Reavis & Panciera 1983; Newsholme *et al.* 1983). In natural and experimental cases of acute monensin poisoning, extensive muscle damage and, frequently, mortality occur within 2–10 d of ingestion of monensin (Confer *et al.* 1983; Van Vleet *et al.* 1983). This corresponds with the period of high AST activity in the sheep in this report, fed maduramycin-containing pellets. On the other hand, chronic muscle lesions resembling those outlined in the animals in the maduramicin trial in this paper have been reported by Nation, Crowe & Harries (1982) in sheep slaughtered approximately 2 months after the ingestion of toxic levels of monensin.

In natural and experimental cases of poultry litter poisoning, cattle and sheep displayed pulmonary and hepatic lesions compatible with congestive heart failure. In cattle, centrilobular necrosis was the most striking hepatic lesion. Congestion and mild dilatation of the central veins were, however, the only noteworthy changes in sheep. Pulmonary lesions indicative of congestive heart failure—such as alveolar and septal oedema and alveolar macrophage accumulation—were pronounced and common in cattle. In sheep, the pulmonary lesions were mild and chronic in nature, being characterized by alveolar wall thickening and fibrosis, focal atelectasis and the accumulation of haemosiderin-laden macrophages in scattered alveoli.

The macro- and microscopic cardiac features of this intoxication, namely, dilatation, myofibre hypertrophy and vacuolation and fibrosis, correspond with the lesions described in dilated cardiomyopathy in humans (Unverferth 1985). It is therefore suggested that this form of poultry litter poisoning represents a toxic form of dilated cardiomyopathy in cattle and sheep.

A preliminary diagnosis of poultry litter poisoning is based on the epidemiological data, clinical symptoms and macro- and microscopic pathology.The clinical and pathological evidence of heart failure and involvement of skeletal muscles suggest that this intoxication represents a chronic form of ionophore poisoning. In the first part of this report it was concluded that maduramicin was the most likely ionophore involved (Fourie *et al.* 1991). In fact, fewer field outbreaks of this form of poultry litter toxicity have occurred in South Africa since 1991, when a recommendation was passed that no poultry litter derived from rations containing maduramicin may be sold for cattle and sheep feed without a warning on the label that maduramicin is highly toxic in these species (Bastianello & Fourie, unpublished observations 1994). Recently, however, an outbreak of ionophore toxicity occurred in which several cattle died suddenly within *c* 21–28 d of being fed a complete ration containing toxic (*c* 90 ppm) levels of the ionophore, salinomycin. The myocardial micropathology in the cattle in this outbreak was highly comparable to that seen in cattle ingesting toxic litter, *viz.* a cardiomyopathy characterized by myocardial fibre atrophy and hypertrophy and scattered foci of fibrosis (Bastianello, unpublished data 1994). The possible role of other ionophores in this form of poultry litter toxicity should therefore not be ignored.

ACKNOWLEDGEMENTS

The authors acknowledge the help of the technical staff and other personnel of the Division of Pathology.

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