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CLINICAL PATHOLOGICAL CHANGES IN GOUSIEKTE, A PLANT-INDUCED CARDIOTOXICOSIS OF RUMINANTS

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ABSTRACT

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Twenty sheep were dosed with either *Pachystigma pygmaeum* or *Fadogia homblei* belonging to the Rubiaceae. The experimentally-induced cardiotoxicoses were monitored by various clinical pathological parameters and heart function tests. Elevated AST (aspartate transaminase) activity in the serum proved to be a more reliable indicator of cardiac damage in gousiekte than either LD (lactate dehydrogenase) or CK (creatine kinase). Persistent increases of AST activity were recorded from c. 14 days after commencement of dosing, and this activity sometimes peaked as late as 30 days after the dosing had ceased. Tachycardia and diminished heart function were registered only terminally. Lesions of gousiekte were present in all the sheep that were exposed to the plants.

In a field outbreak of *P. pygmaeum*, where 60 out of 90 sheep died, 14 out of the 15 animals examined had increased AST levels compared with none of the 15 controls. These results indicated that increased enzyme levels can be of use to identify affected animals during latency in a natural outbreak of gousiekte.

INTRODUCTION

Gousiekte is a poisoning of ruminants characterized by acute heart failure 5–8 weeks after the initial ingestion of certain rubiaceous plants. In natural cases, obvious premonitory signs are seldom seen and animals typically drop dead—hence the popular Afrikaans name gousiekte, meaning 'quick disease' (Kellerman, Coetzer & Naudé, 1988). Gousiekte, which is of great economic importance in southern Africa, is known to be caused by the following plants: *Pachystigma pygmaeum* (Theiler, Du Toit & Mitchell, 1923), *Pachystigma thamnus* (Adelaar & Terblanche, 1967), *Fadogia homblei* (Hurter, Naudé, Adelaar, Smit & Codd, 1972), *Pavetta harborii* (Uys & Adelaar, 1957), *Pavetta schumanniana* (Naudé & Adelaar, unpublished data, 1962) and *Pachystigma latifolium* (T.W. Naudé, personal communication, 1986). Despite considerable effort by several workers, the toxic principle has not yet been isolated from any of these plants. Other members of the Rubiaceae are currently being tested for toxicity.

Gousiekte is primarily a cardiotoxicosis. Theiler *et al.* (1923) found that the majority of animals that die of gousiekte in the veld show various degrees of ventricular dilatation and thinning of the ventrical walls. Extracardiac signs of heart failure, such as effusions in the body cavities and oedema of the lungs, are often present, but in a minority of cases no gross lesions may be evident (Newsholme & Coetzer, 1984).

A diagnosis of gousiekte can only be confirmed histopathologically. Lesions are characterized by a multifocal to diffuse fibrosis accompanied by a mild to moderate round cell infiltration, particularly in the endocardium of the apex, left ventricular wall, and interventricular septum (Theiler *et al.*, 1923; Smit, 1959). Atrophy of the myofibres can occassionally be the most striking lesion seen (Prozesky, Fourie, Neser & Nel, 1988).

Since 1955, when the serious study of cardiac enzymology began, it has become common practice to measure the serum activity of various enzymes as an aid to the diagnosis and prognosis of myocardial infarction in humans. Although not specific for injury to the myocardium, the measurement of the activities of serum aspartate transaminase (AST), serum lactate dehydrogenase (LD) and serum creatine kinase (CK) proved to be useful in determining experimental and natural damage to the myocardium (Kupper & Bleifeld, 1979).

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Statistical comparison of the isoenzyme patterns has revealed that these patterns are distinct for different tissues (Kupper & Bleifeld, 1979; Beatty, 1983; Doxey, 1984). Organ-specific tissue damage in humans can be detected by electrophoretic separation of LD (LD_{1-5}) and CK (MM, MB and BB) isoenzymes. Significant shifts in a serum isoenzyme pattern may occur after injury to a specific organ even without a total increase in the activity of the enzyme having been recorded (Louderback & Shanbrom, 1968). The activity of LD_2 is higher than that of LD_1 in the serum of healthy human beings, but in 95 % of clinically proven cases of myocardial infarction, the activity of LD_1 is elevated, resulting in a shift in the LD_1/LD_2 ratio (Kupper & Bleifeld, 1979). Direct extrapolations between the isoenzyme patterns of humans and sheep, however, cannot be made; for instance, unlike in humans, LD_1 showed the greatest activity in the serum of normal sheep (Beatty, 1983; Doxey, 1984). Furthermore, after an acute myocardial event in a human, an increase in the serum CK–MB isoenzyme activity of more than 6–10 % of the total CK within the first 36 h confirms the diagnosis of acute myocardial infarction (Kupper & Bleifeld, 1979). In sheep, on the other hand, there is no MB isoenzyme present in heart tissue and according to Beatty (1983) and Doxey (1984) all the CK in heart tissue consist of MM isoenzyme.

Because of the lack of overt premonitory signs during the latent period of gousiekte, it is usually impossible to identify affected animals, even shortly before death. In this dosing trial the activity of different enzymes and isoenzymes were monitored to establish when injury to the myocardium sets in and whether naturally affected animals could be identified during latency.

Although release of intracellular enzymes indicates cellular damage, the function of the affected organ need not be impaired. In this study, consequently, an attempt was made to correlate the enzymatic changes with cardiac function measured by means of a cardiopulmonary flow index (Van der Walt & Van Rooyen, 1977; Van der Walt, Van Rooyen, Cilliers, Van Ryssen & Van Aarde, 1981).

FIELD OUTBREAK

In February 1988 a farmer in the Ventersdorp district lost 60 out of 90 sheep on veld sparsely infested with *P. pygmaeum*. Electrocardiograms (ECG) were recorded on 4 of the animals, one of which dropped dead 40 m from the recording site. Blood was collected from the flock and all the sheep that died after handling were necrop-

TABLE 1 Dosing trials with *Pachystigma pygmaeum*

Sheep No.	Initial live mass (kg)	Dosing regimen				Duration of experiment (days)
		Dose (g/kg × n)	Period dosed (days)	Total dose (g/kg)	Total dose (kg)	
1	31	10 × 23	30	230	7,13	31
2	35	10 × 30	39	300	10,5	42
3	31	10 × 31	39	310	9,61	43
4	25	10 × 31	42	310	7,75	51
5	28	10 × 31	42	310	8,68	51
6	28	10 × 31	42	310	8,68	73
7	34	10 × 31	42	310	10,54	90
8	30	10 × 31	42	310	9,3	90

TABLE 2 Dosing trials with *Fadogia homblei* at a total dose of 220 g/kg over 30 days

Sheep No.	Sex R = ram E = ewe	Age mt = milk tooth 2 t = 2 tooth 4t = 4 tooth	Initial live mass (kg)	Total dose (kg)	Duration of experiment (days)
9	R	2t	38	8,36	34
10	R	mt	36	7,92	40
11	E	4t	39	8,58	42
12	E	2t	34	7,48	45
13	E	4t	40	8,8	45
14	R	mt	36	7,92	57
15	E	2t	34	7,48	57
16	E	4t	34	7,48	57
17	E	4t	32	7,04	57
18	R	mt	35	7,70	100
19	E	2t	39	8,58	100
20	E	4t	45	9,90	184

sied. Typical lesions of gousiekte were present in the hearts of all 7 of these sheep. Fifteen control sheep, on the same property, which had not been exposed to *P. pygmaeum*, were also bled.

MATERIALS AND METHODS

Dosing trial

Plant material and experimental animals

P. pygmaeum plants were collected during February, 1986 at Swartrand near Ventersdorp, dried in the shade, ground to a coarse powder, and stored at −10 °C. Eight 6-month old South African Mutton Merino wethers were dosed per stomach tube with the material (Table 1).

Sprouting *F. homblei*, collected in November, 1986 near Bronkhorstspruit, were treated in the same way as the *P. pygmaeum*. The plant was administered per rumen fistula to 12 Merinos of different ages and sexes at a rate of 10 g/kg/day over 30 days (Table 2). During the trials the animals were housed separately and examined daily. Blood was collected for clinical pathological determinations on Day −4, Day 0 and once a week thereafter.

Haematology

Blood haemoglobin (B-Hb) was determined by the cyanmethaemoglobin method (Merck, 1974); haematocrit (B-Ht), by using capillary tubes in a Damon IEC micro haematocrit centrifuge; erythrocyte sedimentation rate (B-ESR), in Wintrobe tubes for 1 h at 20 ± 3 °C. Blood smears stained with RapiDiff[1] were made on Day 0, and then once a week.

Determinations on plasma and serum

Total plasma protein (P-TPP) was determined by the Biuret method (Merck, 1974), plasma glucose using the GOD-Perid method (Boehringer Mannheim) and serum urea (SU) by the Berthelot method (Merck, 1974).

Enzymology

The activities of the following enzymes were determined in the serum using Boehringer Mannheim test kits[2]: LD (EC 1.1.1.27), AST (EC 2.6.1.1), CK (EC 2.7.3.2) and γ-glutamyltransferase (GGT, EC 2.3.2.2). In addition, isoenzymes of LD (LD_{1-5}) and CK (MM, MB and BB) were separated on agarose using the Beckman Paragon isoenzyme electrophoresis kit. All enzyme activity were measured at 25 °C. Isoenzymes were quantified using a Model CDS-200 Beckman Densitometer.

Cardiac function

The electrical activity and function of the heart was monitored once a week using lead II of the electrocardiogram (Schultz, Pretorius & Terblanche, 1972) and by means of a cardiopulmonary flow index (CPFI) (Van der Walt & Van Rooyen, 1977; Van der Walt *et al.*, 1981). The CPFI can be defined as the ratio of the cardiopulmonary blood volume to stroke volume and this ratio is equivalent to the number of heartbeats necessary to pump blood from the right side to the left side of the heart through the lungs.

Pathology

Specimens of various organs were collected in 10 % buffered formalin at necropsy, routinely processed and stained with haematoxylin and eosin. The Masson's trichrome stain for collagen was applied to various myocardial sections (Anon, 1968).

Field outbreak

In the Ventersdorp outbreak, AST activities in the serum of 15 animals that had been exposed to *P. pygmeaum* were compared with the AST activities of 15 sheep on the farm that had not been exposed to the toxic plants. ECG were recorded and serum enzyme activities were measured as for the experimental cases described previously. The gross and microscopical examinations of

[1] Clinical Sciences Diagnostics

[2] Cat. No. for LD = 124885; AST = 124362; CK = 126322; GGT = 125938

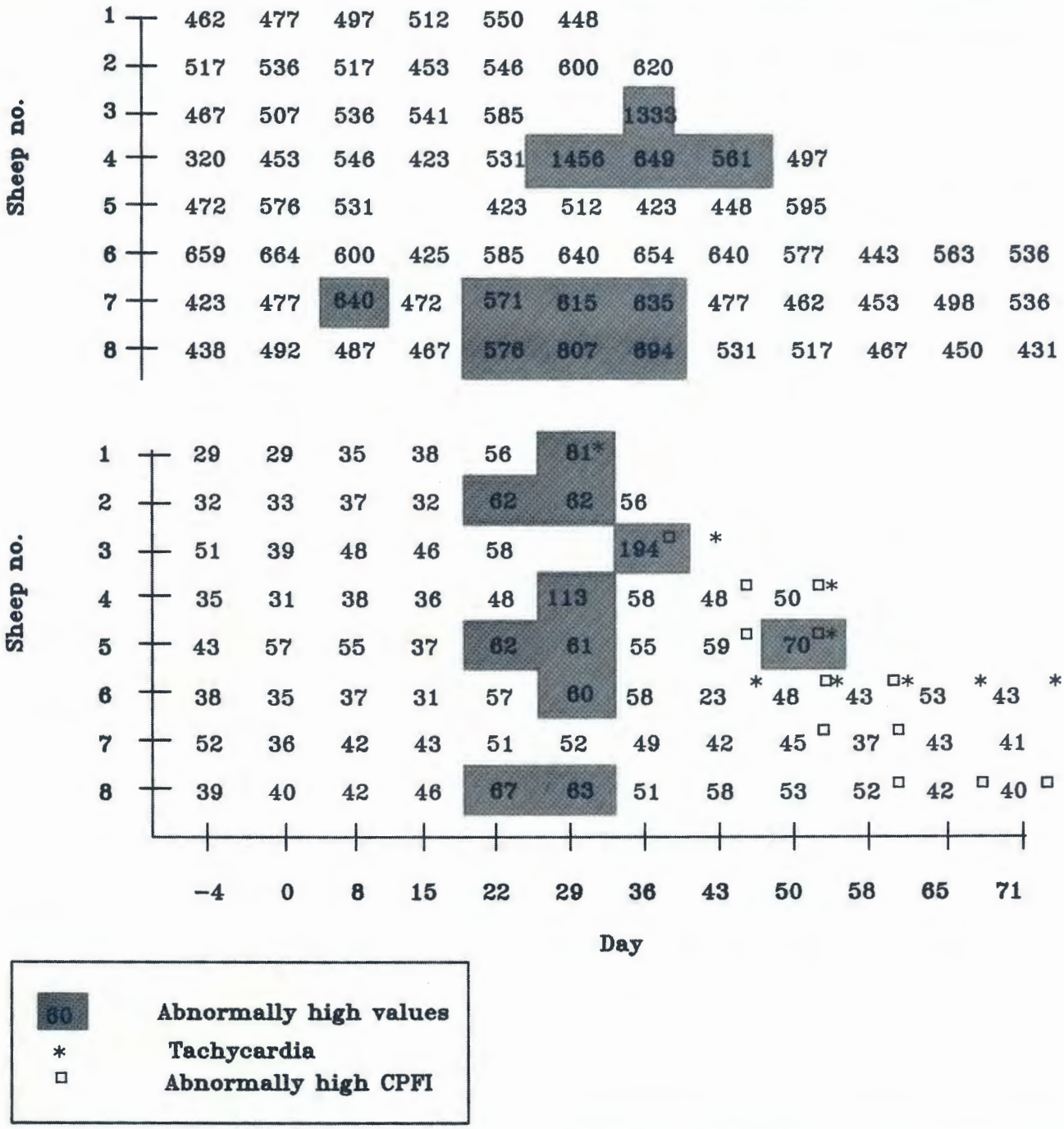

FIG. 1 Clinical pathological and heart function changes in experimental gousiekte induced by *P. pygmaeum*. Serum levels of LD are given above and AST below.

7 sheep that died after being handled, were carried out as described for the experimental cases (Table 5, Sheep 1, 2, 4, 5, 6, 7 & 14).

RESULTS

Dosing trials

The findings are summarized in Tables 3 & 4 and Fig. 1 & 2.

The pattern of the chemical pathological changes induced by the 2 plants were essentially similar. The exception to this rule, however, was the decrease in GGT activity recorded in all the sheep while they were actually being dosed with *P. pygmaeum* (Table 3).

Changes in the activities of AST and LD during the course of the intoxication are given in Fig. 1 & 2. Abnormally high AST activities were noticed in 19 out of 20 sheep from *c.* Day 21 onwards. This increase in AST activity was accompanied by abnormally high LD activity (100 U/ℓ above the initial value) in 14 out of 20 sheep. Transient mild elevations in the activities of isoenzyme LD_1 (8/20 sheep on *c.* Day 14) and LD_2 (11/20 sheep periodically) were sometimes also evident (Tables 3 & 4). The levels of LD isoenzymes (mean + SD) in the serum of 20 sheep (38 estimations) before dosing were LD_1, 66 % ± 7; LD_2, 6 % ± 2; LD_3, 19 % ± 3; LD_4, 4 % ± 2; LD_5, 5 % ± 3. Tachycardia, abnormal heart sounds on auscultation, increased CPFI values and/or arrhythmia were recorded terminally (Tables 3 & 4).

TABLE 3 Observations on sheep intoxicated with *P. pygmaeum*

Sheep No.	Clinical signs	Clinical pathology		Cardiac function (CPFI and ECG)	Fate and pathological findings
		Haematology	Enzymology		
1	Anaemia, tachycardia and polypnoea (Day 30–31)	Increased B-ESR (Day 29), decreased B-Ht, B-Hb (Day 29) and P-glucose (Day 22)	Decreased GGT (Day 8–death) elevated AST (Day 29), CK (Day 29) LD_5 (Day 15) and LD_2 (conspicuously on Day 29)	Inverted T wave (Day 16–26)	Died on Day 31. Pronounced lung oedema
2	No signs	No change	Decreased GGT activity (Day 8–death), elevated AST (Day 22–29)	Inverted T wave (Day 18–37)	Died on Day 42. Pronounced lung oedema and hydropericardium
3	Anaemia (Day 29–36), tachycardia (Day 41–42), systolic murmur (Day 42)	Increased B-ESR (Day 25–32), decreased B-Ht, B-Hb (Day 29–39), and P-glucose (Day 22). High SU (Day 29)	Decreased GGT activity (Day 8–36), elevated AST (Day 36), total LD (Day 36), LD_2 (Day 29), LD_5 (mildly elevated on Day 36) and CK (Day 36)	Inverted T wave (Day 16–23). Elevated CPFI (Day 36)	Died on Day 43. Pronounced lung oedema and hydropericardium
4	Anaemia (Day 29–30), tachycardia, systolic murmur and polypnoea (Day 48–death)	Increased B-ESR (Day 25–36), decreased B-Ht and B-Hb (Day 29–36)	Decreased GGT activity (Day 8–43); elevated activities of AST (Day 29) total LD (Day 29–43), LD_1 (Day 36 and LD_2 (Day 29 and 50)	Elevated CPFI (Day 44–death)	Died on Day 51. Lung oedema and hydropericardium
5	Tachycardia and gallop rhythm (Day 48–death), systolic murmur, polypnoea (Day 51)	Increased B-ESR (Day 15–22 and 36–death) and terminally decreased B-Ht	Decreased GGT activity (Day 8–42), elevated activities of LD_1 (Day 15), AST (Day 22–29 and 50) and CK (Day 50)	Inverted T wave (Day 44–death). Elevated CPFI (Day 43–death)	Died on Day 51. Lung oedema and hydropericardium
6	Anaemia (Day 45), tachycardia (Day 42–death)	Increased B-ESR (Day 11 and 36–58), decreased B-Ht, B-Hb (Day 43–58) and P-glucose (Day 36)	Decreased activity of GGT (Day 8–43), elevated activity of AST (Day 29), LD_1 (Day 15) LD_2 (Day 36–43), LD_5 (Day 43) and CK (Day 43)	Inverted T wave (Day 23–44 and 72). Elevated CPFI (Day 51–72)	Slaughtered on Day 73
7	Transient systolic murmur (Day 48–53)	Increased B-ESR (Day 36–58), decreased B-Ht, B-Hb (Day 36–58) and P-glucose (Day 36–43)	Decreased activity of GGT (Day 8–43), elevated activities of total LD (Day 22–36), LD_2 (Day 36–43), and CK (Day 29–50)	Inverted T wave (Day 23 and 100) Elevated CPFI (Day 44–65)	Slaughtered on Day 190. Myofibrillar lysis
8	Anaemia (Day 30)	Increased B-ESR (Day 25–43); decreased B-Ht, B-Hb (Day 29–32) and P-glucose (Day 22)	Decreased GGT (Day 8–43), elevated activities of AST (Day 22–29), total LD (Day 22–36), LD_2 (conspicuously on Day 22–29), LD_5 (Day 22 and 36) and CK (Day 22, 36–43 and 58)	Inverted T wave (Day 16–44 and 58). Elevated CPFI (Day 58 and 72)	Slaughtered on Day 90. Myofibrillar lysis

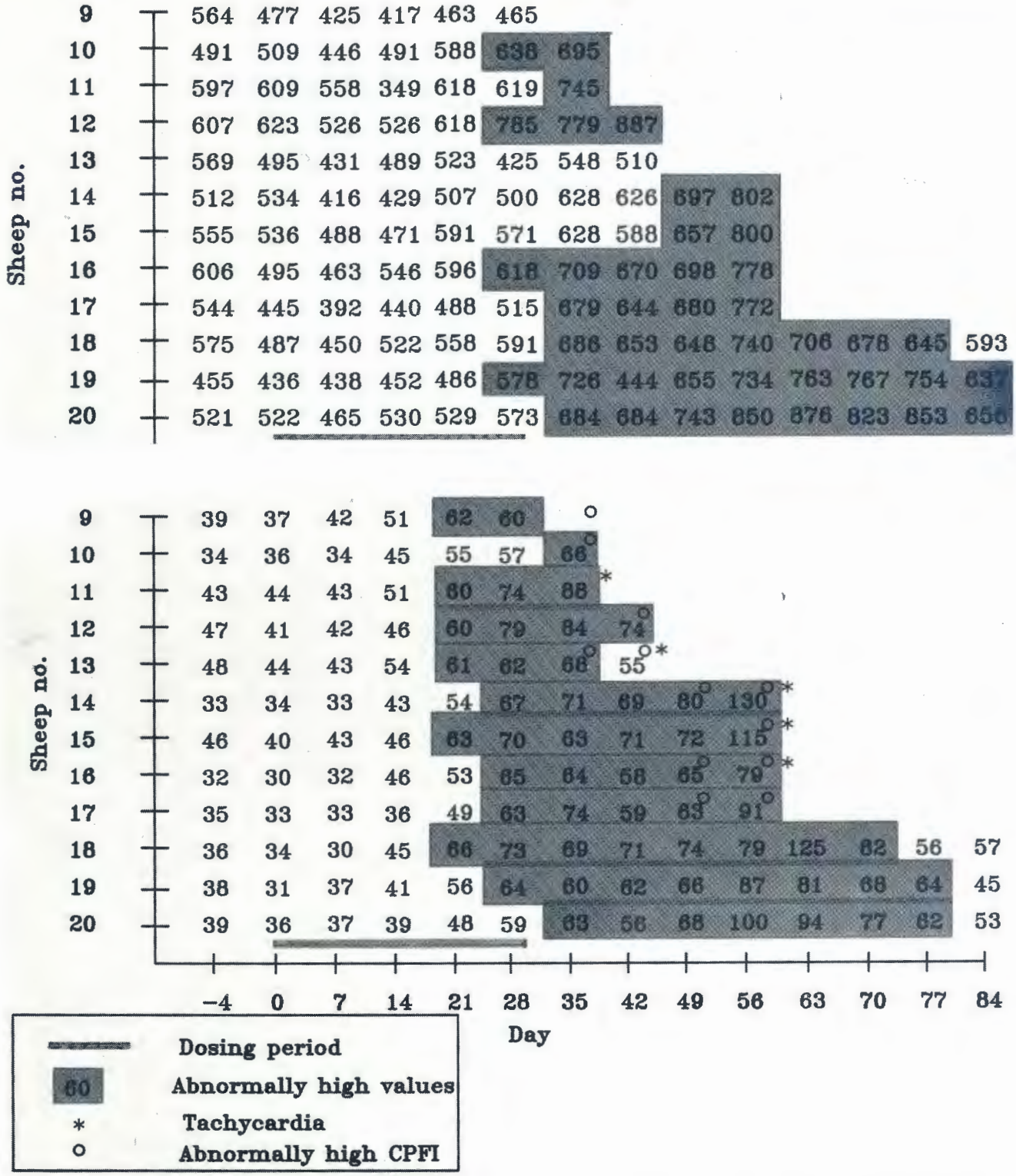

FIG. 2 Clinical pathological and heart function changes in experimental gousiekte induced by *F. homblei*. Serum levels of LD are given above and AST below.

Examination of the blood smears revealed that parasitaemia with *Eperythrozoon ovis* developed in the 8 experimental sheep dosed with *P. pygmaeum*. The affected animals were treated with 10 mg/kg oxytetracycline[3] intravenously for 3 days from Day 28–30.

All the sheep in the *F. homblei* group were similarly treated against *E. ovis* after a positive smear was identified on Day 18. The 3-day treatment was repeated from Day 39–41 when parasitaemia with *E. ovis* again developed.

[3] (Liquamycin, Pfizer.)

TABLE 4 Observations on sheep intoxicated with *F. homblei*

Sheep No.	Clinical signs	Clinical pathology		Cardiac function (CPFI and ECG)	Fate and pathological findings
		Haematology	Enzymology		
9	No signs	Elevated P-glucose (Day 34)	Elevated activity of AST (Day 21–death) and LD_5 (Day 28)	Elevated CPFI (Day 29)	Died on Day 34, Lung oedema and hydropericardium
10	Tachycardia and polypnoea (Day 39–death), pulmonary oedema (Day 40)	Terminally decreased B-Ht	Elevated activities of AST (Day 35–death), total LD (Day 28–death), LD_1 (Day 7–14), LD_2 (conspicuously on Day 40) and CK (Day 21–28)	Elevated CPFI (Day 36–death)	Slaughtered *in extremis* on Day 40. Pronounced lung oedema, hydropericardium
11	Anaemia (Day 38–death) and tachycardia (Day 38–death)	Decreased B-Ht, B-Hb (Day 38) and P-glucose (Day 35–38) Increased B-ESR (Day 38)	Elevated activities of AST (Day 21–death), total LD (Day 35), LD_1 (Day 14 and 42), LD_2 (Day 42) and CK (Day 35)	Inverted T wave (Day 22–37)	Died on Day 42. Pronounced lung oedema and hydropericardium
12	Tachycardia (Day 45)	Decreased P-glucose (Day 7 and 21), increased P-glucose (Day 45)	Elevated activities of AST (Day 21–death), total LD (Day 28–death), and CK (Day 28–35)	Inverted T wave (Day 15–death). Elevated CPFI (Day 43)	Slaughtered *in extremis* on Day 45. Pronounced lung oedema, hydropericardium
13	Tachycardia (Day 42–death), systolic murmur (Day 41–death) and dyspnoea (Day 43–death)	Decreased B-Ht (Day 24–31), and P-glucose (Day 21)	Elevated activities of AST (Day 21–35), LD_2 (conspicuously on Day 21), LD_5 (Day 35)	Configuration changes of the T wave (Day 8–death) and of QRS (Day 22–death). Elevated CPFI (Day 36)	Slaughtered *in extremis* on Day 45. Pronounced lung oedema, hydropericardium
14	Tachycardia (Day 55–death)	No change	Elevated activities of AST (Day 28–death), total LD (Day 45–death), LD_2 (Day 56), LD_5 (Day 28 and 35) and CK (Day 35)	Inverted T wave (Day 14–death). Elevated CPFI (Day 50–death)	Slaughtered *in extremis* on Day 57
15	Tachycardia (Day 55–death)	No change	Elevated activities of AST (Day 21–death), total LD (Day 49–death), LD_5 (Day 28, 35 and 56) and CK (Day 28–35 and 56)	Inverted T wave (Day 15–death) and elevated CPFI (Day 57)	Died on Day 57. Lung oedema
16	Arrhythmia (Day 42–death) and tachycardia (Day 57)	No change	Elevated activities of AST (Day 28–death), total LD (Day 28–death) and LD_1 (Day 14)	Elevated CPFI (Day 50–death)	Slaughtered *in extremnis* on Day 57
17	Arrythmia (Day 49–death), 2:2 coupled rhythm (Day 49–death) and gallop rhythm (Day 57)	No change	Elevated activities of AST (Day 28–death), total LD (Day 35–death), LD_1 (Day 14) LD_5 (Day 28) and CK (Day 28–death)	Elevated CPFI (Day 50–death)	Died on Day 57. Lung oedema
18	No signs	No change	Elevated activities of AST (Day 21–70), total LD (Day 35–77) and transient increased LD_5 activities between Day 28 and 70	Inverted T wave (Day 7–43)	Slaughtered on Day 100. Myofibrillar lysis
19	No signs	No change	Elevated activities of AST (Day 35–77), total LD (Day 28–84), LD_1 (Day 14–21) LD_5 (Day 7, 35, 56 and 70) and CK (Day 34–56)	Inverted T wave (Day 7–death)	Slaughtered on Day 100. Myofibrillar lysis
20	No signs	No change	Elevated activities of AST (Day 35–77), total LD (Day 31–84) and LD_1 (Day 14 and 63–77)	No change	Slaughtered on Day 184

TABLE 5 AST enzyme levels of sheep during a natural outbreak of gousiekte in the Ventersdorp area

Flock exposed to *P. pygmaeum*		Flock not exposed to *P. pygmaeum*	
Sheep No.	AST U/ℓ	Sheep No.	AST U/ℓ
1	41	16	39
2	68	17	47
3	97	18	52
4	77	19	41
5	615	20	57
6	115	21	29
7	105	22	52
8	63	23	47
9	68	24	34
10	62	25	51
11	101	26	47
12	82	27	39
13	64	28	44
14	83	29	45
15	72	30	42
	$\bar{X}$ = 114,2		$\bar{X}$ = 44,4

The pathological changes are summarized in Tables 3 & 4. Lung oedema was the most conspicuous lesion in most of the animals that died. Histopathological lesions of gousiekte occurred in 16 sheep. In 4 animals (Table 3, Sheep 7 & 8; Table 4, Sheep 18 & 19), the predominant lesion was dissociation of myofibrils in the endocardium of the apex, left free ventricular wall and interventricular septum. This lesion was characterized by a loss of striations and a weakly eosinophilic, homogeneous to finely fibrillar cytoplasm.

Field outbreak

The 15 control sheep had a mean serum AST activity of 44 U/ℓ. Fourteen out of the 15 sheep that were exposed to *P. pygmaeum* showed increased AST activity, with an average value of 114 U/ℓ (Table 5); ECG recordings, made on certain of the sheep, were normal. All the exposed sheep died during the following week.

Cyanosis, various degrees of myocardial dilation, thinning of the ventricular walls (6 sheep), lung oedema (5 sheep) and hydropericardium (3 sheep) were recorded in the 7 sheep that had died during our visit to the farm. Typcial lesions of gousiekte were evident in these sheep on histopathological examination.

DISCUSSION

Gousiekte is a difficult toxicological problem to investigate owing to the long latent period without premonitory signs and the individual variation in susceptibility of the animals to the plant toxin. The problem is exacerbated by the great variation in the toxicity of the plants (Kellerman *et al.*, 1988).

This study has shown that certain clinical pathological parameters can be used for the identification of affected animals during latency and in studies of the pathogenesis of gousiekte. Elevation of serum AST activity seems to be the most reliable indicator of cardiac damage in gousiekte (Table 3 & 4, Fig. 1 & 2). In a field outbreak where control animals were available, increased AST activities, with 1 exception, were seen in the affected animals (Table 5). All the animals, including the one with normal AST activity, died during the following days. This observation has important practical application as farmers can now be advised which animals to slaughter when gousiekte breaks out in their flocks. With regard to diagnosis, it should be pointed out that electrocardiagrams recorded near death in some of the sheep were normal, showing that changes in electrical activity do not necessarily occur in gousiekte.

Elevated serum LD activity proved to be relatively less effective than that of AST in determining cardiac damage (Table 3 & 4, Fig. 1 & 2). No specific pattern in the LD isoenzyme changes was observed during the latent period. This lack of change in the isoenzymes may be explained by the fact that the distribution of LD isoenzyme in the serum of normal sheep approximates that of cardiac tissue (Beatty, 1983). Doxey (1984) quotes isoenzyme values (LD_1, 89 %; LD_2, 6 %; LD_3, 3 %; LD_4, 2 %; LD_5, nil) in ovine cardiac muscle which roughly resembled those (LD_1, 66 %; LD_2, 6 %, LD_3, 19 %; LD_4, 4 %; LD_5, 5 %) recorded in the serum of sheep in this study. Increases in the serum LD activity as a result of cardiac damage in sheep, therefore, are not accompanied by notable changes in the iso-enzyme pattern. In humans, on the other hand, where the LD isoenzyme pattern in cardiac tissue and serum differ considerably, cardiac damage is associated with changes in the pattern of the isoenzymes in the serum i.e. LD_1:LD_2 (Kupper & Bleifeld, 1979).

Although AST and LD are widely distributed in the body the elevated serum activity of these two enzymes in gousiekte can be attributed to cardiac damage. In gousiekte only the heart is affected and secondary damage to other organs arising from congestive heart failure is rare (Kellerman *et al.*, 1988). Furthermore, GGT activity remains within normal limits, indicating that hypoxic damage to the liver associated with congestive heart failure is absent. The activities of AST and LD in the serum were also not influenced by the transient parasitaemia with *E. ovis* recorded in some of the sheep.

Measurement of CK activity in the serum proved to be of no diagnostic value in gousiekte as this activity was found to follow an erratic pattern during the course of the intoxication. No CK-MB isoenzyme activity could be recorded in the serum of affected sheep, thus supporting the observation of Beatty (1983) that this enzyme was absent in ovine cardiac muscle.

The determination of routine parameters such as B-ESR, B-Ht and glucose levels proved to be of great value in this study. Changes in the pattern of these values (Tables 3 & 4) were indicative of a developing parasitaemia with *E. ovis* evident in blood smears.

Enzyme activities followed a peculiar pattern in experimental gousiekte; firstly, no increases were found during the first *c.* 2–3 weeks of the latent period; secondly, the peak of activity could occur up to 30 days after dosing with the plant had ceased (Fig. 1, Sheep 18). Tachycardia and cardiac dysfunction were registered only terminally (Fig. 1 & 2) corresponding with the findings of Pretorius & Terblanche (1967) and Van der Walt *et al.* (1981). The fact that progressive damage to the myocardium occurs long after exposure to the plant has ceased, has been confirmed by histopathological examination: detection of newly formed lesions shows that the cardiac injury is an on-going process. It is thus possible that mechanisms, other than a direct toxic effect, may play a role in the pathogenesis of gousiekte.

Immune mechanisms in gousiekte and the role of blood parasites, such as *E. ovis*, the presence of which are indicative of immunosuppression, will now be investigaged.

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