The role of fluorescence polarization immuno-assay in the diagnosis of plant-induced cardiac glycoside poisoning livestock in South Africa

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ABSTRACT

SCHULTZ, R.A., KELLERMAN, T.S. & VAN DEN BERG, H. 2005. The role of fluorescence polarization immunoassay in the diagnosis of plant-induced cardiac glycoside poisoning of livestock in South Africa. *Onderstepoort Journal of Veterinary Research,* 72:189–201

Poisoning with cardiac glycoside-containing plants is collectively the most important plant-associated poisoning of livestock in southern Africa. As a diagnosis of this significant poisoning is currently based on circumstantial evidence, a practical chemical procedure indicating the presence of cardiac glycosides in plants and animal specimens would be of considerable benefit.

The fluorescence polarization immunoassay (FPIA) method, used to determine digoxin plasma levels in humans and dogs, was adapted to estimate cardiac glycoside levels in known cardiac-glycoside-containing plants as well as in the rumen and organs of dosed sheep. Positive FPIA values were obtained with bufadienolide-containing plants, while negative results were obtained with plants not known to contain cardiac glycosides. The FPIA has aided in the diagnosis of cardiac glycoside poisoning in livestock and game in 30 outbreaks examined at the Division of Toxicology, Onderstepoort Veterinary Institute. Each outbreak is briefly described.

As a result of this assay, a better understanding of cardiac glycoside poisoning has been reached.

Keywords: Bufadienolide, cardenolide, cardiac glycoside poisoning, ruminants, toxic plants

INTRODUCTION

Cardiac glycoside-containing plants have a worldwide distribution, yet poisoning of stock with these plants is of significance only in southern Africa, where they collectively cause the most important plant-associated poisoning in the region (Kellerman, Coetzer & Naudé 1988; Kellerman, Naudé & Fourie 1996). South African plants contain two types of cardiac glycosides, viz. cardenolides and bufadienolides. Of these, the cardenolide-containing plants such as *Acokanthera oppositifolia*, *Adenium multiflorum* and *Gomphocarpus fruticosus* are of lesser veterinary importance, because they are seldom ingested by stock. The veterinary important cardiac glycoside-containing plants have bufadienolides as their active principles, and poisoning by them may be either acute or chronic. Amongst those plants that cause acute poisoning, tulp (*Moraea pallida, Moraea miniata* and *Moraea polystachya*), slangkop (*Drimia* spp., formerly *Urginea*) (Germishuizen & Meyer 2003) and witstorm (*Thesium lineatum*) are the most notable. In both bufadienolide and cardenolide poisoning the respiratory, cardiovascular, gastrointestinal and nervous systems are affected (Kellerman *et al.* 1988, 1996).

Joubert & Schultz (1982a, b and c) demonstrated that activated charcoal is an effective treatment for plant-induced cardiac glycoside poisoning of live-

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Accepted for publication 11 April 2005—Editor

stock. Early diagnosis is most important as animals exhibiting advanced cardiac and respiratory dysfunction are high risk cases and handling while treating them may aggravate their conditions.

The diagnosis of cardiac glycoside poisoning is currently based on circumstantial evidence, such as the presence of cardiac glycoside-containing plants that have been ingested, clinical signs and necropsy features consistent with heart failure (Kellerman *et al*. 1988). The diagnoses of field intoxication in the laboratory have been hampered by the diversity of cardiac glycosides and their aglycones in the various plants. Several attempts have accordingly been made to develop a functional direct or indirect chemical method for demonstrating cardiac glycosides in plants and specimens of animal tissues, i.e.:

- The rubidium test was time-consuming and not suitable for routine use (Bourdon & Mercier 1969).
- Thin layer chromatography (TLC) (McVann, Havlik, Joubert & Monteagudo 1992), high performance liquid chromatography (HPLC) and qualitative nuclear magnetic resonance (NMR) studies (H.D. Brandt, Medical University of Southern Africa, personal communication 1993), in which in all cases the lack of standards hampered the interpretation of the results.
- Competitive radioimmunoassay (RIA) with antibodies of broad specificity to cardiac glycosides to screen plants and animal specimens for the presence of immunoreactive cardiac glycosides (Radford, Gillies, Hinds & Duffy 1986).
- Cheung, Hinds & Duffy (1989) observed a good correlation between RIA and that of the Abbott TDx analyzer, used with Digoxin II reagents, for cardiac glycoside-containing tissue samples. This fluorescence polarization immunoassay (FPIA) is an existing medical technique for demonstrating cardenolides in human serum based on antigen/antibody reaction and competitive binding to the commercially available fluorophore for digoxin.

Some of the phytogenous bufadienolides and cardenolides in southern Africa were investigated in our laboratory by modified methods to extract (Bourdon & Mercier 1969) and test for cross immunity with FPIA against the commercially available fluorophore for digoxin (Cheung *et al*. 1989). The aim of the study was to evaluate the role of FPIA in the diagnosis of plant-induced cardiac glycoside poisoning of livestock in South Africa.

MATERIALS AND METHODS

Experimental cases

Plants

Fresh or shade-dried leaves, stems and bulbs of various plants were milled or blended for FPIA (Tables 1–4) or for dosing to sheep (Tables 6–8). Some of the plants were associated with poisonings while others were collected for determination of normal FPIA values.

Animals

Thirty-one milk-tooth to full-mouth Merino and Dorper sheep of various sexes and body mass varying between 19 and 82 kg, were dosed with the dried/fresh plant material (Tables 6–8). Background levels were estimated in non-intoxicated animals (Table 5).

Extractions

Two grams each of plant material, rumenal/stomach contents, minced liver and kidney were homogenized (Sorvall Omni-mixer, stainless steel chamber) with 40 mℓ water. These samples, or 40 mℓ aliquots of clear rumenal fluid, were acidified with 10 drops of concentrated hydrochloric acid. After addition of 80 mℓ dichloromethane (Merck, AR), samples were shaken for 30 min and centrifuged at 3 000 rpm for 30 min in a 250 mℓ screw-top polypropylene bottle. The supernatant water and emulsion phases were discarded. To the organic phase was added 40 mℓ water and 10 drops of concentrated ammonia and the resultant mixture was shaken and centrifuged as before. The supernatant fluid was discarded.

The cleaned-up organic phase was then filtered (Whatman no. 1) through sodium sulphate (Merck, AR Anhydrous) and the solvent evaporated on a Heidolph rotovap at 50 °C. The deposit was dissolved in 2 mℓ methanol at room temperature with the aid of an ultrasonic water bath (Elma transonic 420) and passed through a 0.45 µm polypropylene filter. A 100 µℓ aliquot of the filtrate was mixed with 1 mℓ serum of a healthy sheep. A control serum sample was also submitted.

The presence of cardiac glycosides in the serum was demonstrated by FPIA using an Abbott TDx analyzer (Abbott Laboratories, North Chicago, USA) and a Digoxin II kit (Abbott Laboratories Diagnostic Division). The cardenolide or bufadienolide equivalents of digoxin are expressed in nmol/ℓ of serum.

Field cases

The same procedures for extraction and FPIA, as for experimentally-induced cardiac glycoside poisonings, were followed.

RESULTS

Experimental cases

Normal values in plants

BUFADIENOLIDE-CONTAINING PLANTS

The results of the FPIA values in plant leaves, stems and bulbs are summarized in Table 1.

High values of bufadienolide were recorded in two species of the genus *Moraea:* that for dry leaves of *M. pallida* being 22 230 (n = 7) and for fresh leaves of *Moraea marlothii* 12 440 (n = 1). Much lower values were recorded in other members of this genus, i.e. *Moraea simulans*, *M. polystachya*, *Moraea tripetala* and *Moraea stricta*.

Low levels of bufadienolides were detected in the fresh leaves of *Drimia* spp. which are collectively known as "slangkop", while those of the fresh bulbs were higher.

With the notable exceptions of *Kalanchoe lanceolata, Tylecodon reticulatus* and dried *Cotyledon orbiculata* the values for members of the Crassulaceae

TABLE 1 FPIA values in bufadienolide-containing plants

Plant	FPIA (nmol/ℓ)		
	Leaves or other plant parts as indicated		
	Value/range	Mean	No.
IRIDACEAE			
Moraea marlothii	12 440		1
Moraea pallida	1 976–47 000 (dried)	22 230	7
Moraea polystachya	56–208 (dried)	105	8
Moraea simulans	72 (fresh)		1
Moraea stricta	796		1
Moraea tripetala	300 (dried)		1
HYACINTHACEAE			
Drimia altissima	10–12	11	2
Drimia altissima bulb	60		1
Drimia delagoensis	104–106	105	2
Drimia sanguinea	28		1
Drimia sanguinea bulbs	72–5 460	777	12
CRASSULACEAE			
Bryophyllum delagoense	8 (fresh)		1
Bryophyllum delagoense stems	16		1
Cotyledon orbiculata	11–110 (fresh)	52	9
Cotyledon orbiculata	2 100 (dried)		1
Kalanchoe lanceolata	592–802	697	2
Kalanchoe rotundifolia	3–436 (fresh)	156	3
Kalanchoe thyrsiflora	96–168 (fresh)	142	3
Kalanchoe crenata	80–96 (fresh)	88	2
Tylecodon paniculatus	90 (fresh)		1
Tylecodon reticulatus	22 800 (fresh)		1
Tylecodon ventricosus	2–41 (fresh)	17	7
Tylecodon ventricosus stems	50		1
Tylecodon wallichii	1–57 (fresh)	38	4
Tylecodon wallichii stems	76		2
SANTALACEAE			
Thesium lineatum	4–102 (dried)	37	3
Thesium triflorum	3		1

TABLE 2 FPIA values in cardenolide-containing plants

Plant	FPIA (nmol/ℓ)				
	Leaves (fresh)		Leaves (dried)		
	Value	No.	Value	Mean	No.
APOCYNACEAE					
Acokanthera oblongifolia	2 908	1	5 416		1
Adenium boehmianum	99 600	1			
Acokanthera oblongifolia	2 908	1	5 416		1
Gomphocarpus fruticosus	3 928	1	5 220–9 280	7 250	2
Nerium oleander	7 980	1	4 200–8 630	6 415	2

TABLE 3 FPIA values in plants not known to contain cardiac glycosides

Plant	FPIA (nmol/ℓ)			
	Leaves		Berries/seeds	
	Value/mean	No.	Value	No.
Cucumis myriocarpus	5	1		
Euphorbia gariepina	0	1		
Ficus macrophylla	1	1	1.4	1
Gnidia burchellii	0	1		
Ledebouria spp.			0	1
Melia azedarach (Syringa)	4	1	8	1
Merwilla plumbea formerly *Scilla natalensis*	3	2		
Ornithogalum prasinum	2	1		
Ornithoglossum viride	4	2		
Persea americana (Avocado pear)	30	1		
Senecio latifolius	12	1		
Sophora japonica	1.4	1	0	1
Terminalia sericea (Silver Cluster-leaf)	0.1	3		

TABLE 4 FPIA values in plants parasitic on cardenolide-containing plants and plants not known to contain cardiac glycosides

Parasitic plant	FPIA (nmol/ℓ)	Host plant	FPIA (nmol/ℓ)
Tapinanthus quequensis (Loranthaceae)	8 030	*Nerium oleander* (Apocynaceae)	8 630
Tapinanthus quequensis (Loranthaceae)	0	*Acacia caffra* (Fabaceae)	Not done
Tapinanthus quequensis (Loranthaceae)	0	*Euclea crispa* (Ebenaceae)	Not done
Viscum rotundifolium (Viscaceae)	1 642	*Nerium oleander* (Apocynaceae)	4 200
Viscum rotundifolium (Viscaceae)	11	*Ziziphus mucronata* (Rhamnaceae)	4
Viscum verrucosum (Viscaceae)	5	*Acacia karroo* (Fabaceae)	Not done
Viscum combreticola (Viscaceae)	8	*Croton* spp. (Euphorbiaceae)	Not done

(collectively known as "plakkies") were relatively low. Little difference was found in the values obtained in the leaves and the stems of *Tylecodon ventricosus* and *Tylecodon wallichii*.

CARDENOLIDE-CONTAINING PLANTS

Apart from *M. pallida*, *M. marlothii* and *T. reticulatus* (Table 1), the values obtained for cardenolide-containing plants, in the one or two specimens of each species examined, seemed generally to be higher (2 908–99 600 nmol/ℓ) than those of the bufadienolide-containing plants (Tables 1 and 2). The highest value (99 600 nmol/ℓ, *n* = 1) was recorded in the fresh leaves of *Adenium boehmianum* (Table 2).

PLANTS NOT KNOWN TO CONTAIN CARDIAC GLYCOSIDES

Nil values or extremely low (negligible) levels of "cardiac glycoside" were registered (Table 3).

PARASITIC PLANTS

Parasitic plants collected on *Nerium oleander* had noticeably higher values than either their counterparts or related species growing on plants not known to contain cardiac glycosides (Table 4).

FPIA in experimentally poisoned animals

The background FPIA levels in the serum and organs of non-intoxicated animals are given in Table 5.

SERUM

Noticeable elevations with high FPIA values were recorded in the sera of animals 1–2 h after they were dosed with *M. pallida* and *Drimia sanguinea*. Similar high values were not registered in the sera of sheep to which *M. polystachya* had been administered or in those dosed with members of the Crassulaceae (Tables 6–8).

RUMENAL CONTENTS, KIDNEY AND LIVER

The highest FPIA values were recorded in the rumenal contents followed by the kidneys and liver, of sheep poisoned by *M. pallida* (Table 6). The FPIA values in the sheep that received fresh bulbs of *D. sanguinea*, though still noteworthy, were of a lesser order than those of *M. pallida*. Small to moderate elevations were still discernable in the liver and kidneys 24–72 h after dosing with *D. sanguinea* (Table 7).

Much lower FPIA levels were recorded in rumenal contents and kidneys of the two sheep poisoned with *M. polystachya* (Table 6).

Comments on experimental cases

The findings of this investigation should be interpreted in the light of the small number of plants and animals examined (Tables 1–8).

The FPIA values of tulp were variable with *M. pallida and M. marlothii* in general having higher values than the other *Moraea* spp. (Table 1). This finding is of considerable diagnostic importance as *M. pallida* (yellow tulp) is the species most often incriminated in poisoning of stock (Kellerman *et al*. 1996). To the best of our knowledge *M. marlothii*, *M. stricta* and *M. tripetala* have not been incriminated in poisoning of animals, although there is circumstantial evidence that *M. tripetala* might have poisoned a human (Naudé, Kellerman & Schultz, unpublished data 1995).

The FPIA levels in the fresh leaves of slangkop were of the same order as those of plakkies but considerably lower than that of tulp (Table 1). The fact that fresh bulbs of the various *Drimia* spp. have higher values than the leaves does not affect the incidence of poisoning as only the latter and flowering spikes are eaten by stock. Note that the FPIA of flowering stems of *Drimia altissima* (12 nmol/ℓ)—the part of the plant supposedly eaten most often by stock—did not materially differ from that of the leaves (10 nmol/ℓ) in the one specimen examined. The relatively high FPIA values in *D. sanguinea* may be fortuitous from a diagnostic point of view, as this species is responsible for the most "slangkop" deaths.

With the exception of *T. reticulatus,* the other plakkies (*Bryophyllum, Cotyledon, Kalanchoe* and *Tylecodon* spp.*)* tested registered relatively low values. As can be expected, dessication increased FPIA values: fresh leaves of *C. orbiculata* (44 nmol/ℓ) after dessication registered a *c*. 50-fold increase (2 100 nmol/ℓ).

Conspicuously higher FPIA values were obtained in cardenolide-containing plants than in those containing bufadienolides. Levels in parasitic plants growing on a cardenolide-containing plant and other non-cardiac glycoside-containing plants were comparable to their host plants and is an important factor in the diagnosis of cardiac glycoside poisoning.

In plants not known to contain cardiac glycosides very low FPIA levels were recorded, only *Persia*

TABLE 5 FPIA values in non-intoxicated animals

Animal	FPIA (nmol/ℓ)											
	Serum			Stomach/rumenal contents			Liver			Kidney		
	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.
Cattle	0.2	0–2.4	21	1.4	0.4–3	6	4.0	0–7 (30)	11	3.2	0–10	12
Sheep				0.03	0–0.2	6	1.1	0–4	6	0.3	0–2	12
Dogs				0.5	0–5	10	0	0	10	0.5	0–3	10
Ostriches				1	0–2	2	2	0–4	2	0	0	2

TABLE 6 FPIA values in sheep dosed with dried tulp

Sheep	Plant (g/kg)	Activated charcoal treatment Time (h)	FPIA (nmol/ℓ) Serum Interim (h)							Rumen contents	Liver	Kidney	Fate
			0	1	2	4	6	20	44				
Moraea pallida													
1	0.75	7	0.1		0.8	1.1	1.1						Discharged
2	0.75	20	0.3	0.6	1.6			1.8	0.7				Discharged
3	1									400	9	68	Died 5 h
4	1.25	4	0.1	1	1.5	1.8							Discharged
5	1.25	5			4.5	4.6							Discharged
6	1.25									43[a] 58[b]	4	41	Died 3 h
7	1.25									10[a] 64[b]	30	21	Died c. 6 h
8	2		0.5	2.7	2.4					450	16		Died 2 h
9	2		0	2.3	3.1					480	40		Died 2 h
10	2		0	4.4						1 120	26		Died within 2 h
11	5										24		Died 2 h
Moraea polystachya													
1	5		0.1	0.6	0.4		0.5			13	6	18	Died within 24 h
2	5		0.1	0.3	0.6	0.3				3	4	8	Died within 24 h

[a] Fluid (rumen liquor)
[b] Fibre

americana (avocado pear) and *Senecio latifolius* registering above 10 nmol/ℓ (Table 3). Some species in the families Hyacinthaceae and Crassulaceae also tend to have low levels but it is possible that false negative results can be recorded. It seems probable, however, that high levels found in plants by this method are always an indication of the presence of cardiac glycosides. The higher levels in the wide range of *M. pallida* and *T. reticulatus* samples can be explained as being caused by the effect of diluting the samples. To remain within the range of the TDx analyser, dilution of the plant extracts were necessary but the results were non-linear. Dilutions were necessary when analysing extracts of cardiac glycoside-containing plants that had a very good cross reactivity with digoxin, which affected the upper levels of the range in these plants. When comparing high levels this must also be borne in mind.

From these limited experimental findings it would seem that the possibility of diagnosing *M. pallida* poisoning is better than in the case of other tulp or slangkop species (Tables 6 and 7). The somewhat low FPIA values found in the organs of animals poisoned by *D. sanguinea* probably reflect the low values in the leaves and/or the long interval before clinical signs set in. Similarly, in *M. polystachya* poi-

TABLE 7 FPIA in sheep dosed with fresh slangkop (*D. sanguinea)*

Sheep	Plant (g/kg)	Activated charcoal treatment Time (h)	FPIA (nmol/ℓ) Serum Interim (h) 0	1	2	4	24	Rumen contents	Liver	Kidney	Fate
1	1.5							5	8	12	Died 72 h
2	2	30	0.7	1.9	2.6	2.5	1.6				Discharged
3	2.5	13.5	0		3	2.2	0.6				Discharged
4	2.5	24	0.6	1.8	2.3		2.3				Discharged
5	2.5								15	12	Euthanased 24 h
6	3	30	2.4	2.1	2.8	2.9	1.3				Discharged
7	4			3	3	2.6	1.7	4	8	23	Euthanased 48 h
8	5			2.1	3.3	3.4	2.4	3.4	6	7	Died within 48 h
9	5		0.2	2.3	3	1.4	1.5	2	6	4	Died within 48 h
10	5		0.1	2.8	3.4	2.4	2.4	2	8	16	Died within 48 h
11	5							96	31	18	Died within 24 h

TABLE 8 FPIA values in sheep dosed with plakkies (fresh leaves and stems)

Sheep	Plant g/kg	Activated charcoal treatment Time (h)	FPIA (nmol/ℓ) Serum Interim (h) 0	1	2	3	6	24	48	Fate
Tylecodon ventricosus[a]										
1	2.5 5 10		0			0	0	0	0	Died day 3
2	10	23 48	0	0.11	0	0	0			Krimpsiekte Day 6
Tylecodon wallichii										
1	2		0	0	0	0		0		Discharged
2	7.5	48	0	0	0	0		0	0	Died day 4
Cotyledon orbiculata										
1	5		0	0.24	0	0	0	0		Discharged
2	5		0		0	0	0			Discharged
3	10		0.17	0.13	0.15	0.16		0.13		Discharged

[a] Botha, Kellerman, Schultz, Erasmus, Vleggaar & Retief 1998

soning, the relatively low levels in the organs reflect those in the leaves.

The animal organ of choice for analysis depends on the interval between ingestion of the poisonous plant by the animal and collection of the sample. In acute intoxication a sample of the rumenal contents appears to be the best one to take but if the animals have shown clinical signs for several days, the liver and kidneys are probably preferable (Tables 6 and 7).

Elevated FPIA values were recorded in the sera of most animals poisoned by cardiac glycoside-containing plants except the plakkies (Tables 6 and 8). Most of the determinations were carried out on the sera 1–4 h after dosing, but indications are that these high values can persist for 24 h or longer.

Meaningfully elevated values were registered in the sera both of animals that died and those that survived. Owing to lack of numbers, however, the effect of treatment with charcoal could not be determined.

Field cases (history included)

Cattle

OUTBREAK 1

In July 1993 a recumbent bovine in the vicinity of Vrede in the eastern Free State Province was diagnosed as having been poisoned by tulp. The veterinarian described the clinical signs of the animal, which had been sick for a day, as "typical" for tulp poisoning. Despite treatment with activated charcoal, the animal died the same night. A FPIA value of 4.3 nmol/ℓ in the rumenal contents, in comparison with values in control animals (0.4–3, Table 5), supported the diagnosis.

OUTBREAK 2

During August 1993 16 heifers (220–250 kg) near Vrede developed signs of tulp poisoning after eating the plant. Three of them died before, and seven after, treatment with activated charcoal. According to the veterinarian, the clinical signs varied from slight ataxia to difficulty in rising and the virtual inability to walk. Their hearts were arrhythmic and they were constipated. FPIA values of 292 nmol/ℓ in the rumenal contents, 37.4 nmol/ℓ in the liver and 6.2 nmol/ℓ in a kidney of one of the affected animals were consistent with tulp poisoning.

OUTBREAK 3

In August 1993, again in the Vrede district, 13 heifers died overnight. Two had showed pareses of the hindquarters. The camp in which they grazed was infested with tulp that had been eaten. FPIA values in the rumenal content of 44 nmol/ℓ, in the liver of 40 nmol/ℓ and a kidney of 20 nmol/ℓ attested to the heifers having died of tulp poisoning.

OUTBREAK 4

Five yearling cattle died and six were sick when a herd of 107 head accidentally grazed a heavily tulp infested camp near Bronkhorstspruit in Gauteng Province during September 1993. A large number of the cattle were said to have developed diarrhoea. Fragments of tulp leaves could be identified in the rumenal contents of two of the three carcasses brought to the Onderstepoort Veterinary Institute for post mortem examination. The most notable histopathological lesions were moderate oedema and degeneration of myocardial cells accompanied by diffuse foci of myocardial necrosis. Some of the FPIA values in the carcasses examined (rumen 265 and 3.7 nmol/ℓ; liver 2 and 10 nmol/ℓ; kidney 8 and 2 nmol/ℓ in two of the animals respectively) suggested that the cattle had died of tulp poisoning.

OUTBREAK 5

In October 1993 two cattle died suddenly in a camp containing *Dichapetalum cymosum* (gifblaar) near Naboomspruit (Mookgopong), Limpopo Province. No significant lesions could be found on post mortem examination and fragments of *D. cymosum* were not present in the rumen. However, FPIA values of 1.4 nmol/ℓ in the rumen, 6 nmol/ℓ in the liver and 14 nmol/ℓ in the kidneys indicated that the animals had been poisoned by a cardiac glycoside-containing plant.

OUTBREAK 6

One cow out of 106 died shortly after being translocated to the Ellisras (Lephalale) district, Limpopo Province, in November 1993. The FPIA values of the liver (10 nmol/ℓ), kidney (8 nmol/ℓ) and rumenal contents (0.3 nmol/ℓ) incriminated *D. sanguinea* poisoning as possible cause of death.

OUTBREAK 7

Six heifers died and 25 out of a herd of 134 became sick soon after being introduced into a camp at Louis Trichardt (Makhado), Limpopo Province, during March 1994. The heifers had replaced cows that had grazed the pasture without mishap for 2 months. The affected animals showed typical signs of cardiac glycoside poisoning; namely, weakness of the hindquarters, stilted gait and diarrhoea. Two fell down and had convulsions before they died. Apart from rumenal stasis, the necropsy findings on one animal were essentially negative. No poisonous plants could be identified in the pasture. When other causes of death, such as poisoning with pesticides and arsenic, were eliminated, FPIA was performed on the sera of nine clinically affected heifers. A high value of 38.1 nmol/ℓ in the serum of one justified a putative diagnosis of cardiac glycoside poisoning. The diagnosis was supported by a FPIA value of 12 nmol/ℓ in the liver of a heifer that died.

OUTBREAK 8

Two cattle became sick and three died out of a herd of 37 during June 1994 on veld infested with tulp

near Wonderfontein in Mpumalanga Province. The affected animals were weak, ataxic and had softer faeces than the rest of the herd. Although the cause of death in the heifer presented for necropsy was complicated by *Pasteurella*-pneumonia, the FPIA values in her organs were elevated, namely, kidney, 20 nmol/ℓ; rumenal contents, 6 nmol/ℓ; and liver, 4 nmol/ℓ. In the light of this a putative diagnosis of tulp poisoning was made.

OUTBREAK 9

Four cattle died suddenly and ten became sick in a mixed herd comprising 180 head aged between 8 months to 3 years near Rehoboth, Namibia, in January 1995. The clinical signs were consistent with those of heart failure and the animals had diarrhoea. Their pasture was infested with blue tulp (*M. polystachya*) which had been eaten. The FPIA values in one of the animals (rumenal contents, 18 nmol/ℓ; kidney, 14 nmol/ℓ; liver, 3 nmol/ℓ) corroborated the diagnosis of tulp poisoning.

OUTBREAK 10

During April 1995, 50 calves aged 6–7 months and 17 heifers out of a mixed herd of 120 cattle near Fochville, Gauteng Province developed severe watery diarrhoea. The worst affected calves (25) and all the heifers were treated with activated charcoal. Seven of the treated calves, nevertheless, died. None of the cows was affected. According to the owner, who was unacquainted with tulp, the pasture contained milkweed (*Gomphocarpus* sp.) which had not been grazed. It must be pointed out that, although *Gomphocarpus* spp. contain cardenolides they are unpalatable and seldom eaten. The FPIA values in a dead calf (liver, 12 nmol/ℓ; kidney, 7 nmol/ℓ; rumenal contents, 5 nmol/ℓ) supported a putative diagnosis of cardiac glycoside poisoning.

OUTBREAK 11

Two cattle died suddenly in July 1995 while grazing in a mountain camp infested with *T. lineatum* near Carnarvon, Northern Cape Province. As *T. lineatum* plant material from the supposedly toxic camp registered FPIA values of 102 nmol/ℓ, cardiac glycoside poisoning must be considered as a possible cause of death.

OUTBREAK 12

In July 1995, 11 mature cattle in the Molteno district, Eastern Cape Province, died acutely after showing signs such as swaying gait, constipation and aggression. The dead cattle were found in a gorge where they had sought shelter from the cold. The ground was covered with snow and the tulp in the camp showed evidence of being eaten. A diagnosis of tulp poisoning was supported by a FPIA value of 9 nmol/ℓ in the rumenal contents of one of the dead animals.

OUTBREAK 13

Late in September 1995, near Ventersburg, Free State Province, three weaned calves aged 9 months died in a camp containing mainly sprouting green grass, but a portion of it had been cultivated and was sparsely covered by sorghum regrowth and young wheat. The affected animals had manifested ataxia, paralysis, watery diarrhoea and dehydration before death. Examination of the rumenal contents of the dead calves did not reveal fragments of tulp leaves. Mature cows that had grazed in the camp for weeks were not affected. Tulp poisoning was suspected, but no tulp plants except those growing under bushes, which were beyond the reach of the calves. A diagnosis of tulp poisoning was confirmed by high FPIA values in the rumenal contents (43 nmol/ℓ), although values in the kidney (3 nmol/ℓ) and liver (4 nmol/ℓ) were low.

OUTBREAK 14

Seven Brahman bull calves out of 50 developed signs of cardiac glycoside poisoning near Mooinooi, North West Province, during October 1995. Six of these died. The clinical signs included weakness of the hindquarters and sudden death while being driven. According to the attendant veterinarian necropsy revealed severe heart damage, congestion and oedema of the lungs and hyperaemia of the digestive tract. A tentative diagnosis of cardiac glycoside poisoning or poisoning with gifblaar was made. Although the FPIA value in the rumenal contents was low (0.4 nmol/ℓ), that of a kidney was sufficiently high (14 nmol/ℓ) to support a diagnosis of cardiac glycoside poisoning.

OUTBREAK 15

In November 1995, rumenal contents and kidney from the decomposed carcass of a cow that had died suddenly near Vryburg, Northern Cape Province, were submitted for examination. Although neither of the plants was in evidence, poisoning with slangkop or blue tulp was suspected. High FPIA values of 20 nmol/ℓ in the rumenal contents and 12 nmol/ℓ in a kidney confirmed the suspicion.

OUTBREAK 16

In early February 1996, a number of mature cattle became sick and 12 out of 250 died over a period of 8 days near Potgietersrus (Mokopane), Limpopo Province, after showing clinical signs of colic, a tendency to lie down when driven and paralysis, especially of the hind quarters. The lesions at necropsy were non-specific but included congestion and oedema of the lungs, epicardial haemorrhages and congestion of the gastro-intestinal tract. The animals had recently been introduced into the "toxic" camp, which contained abundant green grass. A FPIA value of 13 nmol/ℓ in the liver of one of the affected animals supported a diagnosis of cardiac glycoside poisoning.

Sheep and goats

OUTBREAK 17

During August 1994 a number of ewes grazing on a harvested maize land near Vrede, Free State Province, died acutely without obvious signs of illness. The non-specific necropsy findings included rumenal stasis, reddening of the gut and, in some, evidence of diarrhoea. A high FPIA value of 52 nmol/ℓ in the specimen of rumenal contents from one animal submitted supported the diagnosis of tulp poisoning made by the local veterinarian. However, the FPIA value in the liver of this animal was zero.

OUTBREAK 18

Thirty out of 200 goats died after passing through a marshy area infested with tulp near Rehoboth, Namibia, in August 1994. Clinical signs, consistent with tulp poisoning, such as posterior pareses and muscle tremors, were described. The FPIA values in the organs of one of the goats (rumenal contents, 14 nmol/ℓ; a kidney, 4 nmol/ℓ; liver, 0 nmol/ℓ) agreed with a diagnosis of tulp poisoning.

OUTBREAK 19

One goat out of 300 died and another became sick in late November 1994 near Windhoek, Namibia. A tentative diagnosis of cardiac glycoside poisoning was made on the strength of the clinical signs of diarrhoea, muscular spasms and bloat. Slightly elevated FPIA values in a kidney (6 nmol/ℓ) supported the diagnosis, although those in the rumenal contents (0.3 nmol/ℓ) and liver (0 nmol/ℓ) did not.

OUTBREAK 20

Twenty-five ewes died on tulp-infested veld near Trompsburg, Free State Province in May 1995. Apart hyperaemia of the rumenal wall, the necropsies on two ewes did not reveal significant lesions. A moderately high FPIA level of 1.1 nmol/ℓ in the rumenal contents was consistent with cardiac glycoside poisoning.

OUTBREAK 21

In July 1995 an unspecified number of sheep died near Vrede, Free State Province, while grazing on a harvested maize land infested with tulp. The owner did not know whether tulp or maize (acidosis, diplodiosis) was responsible for the death of his sheep. A FPIA value of 4 nmol/ℓ in the rumenal contents supported the diagnosis of tulp poisoning.

OUTBREAK 22

Twenty-five out of 90 lambs aged 3–6 weeks died suddenly in August 1995 on a pasture heavily infested with tulp near Fauresmith, Free State Province. The veterinarian suspected pulpy kidney disease, but, as their dams when pregnant had been vaccinated, requested that tests for pesticides and cardiac glycosides be done. Moderately high FPIA values of 1.5 nmol/ℓ in the rumenal contents and 4 nmol/ℓ in the liver implicated cardiac glycosides as the cause of death of the lambs.

OUTBREAK 23

Necropsies were performed on three of 15 goats that died on Kaalplaas, the OVI experimental farm, between March and September 1995. One, a kid aged 5 months, had been born on the farm, while the other two had been introduced from elsewhere about 10 months earlier. The *post mortem* features of the animals, which had died suddenly without clinical signs having been seen, were consistent with cardiac glycoside poisoning. Investigation of the paddocks revealed the presence of two cardiac glycoside-containing plants, milkweed (*Gomphocarpus fruticosus*, FPIA 3 928 nmol/ℓ) and a tulp (*M. stricta*, FPIA 796 nmol/ℓ), but only the milkweed showed signs of having been eaten. Elevations of FPIA values in two of the animals (rumenal contents, 3–4 nmol/ℓ; liver, 2–8 nmol/ℓ and kidney, 6 nmol/ℓ) supported a diagnosis of cardiac glycoside poisoning.

Donkeys

OUTBREAK 24

During September 1995, three donkeys were found dead near Harrismith, Free State Province. Extracardial changes of heart failure such as foam in the

trachea and bronchi and oedema of the lungs were noted on post mortem examination of one of them. Moderately elevated FPIA values in the stomach contents (4 nmol/ℓ) and liver (3 nmol/ℓ) suggested that it might have died from cardiac glycoside poisoning.

Horses

OUTBREAK 25

In October 1995 two mares near Nottingham Road, Kwazulu-Natal Province, died of gas colic while several others refused to eat their rations of lucerne (alfalfa) hay. Examination of the hay revealed that it was heavily contaminated with an unidentified tulp species. The FPIA value of dry tulp leaves from the bales of lucerne hay that were incriminated was 22 040 nmol/ℓ. A putative diagnosis of tulp poisoning was made.

OUTBREAK 26

After failing to demonstrate ionophore antibiotics in the feed of horses suffering from colic near Windhoek, Namibia, a sample of the feed was subjected to FPIA. Three of the worst affected horses had died within 7 h of developing clinical signs. After a value of 293 nmol/ℓ in the feed was obtained it was examined microscopically, revealing tulp fragments with a FPIA value of 2 000 nmol/ℓ. The findings strongly suggested that the colic was the result of cardiac glycoside poisoning.

Other animals

OUTBREAK 27: SUNI ANTELOPE

Thirteen Suni antelope died suddenly over a period in a breeding camp at Skukuza, Kruger National Park, Mpumalanga and Limpopo Provinces, in May 1994. The *post mortem* features, congestion and oedema of the lungs, presence of ulcers in the abomasum and subserosal haemorrhages in the wall of the rumen, resembled cardiac glycoside poisoning. Inspection of the camp revealed large numbers of *K. lanceolata* (592–802 nmol/ℓ) which had been grazed. The deaths ceased after the antelope were moved from the toxic camp. The FPIA values in the frozen organs (liver, 1–1.2 nmol/ℓ; kidney 0–2.4 nmol/ℓ) of two of the Sunis supported the diagnosis of *K. lanceolata* poisoning.

OUTBREAK 28: CAPE FUR SEAL

A sub-adult seal died acutely of apparent heart failure shortly after being moved to a new pen in the Pretoria Zoo during November 1993. The lungs were severely oedematous and the stomach was filled with plant material identified as remnants of *Ficus macrophylla*. FPIA values of 18.6 nmol/ℓ in the stomach content and 2.6 nmol/ℓ in the kidney supported a diagnosis of possible cardiac glycoside poisoning. A list of the plants in or about the pen included *F. macrophylla* (FPIA 1–1.4 nmol/ℓ); *Sophora japonica* (0–1.4 nmol/ℓ), of which the seeds might have fallen in the water; *Brachychiton* sp., of which the flowers littered the floor; and *Duranta repens*, of which the fruit might have been eaten. *Populus* spp. and *Robinia pseudoacacia (*Black Locust*)* were also present, but out of reach of the seal and was thus uninvolved in the poisoning. Although the source could not be determined, a putative diagnosis of cardiac glycoside poisoning was made.

OUTBREAK 29: OSTRICHES

Four incidents of suspected cardiac glycoside poisoning of ostriches were investigated between 1993 and 1995. In the first incident, a necropsy was performed on the last of six ostriches to die out of a flock of ten. The bird succumbed soon after being found with its head on the ground. Apart from oedema of the head and upper neck, no significant lesions were found on post mortem examination, but elevated FPIA values in the liver (20 nmol/ℓ) and a kidney (8 nmol/ℓ) suggested cardiac glycoside poisoning. However, inspection of the trampled camp in which the birds had been held for about a year failed to reveal cardiac glycoside-containing plants. A putative diagnosis of cardiac glycoside poisoning was nevertheless made.

In the second incident, necropsies were performed on three birds that had died out of a flock of 20 breeding pairs. The affected flock had recently been translocated from another farm into a camp, the vegetation of which had been burnt but the grass had begun to sprout. The FPIA values in the stomachs of the dead ostriches were 2–38 nmol/ℓ, in the livers 4–10 nmol/ℓ and the kidneys 0–14 nmol/ℓ. Milkweed (*Gomphocarpus* sp.) collected in the camp registered FPIA values of 1 608–3 928 nmol/ℓ in newly sprouted leaves, 1 700 nmol/ℓ in dry stems and 1 600 nmol/ℓ in mature seeds. Despite the isolation of velogenic Newcastle disease (VND) virus from the dead birds, a diagnosis of milkweed poisoning was made.

In the third incident, the FPIA value in the liver of the second month-old ostrich chick to die within 2 days on a farm near Pretoria were 24 nmol/ℓ. The stomach contents were negative. Since no patho-

genic organisms could be isolated from the tissues of this bird, a diagnosis of cardiac glycoside poisoning was made.

In the fourth incident, three 7-week-old chicks with nervous signs were presented for necropsy. In addition to megabacteriosis and the isolation of VND virus, the FPIA values of the stomach contents were elevated (stomach contents, 2–44 nmol/ℓ; liver 0–4 nmol/ℓ). A diagnosis of VND complicated by megabacteriosis and suspected cardiac glycoside poisoning was made.

OUTBREAK 30: DOG

A dog was necropsied that had died within 15 min of playing with a toad (M. Williams, personal communication 1996). The owner killed the toad, which was subsequently identified as *Schismaderma carens* or red toad by W.D. Haacke, Transvaal Museum of Natural History, Pretoria. Elevated FPIA values of the stomach contents of the dog (30 nmol/ℓ) and the dorsal skin glands of the red toad (60 nmol/ℓ) strongly suggested cardiac glycoside poisoning of the dog. It is known that Bufonidae secretes a bufadienolide. *Schismaderma carens* is the only *Schismaderma* spp. among the South African Bufonidae, the others being 11 *Bufo* spp. (Pantanowitz, Naudé & Leisewitz 1998).

DISCUSSION

The diversity of cardiac glycosides and their aglycones in various plants has hampered the laboratory diagnoses of cardiac glycoside poisoning in livestock. The advantage of FPIA is the broad cross immunity of the commercially available digoxin fluorophore to the large variety of cardiac glycosides found in southern African plant species. In order to eliminate certain chemical compounds in the samples that may interfere with the cross immunity, a simple extraction method is used prior to the assay.

As a result of this assay, a better understanding of cardiac glycoside poisoning has been reached. Cardiac glycoside values (digoxin-equivalent expressed in nmol/ℓ) were estimated in plants known to contain cardenolides or bufadienolides and plants not known to contain cardiac glycosides. Interestingly, parasitic plants not known to be poisonous were found to give positive levels when growing on cardenolide-containing plants. Tulp (*Moraea* spp.), most often incriminated in acute cardiac glycoside poisoning of animals had the highest FPIA levels which is a significant finding, as this increases the usefulness of the assay as a diagnostic tool. In the sheep experimentally poisoned by cardiac glycoside-containing plants, elevated levels were obtained in blood, liver, kidneys and rumenal contents. Approximately 30 field cases, mainly involving cattle, were investigated and successfully diagnosed as cardiac glycoside-related by recording FPIA levels in the stomach/rumenal contents, liver and/or kidneys. Apart from ruminants, intoxication of horses could also be linked to tulp in their feed, and that of ostriches to milkweed. Additionally, the method was used to confirm cardiac glycoside intoxication in a dog that had played with a toad.

FPIA, coupled with circumstantial evidence, allows early diagnosis of cardiac glycoside poisoning, making early treatment possible. It is particularly important to make an early diagnosis as in animals suffering from advanced cardiac and respiratory dysfunction, the stress of dosing activated charcoal to them often leads to fatal heart failure.

In spite of the relative limited number of cases examined, the value of FPIA in the diagnosis of plant-induced cardiac glycoside poisoning of livestock in southern Africa has been established. The assay is now being successfully applied in the routine diagnosis of this form of poisoning in our laboratory. This test would also be useful in the diagnosis of cardiac glycoside poisoning caused by the inadvertent overdosage of traditional herbal medicines containing plants such as *D. sanguinea* (Joubert & Mathibe 1989; Foukaridis, Osuch, Mathibe & Tsipa 1995).

ACKNOWLEDGEMENTS

We acknowledge the assistance given by technical staff (Division of Toxicology, ARC-OVI) and Prof. L. Prozesky and his staff (formerly of the Division of Pathology, ARC-OVI). Our gratitude is extended to veterinarians referring cases of suspected cardiac glycoside poisoning to our laboratory.

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