A MACRO- AND LIGHT- MICROSCOPICAL STUDY OF THE PATHOLOGY OF GOUSIEKTE IN SHEEP

3.1 INTRODUCTION

Gousiekte is characterised by a latent period of approximately four to eight weeks between exposure of animals to the plant material and natural death. Macroscopical lesions indicative of congestive heart failure are present in most cases. A diagnosis of gousiekte is traditionally confirmed by demonstrating the presence of "typical" microscopic lesions, namely necrosis, replacement fibrosis, and round cell infiltrates of varying intensity, especially in the sub-endocardial region of the apex and the left ventricular free wall (Theiler, Du Toit & Mitchell 1923; Newsholme & Coetzer 1984; Kellerman *et al.* 2005).

Some naturally poisoned animals show degeneration of myofibres as the principal lesion (Smit 1959). Marked deviations from the "typical" lesions (i.e. myofibre degeneration) have also been reported in some experimental cases (Hurter *et al.* 1972). However, these changes are not generally recognised as grounds for diagnosis.

Since a diagnosis of the disease can be confirmed only by histopathological examination of the myocardium, it is imperative to appreciate the full spectrum of lesions in order to confirm a diagnosis in animals with either "typical" or "atypical" lesions.

The aims of this study were to investigate the effect of the duration of latency on the nature of the myocardial lesions in the left free ventricular wall in sheep dosed with *Pachystigma pygmaeum* and to characterise macro- and microscopical lesion patterns in animals with different latent periods.

3.2 MATERIALS AND METHODS

3.2.1 Dosing trial

Ten Merino sheep approximately 12 months old (ewes and wethers) were dosed per stomach tube with dried, milled *Pachystigma pygmaeum* plant material (table 3.1). *P. pygmaeum* (hairy gousiektebossie) plants were collected from Swartrand ($26^0$17'S, $26^0$48'E) in the North-West Province of South Africa where gousiekte is rife. The plant material was dried in the shade, milled to a coarse powder and stored at –10 ^{0}C. *P. pygmaeum* was selected for the trial because it was the most readily obtainable of the gousiekte plants and farmers annually reported a high incidence of gousiekte in the area. It was therefore highly probable that the plants would be toxic. The South African National Biodiversity Institute in Pretoria verified the identification of the plants.

All the animals, including two control sheep who did not receive the plant material, were clinically healthy at the beginning of the experiment, routinely vaccinated against enterotoxaemia, dewormed, housed separately and their temperature and cardiac and respiratory rates recorded daily. The animals daily received a balanced ration consisting of hay (*Eragrostis),* oats and lucerne (at a ratio of 2:2:1 - 700 g per 45 kg) and concentrated pelleted feed (600 g per 45 kg) and had free access to water.

Since the toxicity of gousiekte plants is variable and diminishes during drying and storage and animals vary in their susceptibility, it was decided to administer a relatively large dose of plant material of approximately 10 g per kilgram live body weight every week day but not over weekends (table 3.1) (Kellerman *et al.* 2005). The dosage rate was based on results of unpublished trials using gousiekte plants collected and stored in the same way. Tachycardia as

measured by auscultation (>90 beats per minute) was the single most important clinical parameter used during latency to determine whether a lethal dose had been given (Pretorius & Terblanche 1967). As soon as tachycardia was noted the dosing regimen was terminated so that the longest possible latent period could be induced.

3.2.2 Pathology

All treated animals either died naturally or were euthanased with an overdose of pentobarbitone sodium when *in extremis*, between 31 and 51 days after the commencement of dosing (table 3.1). The control animals were euthanased at the time when the last experimental animal was necropsied (day 51). Animals were necropsied immediately after euthanasia. Animals that died naturally were necropsied as soon as possible after death but no later than two to three hours after death. At necropsy, for this study, three to four transmural blocks of tissue measuring approximately 1 cm^3 were collected from the middle of the left free ventricular wall of all experimental and control animals and preserved in 10 % buffered formalin. Specimens from various organs, including the lungs, liver, spleen, kidney, gastrointestinal tract and brain, were also collected in 10 % buffered formalin from each case following a complete necropsy. The samples were routinely processed for histopathological examination and stained with haematoxylin and eosin (HE). Two transmural planes were sectioned from each myocardial block to allow examination of both the endo- and the epicardium. Selected sections were stained with Masson's trichrome stain for collagen (Armed Forces Institute of Pathology 1968).

3.2.3 Imaging analysis

For imaging analysis, stained sections (HE and Masson's trichrome) from two control animals (control group) and three of the treated animals (sheep 1, 6 and 10) were photographed with an Olympus BX 50 microscope using a CC12 soft imaging system. The scanned photomicrographs were imported to a drawing template of the 1TEM software imaging system and scaled to the original print of the photograph by using the "bar". Measurements were taken with the 1TEM

soft imaging system. The three treated animals were selected on the basis of their latent periods, namely 31, 42 and 51 days respectively, which represented the entire spectrum of the latent period (table 3.1). The following measurements were taken of not fewer than 15 randomly selected fibres that had full nuclear profiles in each animal in the subendocardial region of the left free ventricular wall: myofibre diameter at the level of the centre of the nucleus (fig. 3.1), nucleus perimeter, and area.

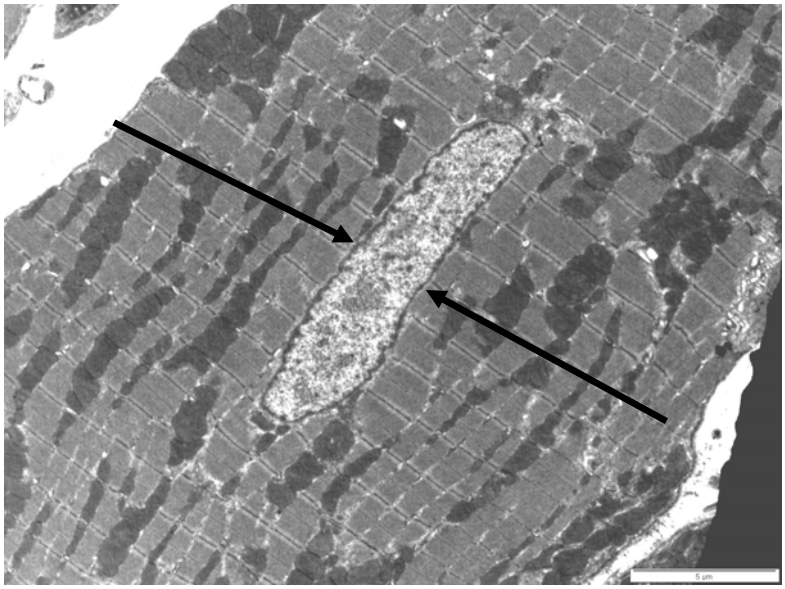


Figure 3.1 Transmission electron microscopical picture of a cross-section of a myofibre to illustrate the measurement of the myofibre diameter at the level of the centre of the nucleus (arrows). (Bar = 5 µm)

3.3 RESULTS

Table 3.1 Sheep examined after dosing with *Pachystigma pygmaeum*

Sheep no.	Gender E: ewe W: wether	Initial live mass (kg)	Dosing regimen (g/kg x no. of days)	Total dose (kg)	First day with tachycardia	Day of death	Days from tachycardia to death
1	W	31	10 x 23	7,13	30	31	1
2	E	22	10 x 30	6,60	34	34	0
3	E	27	10 x 30	8,10	34	35*	1
4	E	25	10 x 30	7,50	34	36	2
5	E	33	10 x 30	9,90	34	38	4
6	E	27	10 x 30	8,10	34	41*	7
7	W	35	10 x 30	10,5	39	42	3
8	W	31	10 x 31	9,61	42	43	1
9	W	25	10 x 31	7,75	42	51	9
10	W	28	10 x 31	8,68	42	51*	9
11	W	26	Control animal			51	
12	W	28	Control animal			51	

Key

***** Animals that were euthanased

3.3.1 Macropathology

Table 3.2 Macroscopical pathological features in ten sheep dosed with *Pachystigma pygmaeum*

Sheep no.	Latent period (days)	Pulmonary oedema and hydro-pericardium	Hydro-thorax	Generalised congestion and hepatosis	Cardiac dilatation
1	31	–	–	–	–
2	34	+	–	–	–
3	35	–	–	–	–
4	36	+	–	–	–
5	38	+	–	–	–
6	41	+	+	–	–
7	42	+	–	–	–
8	43	+	+	+	–
9 *	51	+	+	+	+
10 **	51	+	+	+	+

Key to other lesions

* Subendocardial fibrosis and ascites

** Myocardial mottling, ascites and oedema of the mediastinum, mesenterium, abomasum and wall of the gall bladder

– Lesion absent

\+ Lesion present

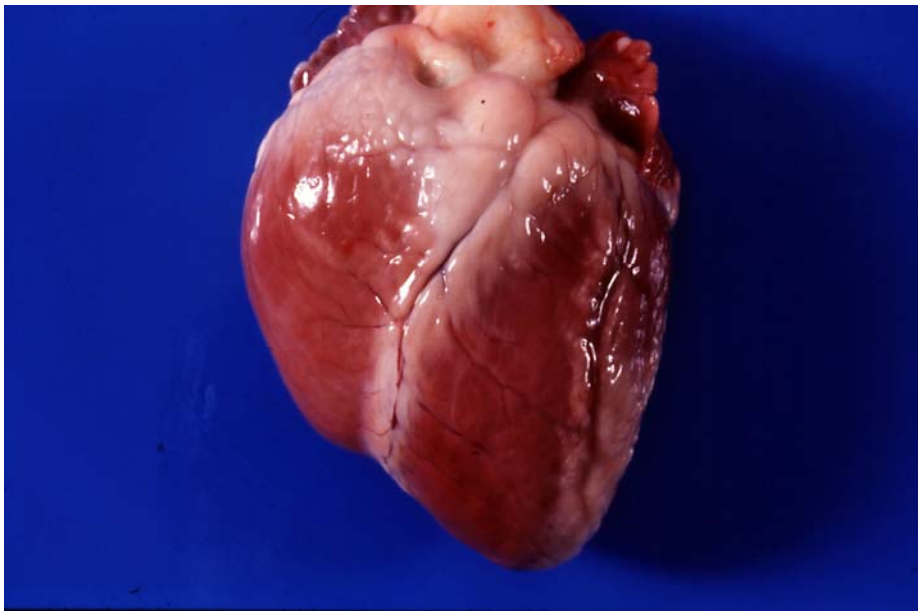


Figure 3.2 Normal heart

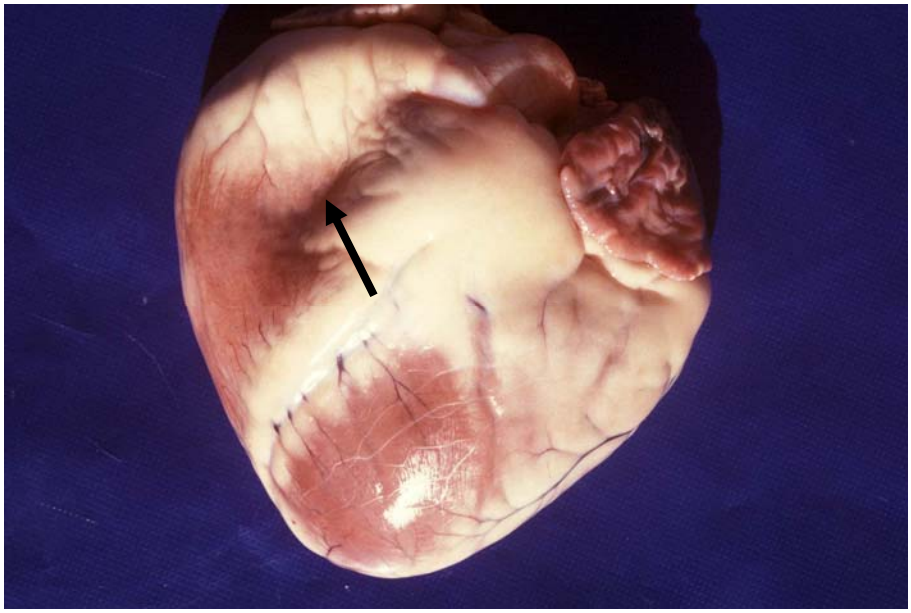


Figure 3.3 Dilated heart in sheep 10 with a long latent period. Note round shape and flabby appearance with collapse of right ventricle because of loss of tone (arrow)

In two sheep (9 and 10) cardiac dilatation was evident (table 3.2). For comparative purposes the heart of a control animal is depicted in figure 3.2. Subjective criteria used in the identification of a dilated heart included the size and shape of the heart. Affected hearts tended to be flabby, rounded in shape with no defined apex (fig. 3.3), and showed attenuated papillary muscles, thickening of the endocardium with opaqueness of the subendocardial myo-

cardium owing to fibrosis, and thinning of the free wall of the dilated chamber. Subendocardial pallor (fibrosis) in sheep 9 and transmural myocardial mottling in sheep 10 (table 3.2) extended with decreasing severity from the apex and the left free ventricular wall (most severe lesions) to the interventricular septum and the right free ventricular wall.

Pulmonary oedema (fig. 3.4) and hydropericardium (fig. 3.5) were present in eight sheep (table 3.2). The lungs were wet and heavy, did not collapse completely when the thorax was opened, were firmer and doughy in consistency, pitted on pressure, and crepitation was reduced. The interlobular septae were dilated, particularly at the edges of the lobes. Fluid oozed from the cut surfaces and the bronchi and trachea were filled with varying amounts of white foam. Multifocal areas of atelectasis were scattered throughout the lungs.

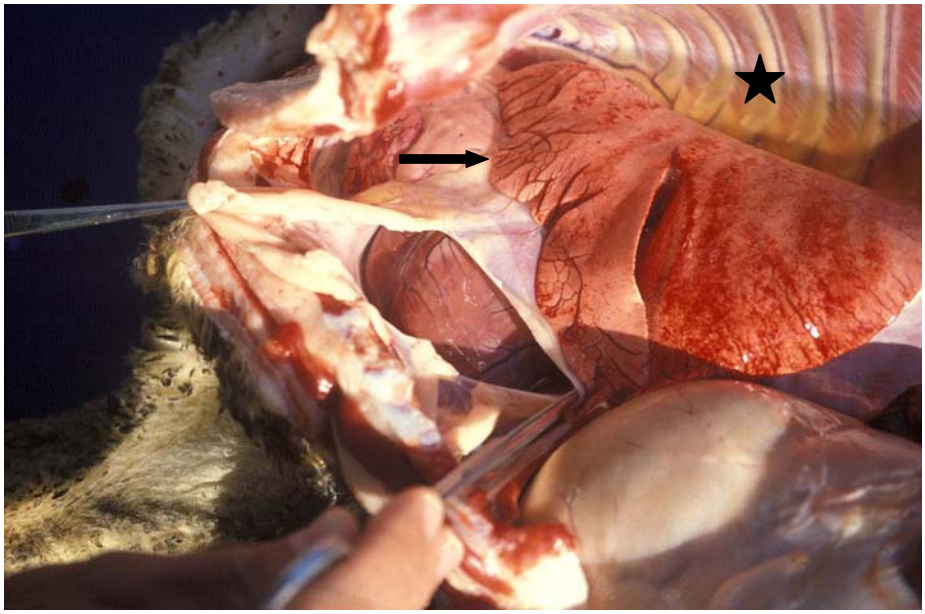


Figure 3.4 Pulmonary oedema depicted as dilatation of the interlobular septae (arrow) and hydrothorax (star) in sheep 10 that died of gousiekte after a long latent period of 51 days

Hydropericardium was characterised by a serous, light yellow fluid that varied in amount from approximately 40 ml to 100 ml. Hydrothorax was noted in sheep 6, 8, 9 and 10 and ascites was evident in two cases (sheep 9 and 10). In all the animals the kidneys were bilaterally symmetrically slightly enlarged, oedematous and variably congested, and the capsule was stripped easily and

showed moderate cortical pallor. The most striking hepatic lesions included mild swelling with round edges, a taut capsule and a dull appearance (hepatosis). In one animal (sheep 10) the liver on cut section had a mottled appearance (suspected centrilobular necrosis). Other lesions noted included generalised congestion in sheep 8, 9 and 10, and oedema of the mediastinum, mesenterium, abomasum and the wall of the gall bladder in sheep 10 (table 3.2).

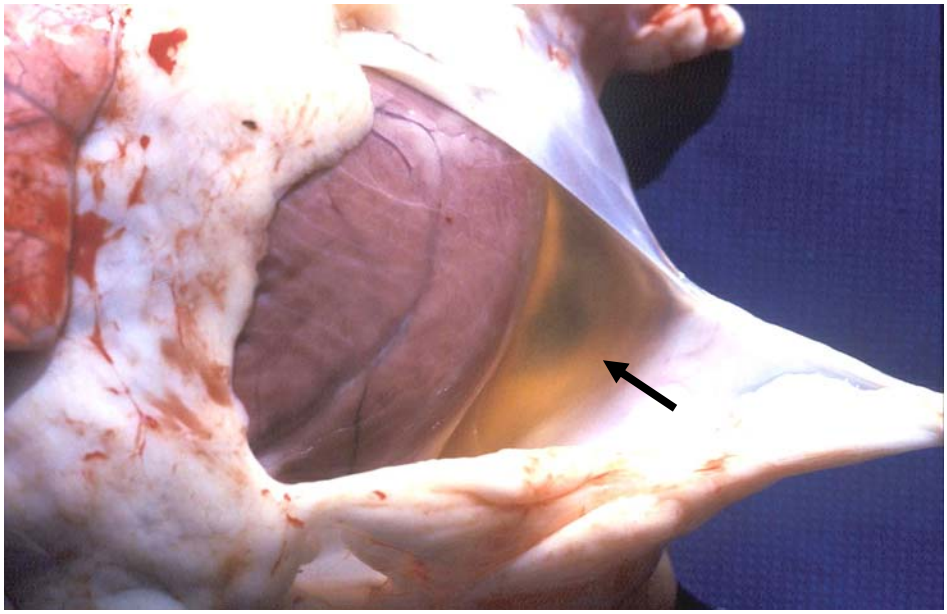


Figure 3.5 Hydropericardium (arrow) in sheep 9 that died after a long latent period of 51 days

3.3.2 Histopathology

Although macromyocardial changes were apparent only in sheep 9 and 10 (table 3.2), light-microscopical lesions were evident in all the animals (table 3.3).

Table 3.3 Histopathological lesions in the subendocardial region of the left ventricle of ten sheep dosed with *Pachystigma pygmaeum*

Sheep no.	Myofibre hyper-trophy	Mono-nuclear cell infiltration	Myofibre necrosis	Replace-ment fibrosis	Endo-cardial thicken-ing	Myofibre atrophy	Arterial medial hyper-trophy and oedema
1	+	+				+++	
2	+	+	+	+			+
3	+	+	+	+	+	+	+
4	+	+	+	+		+	
5	+	+	+		+		+
6	+	+	++	++	+	++	
7	+	++	+		+	+	+
8	+	+		+++	+	++	
9	+	++	+	++	++	++	++
10	+	++		+++	++	++	++

Key

□	Lesion absent	++	Moderate lesion
+	Mild lesion	+++	Severe lesion

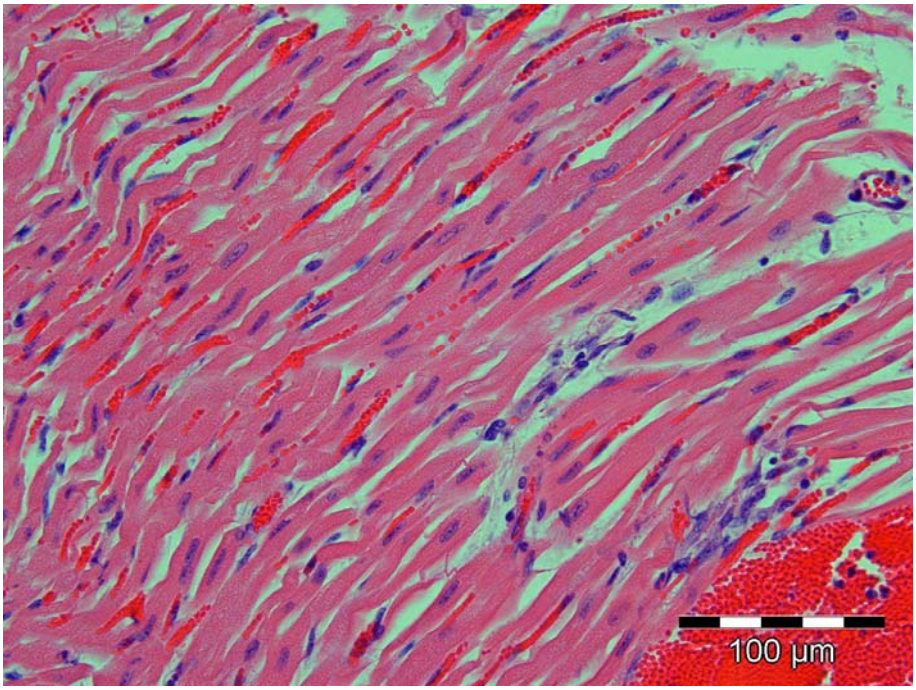


Figure 3.6 Normal myofibres in the subendocardial region of the left free ventricular wall of a control animal. HE

The main histopathological lesions in the experimental animals are outlined in table 3.3. A longitudinal section of a control (normal) heart is depicted in figure 3.6. The lesions were located primarily in the subendocardial region (inner approximately 200-300 µm) and extended to the inner third of the myocardium. Lesions were, in order of prevalence, myofibre hypertrophy, mononuclear cell infiltration, replacement fibrosis, myofibre necrosis, oedema and medial hypertrophy of arterioles and arteries, endocardial thickening and myofibre atrophy.

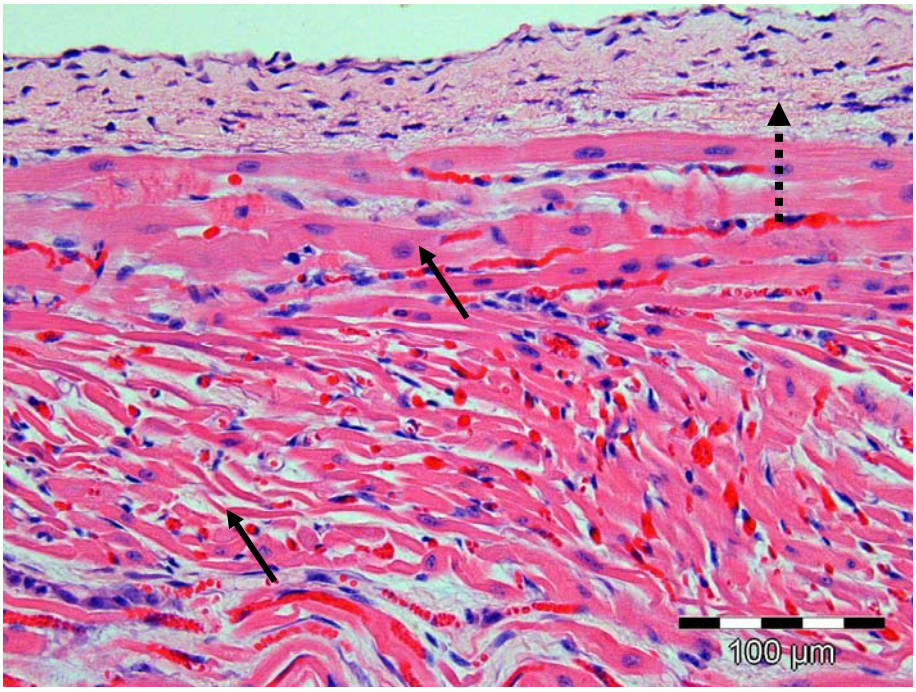


Figure 3.7 Fibre hypertrophy (top solid arrow) and atrophy (bottom solid arrow) in the subendocardial region of an animal with a long latent period (sheep 10). Note the thickened endocardium (dotted arrow). HE

Multifocal to diffuse myofibre hypertrophy and hyperplasia of the myocardial fibre nuclei (characterised by large vesicular, round, oval or elongated nuclei, many with indented or wavy outlines), were recorded in all the sheep (figs 3.7, 3.8). Two to three nuclei, occasionally more, were frequently arranged in rows. Hypertrophy was mainly mild in nature and multifocal in distribution in sheep 1 and 2 and multifocal to diffuse in the remaining animals.

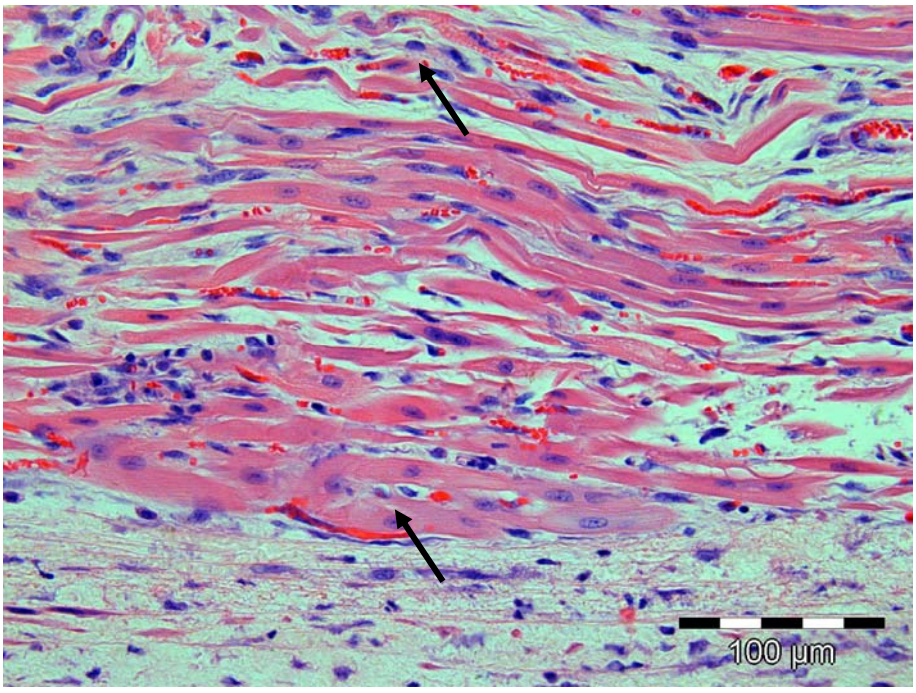


Figure 3.8 Atrophic fibres (top arrow) intermingled with hypertrophic fibres (bottom arrow) in the subendocardial region of a sheep with a long latent period (sheep 9). HE

Multifocal mononuclear cell infiltration was recorded in all the experimental sheep (fig. 3.9). The foci were generally small, contained few cells and were composed mainly of small lymphocytes and macrophages (mononuclear cells). In sheep 7, 9 and 10 the foci were prominent and contained moderate to large numbers of mononuclear cells. In all cases the foci were widely distributed throughout the interstitium, especially perivascularly, and the majority of foci were found closely associated with areas of fibrosis and necrosis.

Foci of replacement fibrosis were present in seven sheep. Sheep 2, 3 and 4 had small, indistinct, multifocal fibrosis. In sheep 6, 8, 9 and 10 the fibrosis was multifocal to diffuse and varied from moderate to severe in extent. Masson's trichrome stain was useful in appreciating the extent of the fibroplasia (figs 3.10, 3.11).

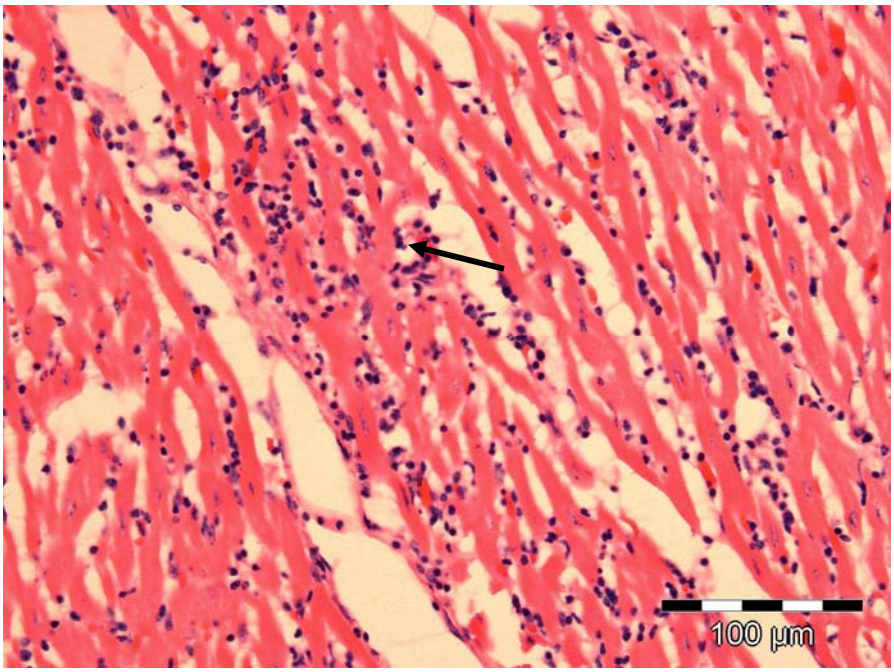


Figure 3.9 Moderate multifocal to diffuse round cell infiltration (arrow) in sheep 7. HE

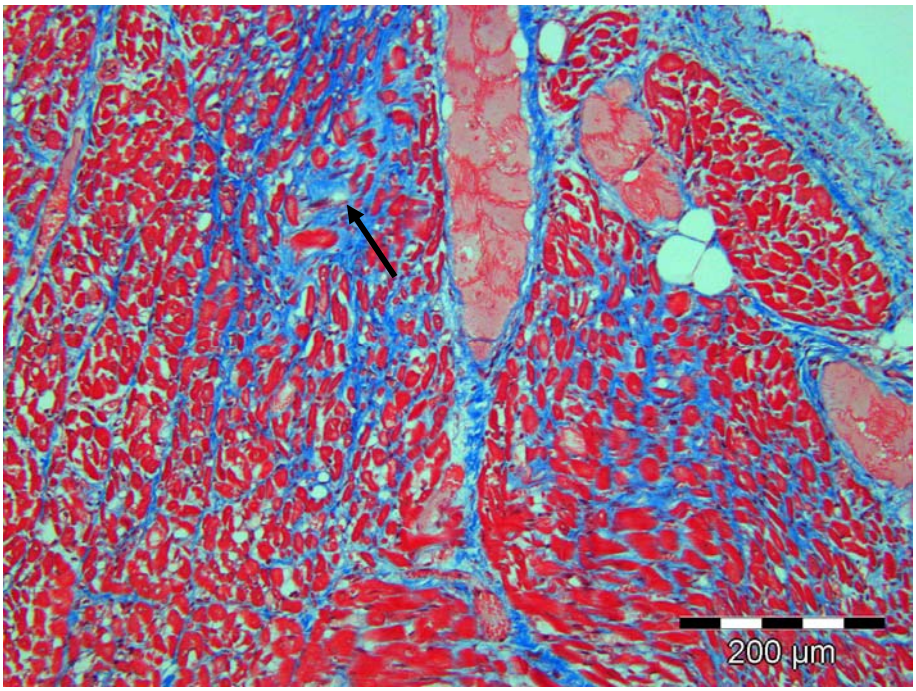


Figure 3.10 Cross-section of myocardial fibres with multifocal to diffuse severe replacement fibrosis (arrow) in the inner third of the myocardium of sheep 8. Masson's trichrome

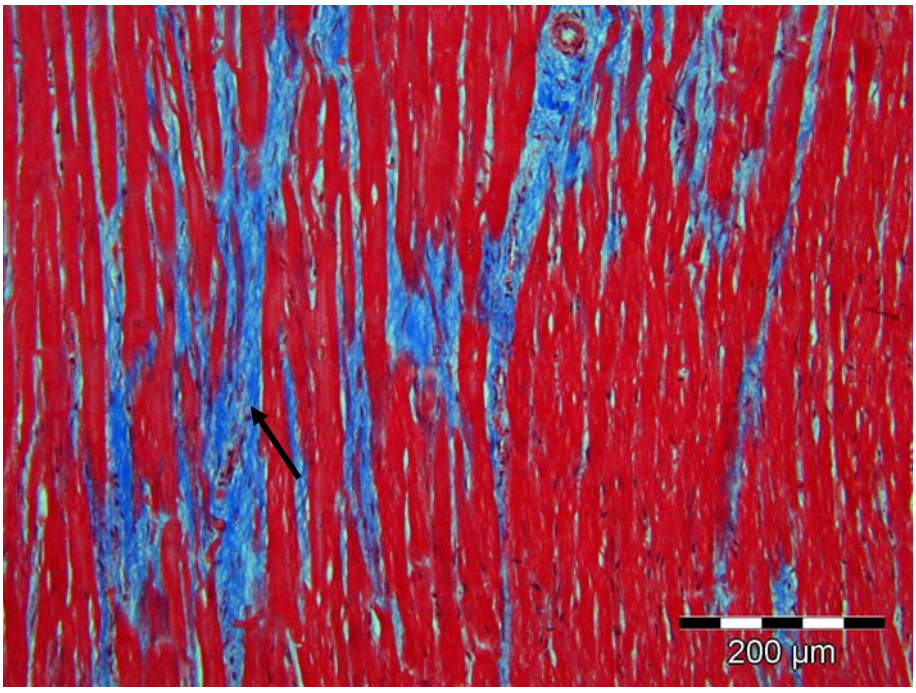


Figure 3.11 Longitudinal section of myofibres with multifocal replacement fibrosis (arrow) in sheep 8. Masson's trichrome

Multifocal coagulative necrosis of myofibres with hyalinisation of single or small to large groups of fibres was evident in seven sheep (sheep 2, 3, 4, 5, 6, 7 and 9). Affected fibres had highly eosinophilic sarcoplasm, striations were indistinct or absent, and nuclei were either unaffected or necrotic (fig. 3.12). In sheep 2, the foci were small and distributed throughout the left ventricular wall. In the remaining animals the foci were either evenly scattered throughout the ventricular wall or were more obviously associated with the areas of fibrosis in the subendocardial region.

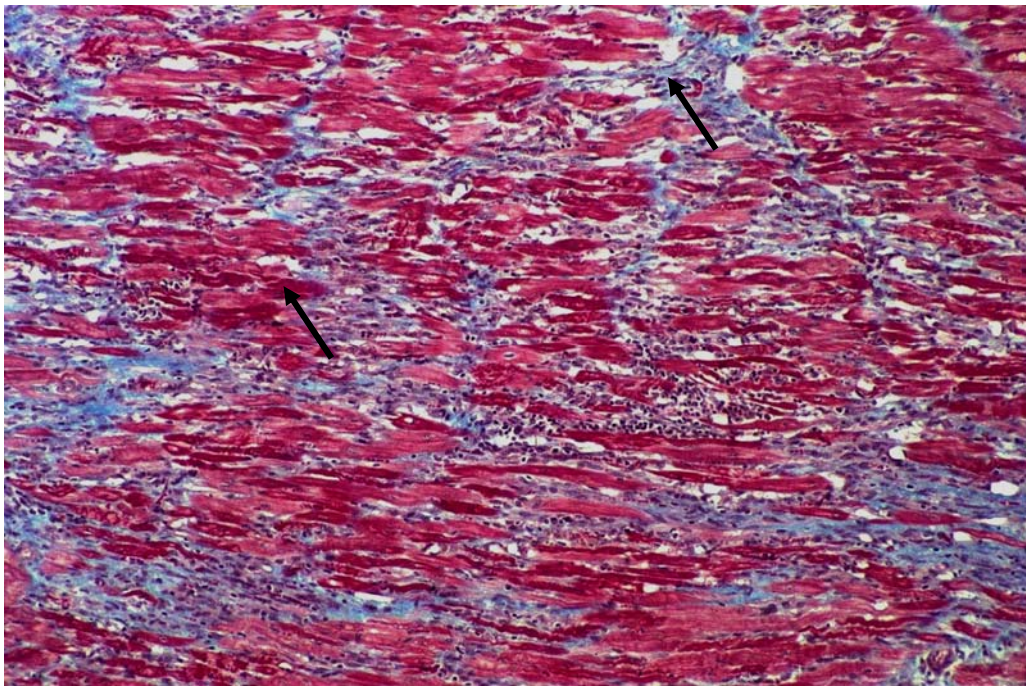


Figure 3.12 Multifocal necrosis (bottom arrow). Also note the interstitial fibrosis (top arrow) in sheep 6. Mason's trichrome X 100

Multifocal to diffuse, mild to moderate, thickening of the endocardium owing to deposition of collagen and elastic fibres was evident in seven sheep (sheep 3, 5, 6, 7, 8, 9 and 10). For the purpose of comparison the endocardium of a control animal is depicted in figure 3.13. In sheep 3, 5, 6, 7 and 8 thickening of the endocardium with disorganisation and disruption of the collagen and elastic fibres was usually mild and either multifocal or diffuse in nature. In contrast, sheep 9 and 10 exhibited diffuse, moderate to severe thickening of the endocardium (fig. 3.14).

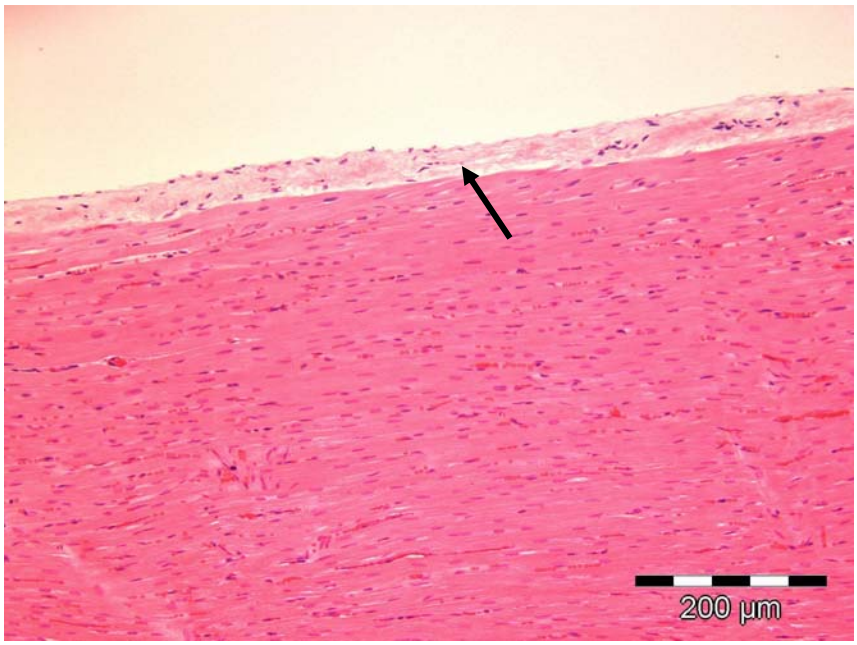


Figure 3.13 Normal endocardium (arrow) in a control animal. HE

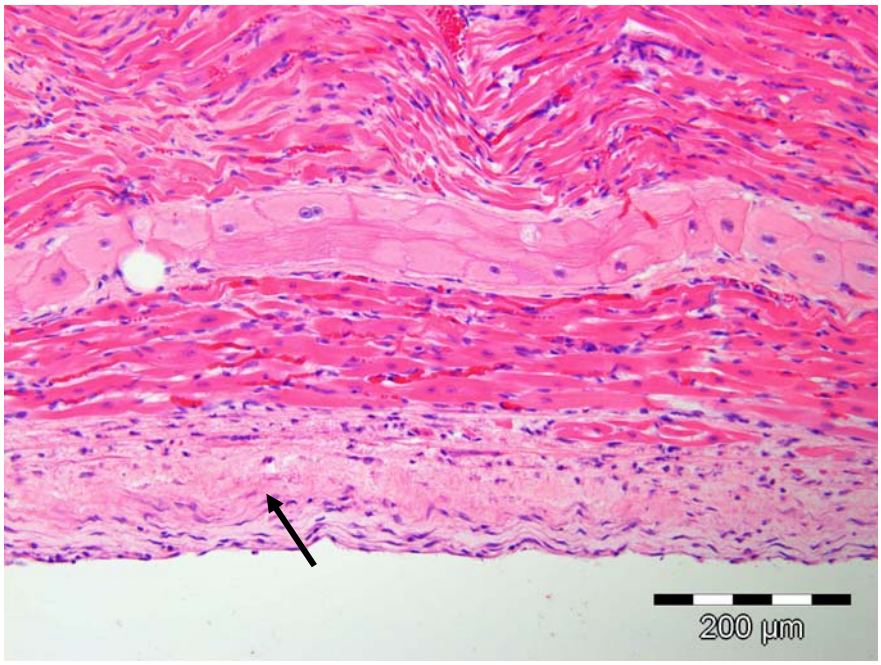


Figure 3.14 Note the thickened endocardium (arrow) in sheep 10. HE

Diffuse and occasionally segmental hypertrophy of the *tunica media* of arteries and arterioles often associated with oedema was evident in six cases (sheep 2, 3, 5, 7, 9 and10; fig. 3.15). Hypertrophy was particularly prominent in sheep 9 and 10.

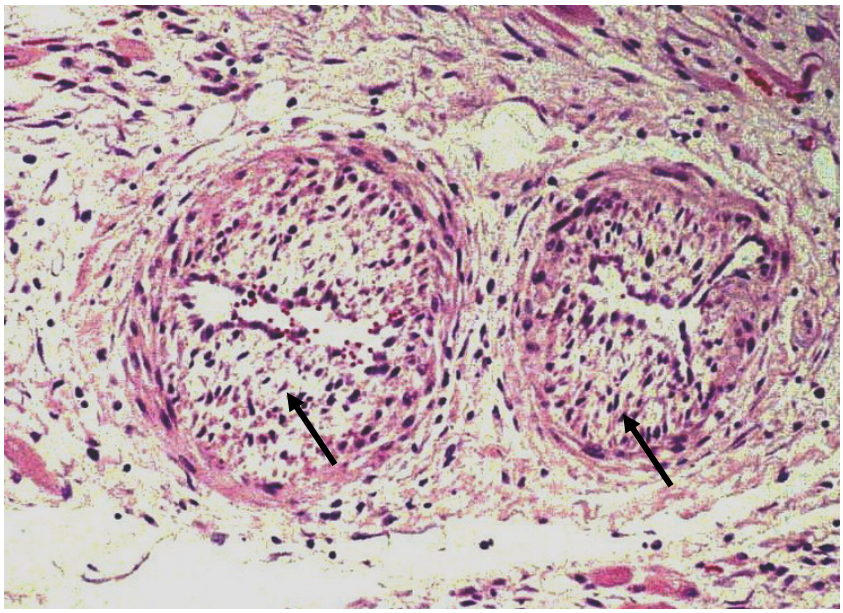


Figure 3.15 Severe medial oedema in two arteries in sheep 10 (arrows). HE X 400

Atrophy of myocardial fibres was present in eight sheep (sheep 1, 3, 4, 6, 7, 8, 9 and 10) and was generally multifocal, involving individual fibres or small groups of fibres (fig. 3.8). Hyaline degeneration of a few haphazardly scattered myofibres was often noted between atrophic fibres. In sheep 6, 8, 9 and 10 prominent tracts of atrophic fibres were present in the subendocardial region. In sheep 1 diffuse atrophy was evident throughout the myocardial wall (fig. 3.16).

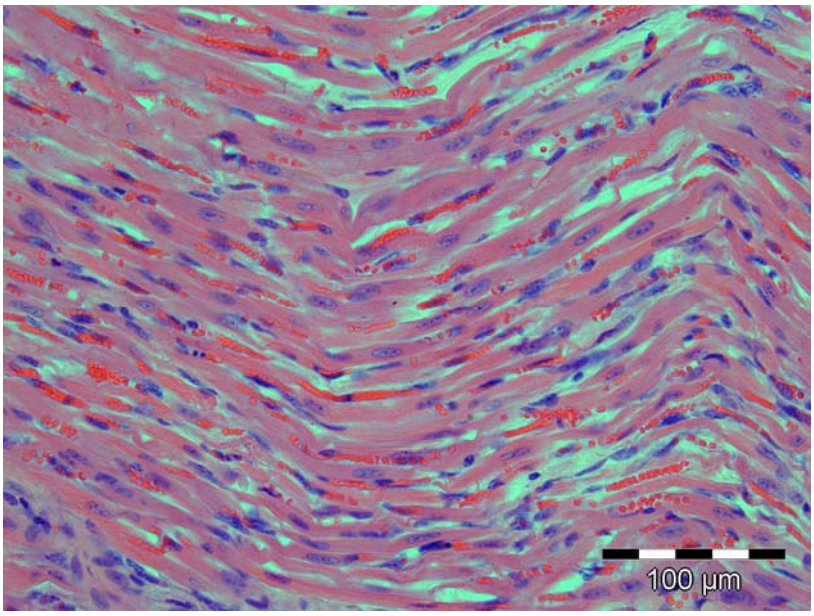


Figure 3.16 Diffuse atrophy of fibres throughout the myocardial wall in sheep 1. HE

Lung lesions were characterised by congestion, scattered alveolar emphysema, multifocal to diffuse alveolar collapse (atelectasis) and the presence of protein-rich intra-alveolar and interstitial fluid (lung oedema), leucocytosis (predominantly mononuclear cells), and thickening of the alveolar walls owing to the presence of mononuclear cells (fig. 3.17). Scattered macrophages were present in the alveolar lumens.

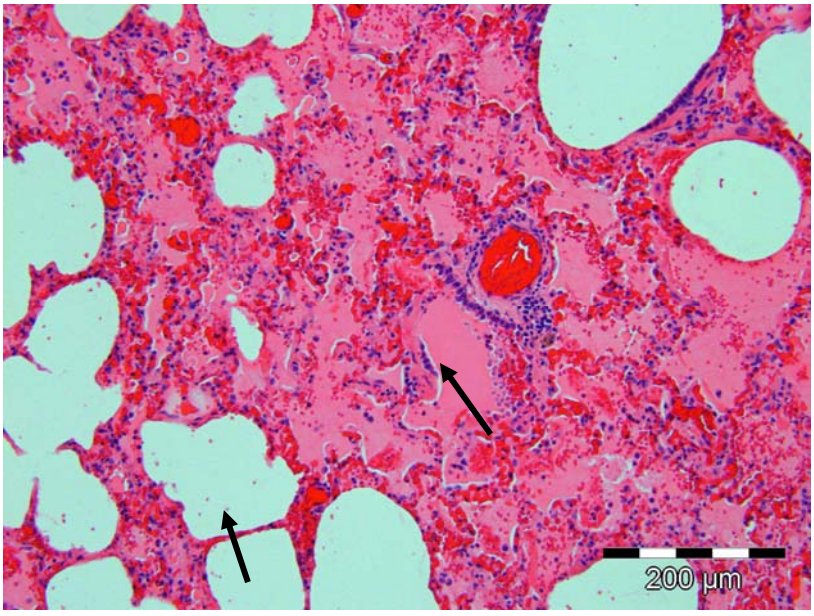


Figure 3.17 Severe lung oedema (top arrow) with emphysema (bottom arrow) in sheep 10. HE

The most striking hepatic lesions were swelling of hepatocytes with dilatation of the central veins and particularly the centrilobular sinusoids. In sheep 10 centrilobular necrosis was evident (fig. 3.18). Renal lesions comprised swelling with increased granularity of the epithelial cells lining the proximal convoluted tubules. Scattered among the swollen epithelial cells were a few necrotic cells (nephrosis).

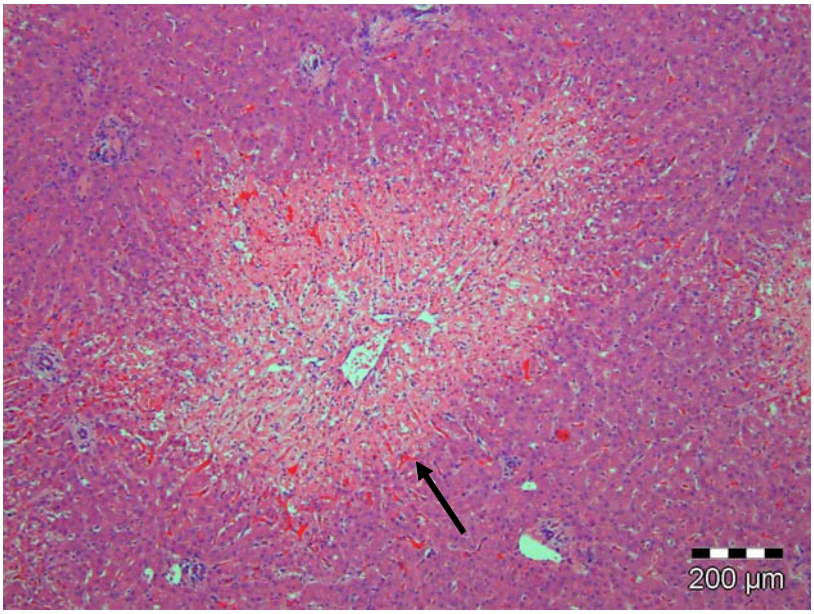


Figure 3.18 Centrilobular hepatic necrosis (arrow) with dilatation of sinusoids in sheep 10. HE

3.3.3 Imaging analysis

3.3.3.1 Descriptive statistics

The myofibre diameter, nucleus perimeter and nucleus area of the affected (gousiekte) and control groups are depicted in tables 3.4 and 3.5

Table 3.4 Affected group

Variable	Number of observations	Mean	Standard deviation	Minimum	Maximum
Myofibre diameter (µm)	52	14,33	3,08	8,12	21,84
Nucleus perimeter (µm)	47	35,81	5,04	25,52	45,11
Nucleus area (μm^2)	47	75,36	18,36	41,44	118,4

Table 3.5 Control group

Variable	Number of observations	Mean	Standard deviation	Minimum	Maximum
Myofibre diameter (µm)	60	13,05	2,29	8,93	19,3
Nucleus perimeter (µm)	41	30,34	4,36	22,08	38,58
Nucleus area (μm^2)	41	47,95	11,11	30,91	75,27

The standard deviation of each variable was then compared for the affected sheep and the control group using Levene's test for equal variance. This showed that the myofibre diameter differed significantly between affected and control animals (P = 0,029). The same was true for nucleus area (P = 0,002). However, there was no significant difference between the two groups in terms of nucleus perimeter (P = 0,36). These differences can be illustrated by means of histograms comparing the distributions of the three variables between the two groups (figs 3.19, 3.20, 3.21).

Figure 3.19 Comparison of myofibre diameter distribution between control and affected animals

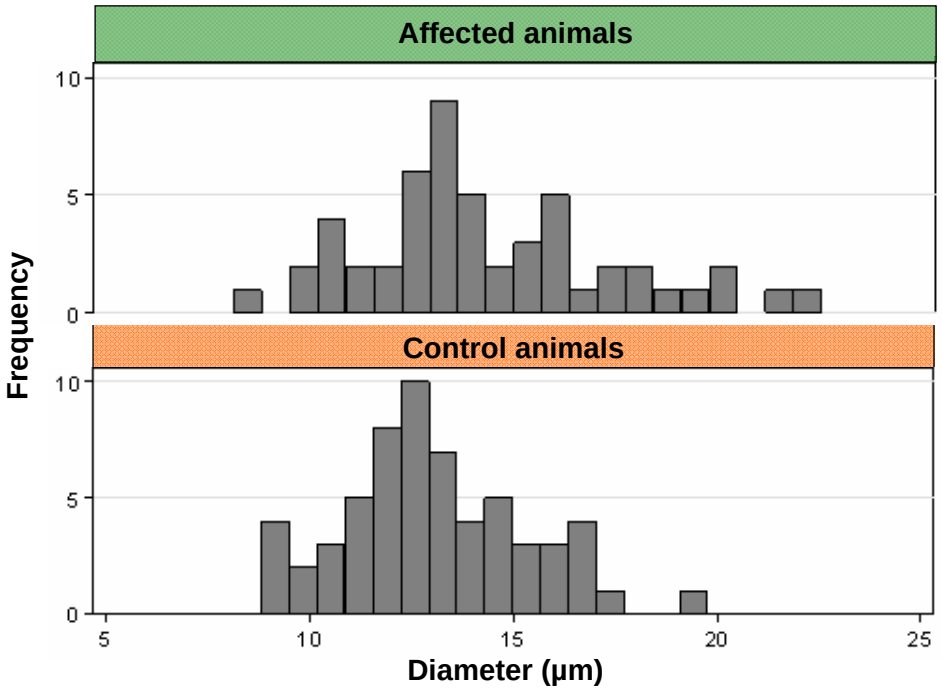


Figure 3.20 Comparison of myofibre nucleus perimeter distribution between control and affected animals

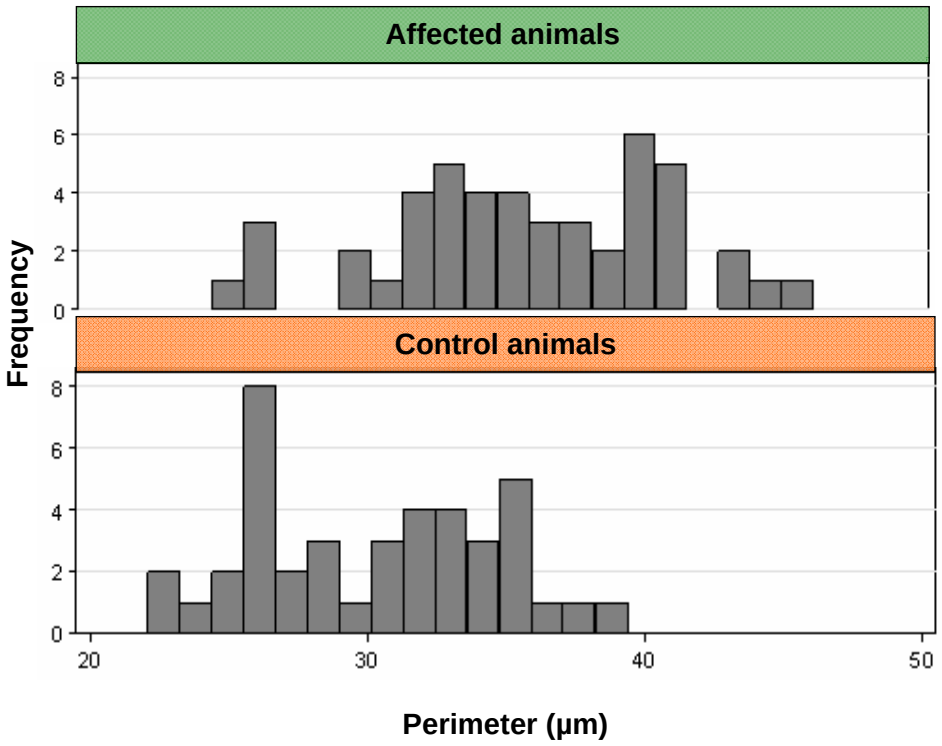
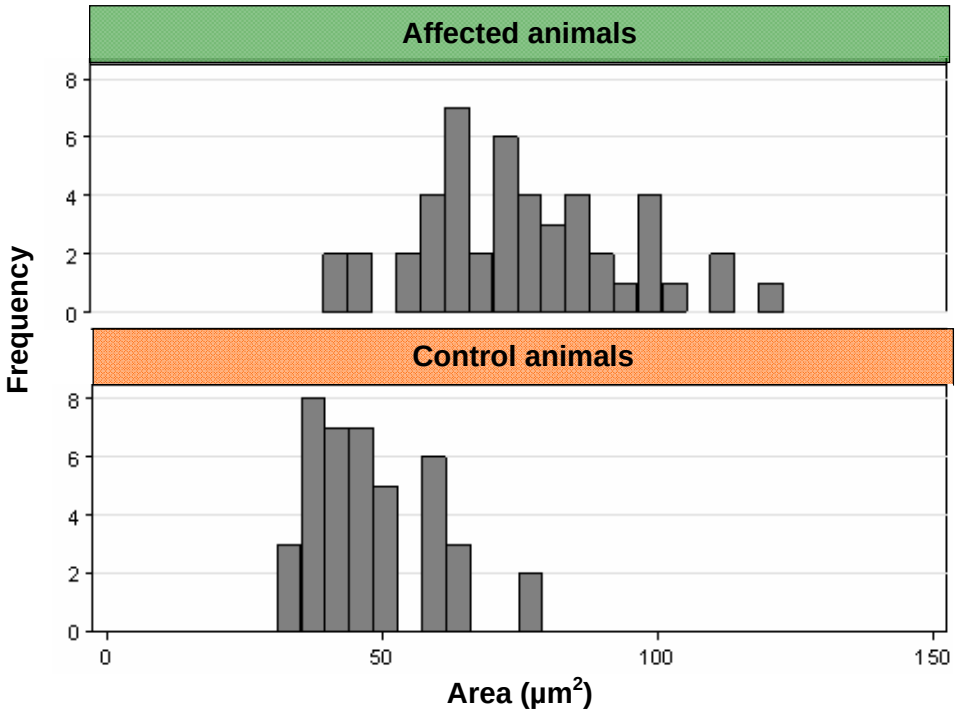


Figure 3.21 Comparison of myofibre nucleus area distribution between control and affected animals



3.4 DISCUSSION

The purposes of this study were, amongst others, to investigate the effect of the duration of latency on the nature of the myocardial lesions and to study the entire spectrum of light-microscopical lesions associated with gousiekte since this could have a profound effect on the criteria used in the diagnosis of natural and experimental cases of the poisoning.

In the majority of animals that die naturally or are euthanased terminally after exposure to plants associated with gousiekte, certain macrolesions are suggestive of the disease as the cause of death. These include signs of congestive heart failure, such as pulmonary oedema, hydropericardium, hydrothorax, generalised congestion and ascites, cardiac dilatation and subendocardial fibrosis. In a low percentage of animals extra-cardiac signs of congestive heart failure may be very subtle or absent (Theiler, Du Toit & Mitchell 1923).

The presence of pulmonary oedema and hydropericardium in eight of the ten treated animals (80 %) suggests that gousiekte causes left-sided congestive heart failure, and corroborates the findings of previous workers (Pretorius *et al.* 1973; Van der Walt & Van Rooyen 1977; Van Rooyen *et al.* 1984; Pipedi 1999). Features suggestive of biventricular heart failure, including the macroscopical lesions outlined for left-sided heart failure and generalised congestion with ascites and centrilobular hepatic necrosis, were less common. Three sheep (sheep 8, 9 and 10) had generalised congestion and two of them developed ascites (sheep 9 and 10). In sheep 10, myocardial mottling was evident and extended from the apex and the left free ventricular wall to the septum and the right free ventricular wall. This suggests that biventricular heart failure occurs mainly in cases with long latent periods where the pathological process extends beyond the initial predilection site, i.e. the subendocardial region of the left free ventricular wall and apex of the heart. In two animals with short latent periods (sheep 1 and 3) no specific macroscopical lesions were noted, which emphasises the variation in the range of lesions associated with the disease.

There are various definitions of heart failure. In essence congestive heart failure is chronic failure of the heart, as a pump, to meet the circulatory requirements of the body, and is characterised by expansion of the extracellular fluid volume and accumulation of oedema fluid in the body cavities. The term heart failure denotes a situation in which the heart is diseased, all compensatory mechanisms have been exhausted, and characteristic clinical and pathological signs are present.

The body's major compensatory mechanisms for heart failure include the intrinsic cardiac response of dilatation and hypertrophy and the systemic response, which includes an increase in heart rate and peripheral resistance, a redistribution of blood flow, venular constriction, and an increase in blood volume. In each case, the compensatory responses are at least temporarily beneficial and directed at increasing cardiac output to meet the metabolic needs of the animal (De Morais & Schwartz 2002; Hamlin & Stokhof 2004; Mohrman & Heller 2006).

In all the treated animals tachycardia (>90 heart beats per minute) was noted 30 to 42 days after receiving plant material. The interval between the recording of tachycardia and death ranged from nought to nine days and tended to be longer in animals with long latent periods compared to animals with short latent periods, although there were exceptions, for example sheep 7 and 8 (table 3.1).

It may be difficult to detect cardiac dilatation macroscopically, particularly during the early stages of its development (Jubb, Kennedy & Palmer 2007; Kumar, Cotran & Robbins 2003). Furthermore, cardiac dilatation may be a pathological or a physiological response to increase cardiac output (Dec & Fuster 1994; Weekes *et al.* 1999). Based on the subjective macroscopical criteria used for the identification of dilated hearts in this study, namely a flabby appearance, rounded shape with thinning of the free wall of the dilated chamber, attenuation of papillary muscles and opaqueness of the endocardium, the hearts of only two of the ten animals (20 %) with extended latent periods were affected (table 3.2).

The endocardium consists of a monolayer of endothelium on a continuous basement membrane, which covers the inner subendothelial layer of dense collagen, and an outer subendothelial layer composed of collagen, elastin, blood and lymph vessels (Jubb, Kennedy & Palmer 2007). Thickening of the endocardium that varied in extent and distribution, with disorganisation and disruption of the collagen and elastic fibres, was evident in seven of the ten (70 %) experimental animals (table 3.3). Diffuse endocardial thickening is seen whenever a ventricle or an atrium is dilated for a prolonged period (Jubb, Kennedy & Palmer 2007) and is not a specific lesion associated with gousiekte.

Altering the end-diastolic volume, which within certain limits results in an increase in stroke volume, can modify the contractile force of the heart. The consequent increased stretching of the myofibres increases the contractile force and results in dilatation of the heart. This is known as the Frank Starling mechanism. Continued stretch increases contractile force to a limit after which increased stretch will result in a decrease in tension developed and eventually in heart failure (King 1999; Mohrman & Heller 2006; Rowell 1993). Cardiac

dilatation and endocardial thickening in animals exposed to plants associated with gousiekte are therefore most likely a response to congestive heart failure resulting in a volume overload. It is postulated that the diseased heart dilates owing to the irreversible nature of the myocardial lesions.

Irrespective of the length of the latent period, the most consistent of the various histopathological lesions recorded in the subendocardial region were hypertrophy of myocardial fibres and mononuclear cell infiltration (table 3.3). In sheep 1 and 2 lesions were more of an acute to subacute nature, for example hypertrophic fibres with small, scattered foci of necrosis accompanied by mild mononuclear cell infiltration. Sheep 1 exhibited extensive myofibre atrophy throughout the ventricular wall without evidence of necrosis. Lesions in the remaining animals were compatible with what has been reported in field cases and were characterised by chronic active lesions, for example multifocal necrosis, replacement fibrosis associated with a mononuclear cell infiltration and, occasionally, atrophy in the inner third of the myocardial wall (Theiler, Du Toit & Mitchell 1923; Newsholme & Coetzer 1984). This study clearly demonstrated that multifocal to diffuse myofibre hypertrophy was a consistent finding in all the treated animals and should be included as a “typical lesion” of gousiekte. Lesions in the two animals with short latent periods in this study differ from those reported by Smit (1959) and Hurter *et al.* (1972), but could still be considered to be “atypical”, since the most conspicuous lesions in these animals were either hypertrophy of myofibres with multifocal coagulative necrosis or myofibre atrophy.

Based on the histopathological lesions recorded in this study it is suggested that the treated sheep fell naturally into two groups on the basis of the duration of the latent period and the histopathological lesions, namely sheep with a short latent period (<35 days) in which fibrosis is not a feature and sheep with an intermediate to long latent period (35 to 51 days) in which fibrosis becomes progressively more severe (tables 3.2 and 3.3).

The myofibre diameter and nuclear area in the affected animals differed statistically from those of the control animals (P = <0,03). However, there was no significant difference when the nuclear perimeter of the two groups was compared. Imaging analysis therefore confirmed the significance of the anisocytosis and anisonucleosis noted light-microscopically in sheep in this study. Anisonucleosis was particularly striking in sheep with intermediate to long latent periods.

In the past, mononuclear cell infiltration in the subendocardial region was regarded as a feature of gousiekte (Theiler, Du Toit & Mitchell 1923; Smit 1959; Newsholme & Coetzer 1984; Kellerman *et al.* 2005). Although present in all cases in this study, these infiltrations were prominent in only three sheep with long latent periods. As a rule, mononuclear cells occurred in small foci in the myocardial interstitium, especially around blood vessels or in association with foci of fibrosis or necrosis. Focal mononuclear cell infiltrates may be present in a variety of cardiac conditions including cases of *Tylecodon* and *Cotyledon* spp. poisoning, and should not be regarded as a specific diagnostic feature of gousiekte (Kellerman *et al.* 2005).

Myocardial damage following exposure of animals to pavetamine provokes an inflammatory reaction that is an integral part of the healing process. In animals with more advanced lesions the inflammatory reaction is histologically characterised by the presence of necrosis and an infiltration of predominantly lymphocytes, macrophages and fibrosis. Lymphocytes are mobilised in both antibody-mediated and cell-mediated immune reactions and also in non-immune-mediated inflammation. Lymphocytes have a reciprocal relationship to macrophages in chronic inflammation and can be activated by contact with antigen. One of the lymphokines, IFNy, is a major stimulator of monocytes and macrophages. Monokines produced by activated macrophages activate lympho-cytes, which themselves produce inflammatory mediators and in the process set the stage for persistence of the inflammatory response. Plasma cells produce antibody directed either at persistent antigen in the inflammatory site or at altered tissue components (Cotran, Kumar & Collins 1999).

In humans, following myocardial infarction, trauma and some forms of myocardial disease, endogenous cardiac antigens are released, evoking a non-specific immunological response (Kaplan 1976). According to Schultheiss *et al.* (1986), sera of patients with dilated cardiomyopathy contained circulating auto-antibodies directed at the ADP/ATP carrier of the inner mitochondrial membrane. In sheep suffering from gousiekte, neither a humoral nor a cellular immune response could be demonstrated against prepared cardiac antigens, namely mitochondria, actomyosin, crude myocardial extract, and sarcolemmal and sarcoplasmic reticular antigens. It was concluded that, owing to the absence of anti-heart antibodies in sheep that died of gousiekte, this was not an autoimmune disease (Fourie 1994).

Historically, histopathological confirmation of gousiekte was based on the presence of distinct fibrosis in the subendocardial region of the apex and left free ventricular wall (Theiler, Du Toit & Mitchell 1923; Newsholme & Coetzer 1984; Kellerman *et al.* 2005). In three sheep in this study (sheep 1, 5 and 7) no fibrosis was present. Furthermore, in sheep 2, 3 and 4, all of which had short to intermediate latent periods, fibrosis was indistinct. The presence or absence of fibrosis on its own can therefore not serve as a diagnostic criterion for the confirmation or exclusion of gousiekte, particularly in cases with shorter latent periods.

Multifocal areas of coagulative necrosis were seen in seven out of ten experimental animals (70 %) in this series, regardless of whether the latent period was short or intermediate (table 3.3). This feature, together with the presence of hypertrophy, can be regarded as a significant microscopical feature in the histopathological diagnosis of gousiekte. The variable extent of necrosis noted in the experimental animals could be ascribed to an individual variation in susceptibility to the toxin.

Examination of the coronary arteries and arterioles revealed medial oedema and hypertrophy in 60 % of the experimental animals (table 3.3). The lesions were present in animals irrespective of the duration of the latent period. Fine

vacuolation of the *tunica media* and thickening of the *tunica intima* of medium and large coronary arteries owing to the presence of a fine fibrinoid material have been described in field cases of sheep with gousiekte (Prozesky *et al.* 1988). Similar changes have been described in humans with subendocardial fibrosis, and it has been suggested that the vascular lesions play a significant role in the pathogenesis of subendocardial fibrosis (Andrade & Teixeira 1973). This aspect is discussed in more detail in chapter 6.

Myocardial fibre atrophy was present in 80 % of experimental animals (table 3.3), and should be regarded as an additional diagnostic feature of gousiekte. It was especially significant in one case with a short latent period (sheep 1), where it had a transmural distribution. In the majority of cases, however, myocardial fibre atrophy was usually focal in nature and involved only individual or small groups of fibres. Occasionally diffuse atrophy can be the most striking histological feature in field cases of gousiekte (Prozesky *et al.* 1988).

3.5 CONCLUSIONS

This study confirmed that the myocardial lesions in animals exposed to gousiekte-inducing plants have a predilection for particularly the subendocardial fibres of the left free ventricular wall. In some animals with long latent periods the lesions extend to the interventricular septum and the right free ventricular wall. On the other hand, in some animals, particularly those with a short latent period, the necrosis or atrophy extends throughout the ventricular wall.

Furthermore, the study clearly demonstrated that irrespective of the length of the latent period, myofibre hypertrophy is a hallmark of gousiekte and was present in all the experimental animals. Lesions in animals with intermediate latent periods ranged in severity but to a large extent complied with the criteria laid down by previous researchers for “typical” lesions. Lesions in animals with a short latent period can be classified as “atypical lesions”. This emphasises the wide variation of possible lesions and highlight the importance of describing the

entire spectrum of lesions associated with the intoxication so that even "atypical" cases can be diagnosed accurately.

The reason why more cases of the "atypical form" of the intoxication have not been reported in sheep and cattle may be the notion amongst veterinarians that the disease is associated only with "typical" myocardial lesions. If these lesions are not present, death may be attributed wrongly to other causes. Diagnostic pathologists have not given serious consideration to the concept of "atypical" cases of gousiekte, and the diagnosis of the disease is still based on the presence of "typical" histological lesions. A possible explanation for this is that the variation in lesions associated with intoxication, particularly in cases with a short course, has not been properly documented and adequately emphasised. In addition, the identification of early lesions, and of myofibre hypertrophy in particular, can be problematic especially if appropriate controls are not available. Another contributing factor may be that myocardial necrosis and an associated inflammatory response are not confined to gousiekte and that the presence of an associated inflammatory response is considered indicative of other intoxications, for example *Tylecodon* and *Cotyledon* spp., rather than gousiekte.

One of the problems with studying cardiac pathology is the assessment of the functional significance of lesions. Furthermore, early lesions that may be difficult to detect by light-microscopical examination may be responsible for severe cardiac dysfunction and death. In an attempt to study the pathogenesis of the cardiac lesions in more detail it was decided to conduct a transmission electron microscopical study of the lesions in sheep associated with the disease.