Onderstepoort Journal of Veterinary Research, 63:97–108 (1996)

First report of field outbreaks of ergot-alkaloid toxicity in South Africa

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ABSTRACT

SCHNEIDER, D.J., MILES, C.O., GARTHWAITE, I., VAN HALDEREN, A., WESSELS, J.C. & LATEGAN, H.J. 1996. First report of field outbreaks of ergot-alkaloid toxicity in South Africa. *Onderstepoort Journal of Veterinary Research*, 62:97–108

Outbreaks of clinical disease caused by the ingestion of ergotized *Lolium rigidum* (annual ryegrass), which resulted in a substantial loss in production, have been reported. A number of outbreaks of a hyperthermia syndrome in cattle, characterized by severe loss in milk production, loss of body mass and reduced fertility, are described. In one major outbreak in March to April 1994, a milling company reported that 2 646 dairy cows on 29 farms had developed clinical signs. In this outbreak, significant levels of ergotamine, ergosine, ergocornine and ergocryptine were found in the milled dairy rations fed to the affected cows. Barley screenings containing ergotized annual-ryegrass seed was identified as the toxic component and probable source of the ergot alkaloids in the ration. The clinical syndrome was reproduced experimentally by feeding suspected feed to a group of nine high-producing Ayrshire cows.

An outbreak of gangrenous necrosis of the extremities in young cattle in the winter of 1987 was also suspected of having been caused by ergot alkaloids in grain screenings.

Keywords: Annual ryegrass, ergot-alkaloid toxicity, field outbreaks, *Lolium rigidum*

INTRODUCTION

It has been known for centuries that the fungus *Claviceps purpurea* can parasitize most cereals, especially rye, and also many other grasses. Campbell (1957) infected 46 grass species in the field and in a greenhouse. The fungus produces sclerotia which replace some of the seed in the seedheads. These sclerotia with their elongated dark-brown bodies of variable size, often somewhat larger than the seed of the host plant, usually contain ergotamine, ergosine, ergocryptine and a range of other, less important, ergopeptine alkaloids.

Poisoning of man and animals by ergot sclerotia has been reported from many parts of the world. The range of clinical signs described includes gangrenous necrosis of extremities, convulsions, hypersensitivity, lameness, abortion and agalactia (Woods, Bradley, Jones & Mantle 1966). In recent years, another syndrome has been recognized in cattle ingesting ergot sclerotia in summer. The clinical signs include hyperthermia, reduced feed intake, lethargy, drop in milk production, loss of body mass, increased respiratory rate, breathing with an open mouth and protruding tongue, excessive salivation, increased water

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Accepted for publication 4 March 1996—Editor

intake and urination, seeking shade, wading in water or frequenting moist areas, as well as reduced reproductive efficiency. Long, rough hair, seen mostly in chronic cases, is more pronounced in young animals. Severely reduced serum-prolactin levels constitute the most important biochemical change. (Jessep, Dent, Kemp, Christie, Ahrens, Burgess & Bryden 1987; Ross, Bryden, Bakau & Burgess 1989; Rottinghaus, Schultz, Ross & Hill 1993; Scrivener & Bryden 1993).

During the past 10–15 years, strikingly similar signs of hyperthermia have been described in cattle ingesting *Festuca arundinacea* Shreb (tall fescue) grass, silage, hay or seed infected by the endophytic fungus *Acremonium coenophialum*. The fungus lives intercellularly in symbiosis with the plant. The gangrenous syndrome, called "fescue foot", occurs less often and in cold weather, whereas the hyperthermia or "summer syndrome" is more common in hot weather. (Siegel, Latch & Johnson 1985; Bacon, Lyons, Porter & Robbins 1986; Zanzalari, Heitmann, McLaren & Fribourg 1989; Shelby & Kelly 1990; Aldrich, Paterson, Tate & Kerley 1993). Ergot alkaloids can also be produced by other grass-endophyte combinations. (Bacon *et al.* 1986; Leuchtmann 1992).

A combination of ergopeptine alkaloids, of which ergovaline is the most important, was identified in endophyte-infected tall-fescue herbage and seed. It is suspected that these ergot alkaloids have an even greater, world-wide economic impact on animal productivity than those present in the sclerotia of *C. purpurea* on grain and grasses. (Bacon *et al.* 1986; Rowan & Shaw 1987; Shelby & Kelly 1990; Cheeke, Luick & Debessai 1993).

The clinical signs caused by both groups of ergot alkaloids are the results mainly of vasoconstriction, inhibition of prolactin secretion and possibly also of increased production of heat by the energy-wasteful mixed-function oxidase (MFO) system, essential in the detoxication of ergot alkaloids (Zanzalari *et al.* 1989).

The vasoconstriction reduces the ability of animals to dissipate excess body heat through the skin during hot weather, and to transfer blood and heat to the extremities during cold weather. It has also been suggested that the consumption of ergot alkaloids decreases mass gains by reducing feed intake and by restricting blood flow to internal organs (Zanzalari *et al.* 1989; Aldrich *et al.* 1993).

In this paper we wish to report an outbreak of gangrenous necrosis in young cattle on two neighbouring farms, and four outbreaks of a hyperthermia syndrome in cattle, one of which affected more than 2 600 high-producing dairy cows. As far as is known, this is the first confirmed report of ergot-alkaloid toxicosis due to *Claviceps purpurea* in South Africa.

FIELD OUTBREAKS OF DRY GANGRENE AND HYPERTHERMIA IN CATTLE

A. GANGRENOUS SYNDROME

History

Seventy one-year-old steers, brought in June 1987 from the southern Cape to farm A in the district of Bredasdorp, were injected with Imidocarb dipropionate (Forray 65, Hoechst Ag-Vet) according to instructions, and put on good, mixed, green pastures consisting mainly of oats, *Medicago* spp. and annual ryegrass. After 10 d, four steers became lethargic and registered elevated rectal temperatures. Anaplasmosis was diagnosed, and they were injected intramuscularly with 20 mℓ of Oxytetracycline hydrochloride (Engemycin 10% Intervet SA) and thioctic acid (Tioctan-Vet, Truka-Panvet) daily for 3 d. The affected animals were stabled and fed lucerne hay and a calf meal, prepared on a neighbouring farm (farm B).

Two of these animals died, and after 12 d the two surviving steers were allowed to graze a small paddock consisting mainly of annual ryegrass, kikuyu (*Pennisetum clandestinum*) and *Trifolium* spp. After a further 10 d, the two steers showed signs of lameness and mild diarrhoea. Oxytetracycline hydrochloride was injected daily for 7 d. At that stage gangrenous necrosis of some extremities was noticed by the owner.

During the same period, six of 30 calves (2–3 months of age) being hand-reared on farm B, also developed signs of gangrenous necrosis of extremities and mild diarrhoea. Four died, but the two surviving calves were brought to the Regional Veterinary Laboratory (RVL), Stellenbosch, together with the two steers from farm A.

The calves from farm B were fed 2 ℓ of cow's milk twice daily and a home-mixed calf meal consisting of unspecified quantities of wheat, barley screenings (bought from the local farmers' co-operative), maize, molasses and a protein-mineral mixture. This meal mixture was also fed to the two steers while they were stabled at farm A. Wheat and barley straw were available *ad libitum* and the calves were allowed to graze in the farmyard and adjacent paddocks.

Materials and methods

At the RVL Stellenbosch, blood was drawn in lithium heparin (14,3 U.S.P./mℓ) from the anterior vena cava of these four animals for biochemical analyses. The animals were then euthanased and necropsied. Liver samples were collected in 10% analytical formalin for trace element analyses. For histopathological examination, specimens of brain, heart, liver, spleen, kidney and intestine were fixed in 10% buffered formalin, processed according to standard procedures,

and stained with haematoxylin and eosin (H.E.). Blood smears from peripheral blood were stained with Cam's Quick-Stain (C.A. Milsch Pty Ltd) and examined for protozoal parasites. Routine chemical pathological analyses of blood included phosphorus, calcium, magnesium, total bilirubin, blood urea nitrogen, albumin, globulin and haematocrit. The trace elements tested for in the livers were copper, iron, zinc, manganese and molybdenum.

Results

No protozoal parasites were evident on microscopical examination of stained blood smears. All the clinical-pathological and trace-element values in the blood and liver samples were within the normal range.

All the animals were in fairly poor condition. The extent of the gangrenous lesions of extremities are given in Table 1. The gangrenous lesions of all affected feet extended up to the level of the fetlock joints.

No signs of ergot sclerotia were found in the green sward on these two farms in August 1987. The calf meal was not examined. No significant changes were found on histopathological examination of sections of organs from affected steers and calves.

B. Hyperthermic syndrome

1. LOCALIZED OUTBREAK AT MALMESBURY

History

In late summer and autumn of 1988, 1990 and 1993, a dairy farmer, milking about 240 Friesian cows, observed clinical signs of hyperthermia and production loss in approximately 50% of his cows. The cows were kept in groups of 40 in open pens throughout the year. The feed consisted of a commercial dairy ration supplemented by lucerne and oat hay. The oat hay was produced on the farm and contained 5–70 % annual ryegrass.

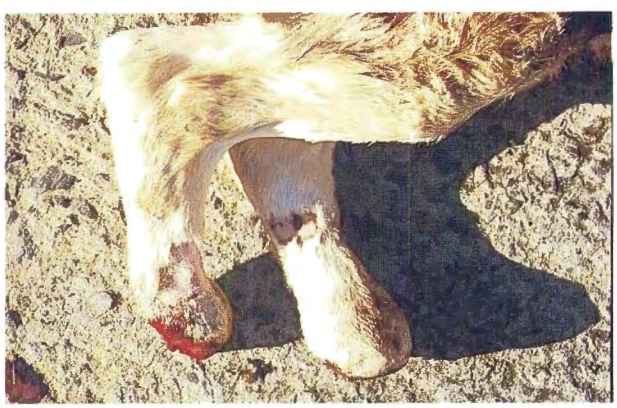

FIG. 1 Loss of hind feet and tail in calf from farm B as a result of gangrenous necrosis

The symptoms included rapid and laboured breathing, elevated rectal temperature (up to 40,5 °C), open-mouthed breathing with protruding tongue, excessive salivation and loss of body condition. In some cases, milk production dropped by up to 50%. The average daily milk production per cow decreased by 20% on this farm during summer and autumn. The cows sought shade and preferred to lie on moist areas, and drank more water. Some even stood with their front feet in the water troughs. In chronic cases, the cows developed long, rough hair. The clinical signs were characteristic of ergot-induced hyperthermia. However, since this syndrome had not previously been reported in South Africa, it was not initially recognized by the local veterinarians.

The signs were most striking during hot weather and were dramatically aggravated by exercise. Most cows appeared to be normal on cold days, but the typical signs of hyperthermia reappeared with hot weather—even during May and June.

Materials and methods

Blood samples from ten affected cows were collected in tubes containing lithium heparin 14,3 U.S.P./mℓ of blood (Vac-U-Test) on 15 February 1988, and from five affected cows on 8 May 1990 and 11 June 1990. Routine biochemical analyses were performed on these 15 blood samples at the RVL Stellenbosch.

On 24 February 1988, one affected cow was sacrificed and necropsied on the farm; on 25 April 1990, five; and on 2 August 1990, four chronically affected cows were slaughtered at the Malmesbury abattoir. The lungs, hearts, livers, kidneys and spleens of all the animals, and the brains of two were examined at the RVL Stellenbosch.

After macroscopical examination, specimens of all the organs were fixed in 10% buffered formalin, processed according to standard procedures and stained with H.E. according to Masson's trichrome methods for histopathological examination, at the Faculty of Veterinary Science, University of Pretoria. Liver samples from eight cows were preserved in analytical formalin for trace-element analyses by the RVL Stellenbosch.

TABLE 1 The distribution of gangrenous lesions in the four animals

Calf No.	Age (months)	Front feet	Hind feet	Ears	Tails
1	12	N	A	A	A
2	12	N	A	A	N
3	3	A	A	N	N
4	3	N	A	A	A

A = Affected
N = Not affected

Four lung samples were subjected to routine bacteriological examination, and five lung and mediastinal lymph-node samples were processed and inoculated in calf-foetal-kidney (CFK) cell cultures for virus-isolation purposes.

Five samples of the dairy feed collected on 11 June 1990, were tested for aflatoxin by means of thin-layer chromatography and for heavy metals by atomic absorption (A.A.S.) at the RVL Stellenbosch. Gossypol levels were assessed in three dairy-feed samples and five cotton-seed samples incorporated in the dairy feed, by the Onderstepoort Veterinary Institute (OVI) in June 1990.

On November 1993, 20 bales of oat hay contaminated by ryegrass, were collected from each of four lands. The bales from each land were pounded on a plastic sheet on a concrete surface, and the material on the sheet, collected. The coarse material was removed by screening through a sieve with 2,1-mm mesh, and the fine, dusty material, through a 200 mesh sieve. The remaining material, containing practically all the ryegrass seed and ergot sclerotia, was examined macroscopically for sclerotia by spreading it out in a thin layer on a flat, white surface. These four samples were also examined for fungal endophytes by use of the rose-bengal staining technique of Saha, Jackson and Johnson-Cicalese (1988). A sample of ryegrass plants was collected out of the oat hay for identification by the Stellenbosch Herbarium (Fellingham 1666 STE).

Results

Chemical pathological findings

All values obtained by biochemical analyses of blood from 15 chronically affected cows, and liver samples of eight of these cows, were within the normal range.

No significant bacteria were isolated by routine bacteriological culturing of four lung samples. Virus isolation from five lung and lymph-node samples from affected cows gave negative results on CFK cell cultures.

Plant identification

The Herbarium, Stellenbosch, identified the ryegrass plants as *Lolium rigidum.*

TOXICOLOGICAL EXAMINATION

No aflatoxins or heavy metals were found when five samples of dairy feed were tested.

Free gossypol levels in the cotton seed were between 0,01% and 0,008%. The levels found in the feed mixture ranged from 0,001–0,0007%.

Low levels of ergot sclerotia were found in the ryegrass seed in each of the four samples collected out of the oat hay. Fungal endophytes were also seen microscopically in all the ryegrass-seed samples examined.

Pathological findings

No conspicuous, gross pathological changes could be found in any of the organs of the ten cows examined, except for emphysema in the dorsal aspect of the diaphragmatic lobes of the lungs.

Histopathology

Significant abnormalities were present in the heart and lungs of all animals examined. The lesions were similar in nature, but varied in extent; the severity of the lesions being classified as mild or moderate. The changes in the heart were consistent with chronic multifocal interstitial myocarditis and were characterized by focal infiltration of leukocytes, predominantly lymphocytes and macrophages, but with occasional neutrophils, in conjuction with fibrosis—particularly of the interstitium in the subepicardium and around the Purkinje fibres and blood vessels in the subendocardium. Additional lesions were randomly distributed, and consisted of atrophy that alternated with hypertrophy, together with hyaline degeneration and necrosis with "replacement" fibrosis of individual or small groups of myocardial fibres. The vascular changes included mild medial proliferation with segmental sclerosis of the mural muscular arteries in the hearts of four animals.

The pulmonary lesion comprised chronic multifocal interstitial pneumonia, characterized by mild interstitial infiltration of neutrophils and/or eosinophils in association with a mononuclear leukocytic reaction, and moderate fibrosis, hyperplasia of type II pneumocytes of alveoli, and hyperplasia of smooth-muscle tunics of bronchioles. The interstitial reaction was accompanied by focal atelectasis that alternated with focal alveolar and interstitial emphysema. The vascular changes included mild segmental intimal and prominent, diffuse medial proliferation, in association with segmental sclerosis of the muscular ateries in the lungs of all affected animals. (Prof. J.W. Nesbit, personal communication 1995).

2 and 3. LOCALIZED OUTBREAKS AT BREDASDORP

History

During February 1993, dairy and beef cattle on two farms approximately 30 km apart in the Bredasdorp district, showed clinical signs typical of the hyperthermia syndrome. About 120 dairy and beef cattle were kept on each of these farms.

About 60% of the cattle on these farms showed signs of hyperthermia, but in young animals, poor growth and long, rough hair were more conspicuous.

FIG. 2 Poor growth and long rough hair in calves, suspected to be caused by the ingestion of ergot sclerotia in summer

FIG. 3 Fungal hyphae of "Acremonium-like" endophyte in *Lolium rigidum* seed

The clinical signs were identical to the hyperthermia syndrome seen in the Malmesbury outbreak, and to ergot-alkaloid toxicosis associated with the ingestion of endophyte-infected tall fescue or *C. purpurea* sclerotia (Jessep *et al.* 1987; Rowan & Shaw 1987; Scrivener & Bryden 1993).

The feed consisted of home-mixed dairy meal, hay and straw. Lucerne grazing was also utilized when available. The beef animals grazed on lucerne and stubble lands, and grain and hay were supplemented when necessary. Annual ryegrass was abundantly present in pastures grazed by the animals, as well as in hay, straw, grain and the screenings incorporated in the dairy feed.

Materials and methods

A sample of ryegrass was collected for identification by the Stellenbosch Herbarium (Fellingham 1667 STE).

Ryegrass-seed samples from a number of bales of lucerne hay and from barley screenings on both farms, were examined at the RVL Stellenbosch, for ergot sclerotia and endophytes. Samples of ryegrass seed and herbage from one Bredasdorp farm was sent to the New Zealand Pastoral Agricultural Research Institute (AgResearch, Ruakura, Hamilton) and to L.P. Bush (University of Kentucky, USA) for demonstration of endophytes and alkaloid analyses. HPLC analyses were done by G.A. Lane, AgResearch Grasslands, Palmerston North, New Zealand. Herbage samples were extracted with chloroform/methanol/ammonia, and seed and ergot samples with methanol/lactic acid. Ergopeptides were separated by reverse-phase HPLC with an acetonitrile/aqueous-ammonium acetate gradient, and detected by fluorescence (excitation 310 nm, emission 410 nm). Ergotamine was identified by co-chromatography, with authentic, ergotamine tartrate (Sigma). Other ergopeptides were tentatively identified by co-chromatography with wheat-ergot components. In the absence of standards, no attempts were made to estimate quantities of the ergopeptides.

Results

The ryegrass plants were identified as *Lolium rigidum* by the Stellenbosch Herbarium.

Variable quantities of annual-ryegrass seed and ergot sclerotia, morphologically resembling the sclerotia of *C. purpurea*, were evident in hay, straw and barley screenings from both farms.

Microscopical examination of annual-ryegrass seed from both farms at the RVL Stellenbosch, revealed heavy infection with endophytic fungal hyphae.

The ryegrass-seed sample was found to be heavily infected with an "acremonium-like" endophyte and contained ergot sclerotia identified from their morphology (Mantle 1977) as *C. purpurea*. (Margaret E. diMenna, AgResearch Ruakura, personal communication 1993). When tested by ELISA, (Garthwaite, Sprosen, Briggs, Collin & Towers 1994), the sample was found to contain 9,5 ppm of paxilline equivalents, and approximately 7 ppm of lysergol equivalents.

ELISA indicated that high levels of ergot alkaloids, as well as several other alkaloid groups often associated

with endophyte infection of grass, were present in the seed sample. N-acetylloline (1 885 ppm), and possibly low levels of ergovaline (0,17 ppm) were present (L.P. Bush, University of Kentucky, personal communication 1994).

H.P.L.C. analyses of ryegrass seed and herbage revealed that the seed samples contained ergosine, ergotamine, ergocornine, and alpha- and beta-ergocryptine and ergocristine (in varying ratios). Ergots which contaminated the seed were analysed and found to contain the same pattern of ergopeptides. Ergopeptides were not detected in clean seed separated from the sample. (G.A. Lane, AgResearch, Palmerston North, personal communication 1995).

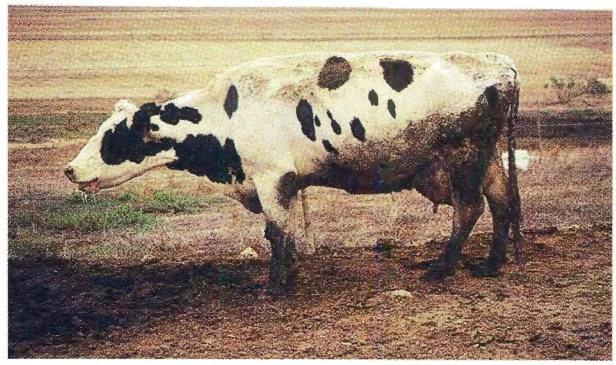

FIG. 4 Dairy cow showing signs of laboured breathing and salivation in the major outbreak

4. MAJOR OUTBREAK IN DAIRY COWS

History

During March and April 1994, severe outbreaks of typical hyperthermia syndrome suddenly occurred on a number of farms in several districts of the Western Cape. The milling company reported that 29 dairy herds, comprising 5 434 cows in lactation, were involved. A total of 2 664 out of 3 113 high-producing cows ingesting the suspected feed, were clinically affected. Daily milk production on affected farms dropped by from 10% to as much as 60%. The clinical signs were typical of hyperthermia as described in the Malmesbury and Bredasdorp outbreaks. On some farms, all animals in the high-producing groups were affected, with many cows obviously distressed on hot days. Housing or shade, as provided on some farms, did offer some relief, but did not prevent serious economic loss and clinical signs. A few farmers installed cold-water sprinklers in the yard at the entrance of the milking parlour to cool the heat-stressed cows. This gave almost immediate relief which lasted a few hours.

Virtually all affected cows were in high-producing groups ingesting ± 30 kg of a "complete dairy feed" bought from the same company. In addition, they received 3–5 kg of hay and, on some farms, also a few kg of silage.

At the time of this outbreak, ergot alkaloids, associated with annual ryegrass, were strongly suspected of being responsible for the hyperthermia syndrome. As no annual ryegrass could be found in the hay or silage on the farms investigated, all the components of the complete dairy feed were examined to establish the source of the suspected ergot alkaloids. It was found that the "complete dairy feed" contained 25–30% of barley screenings in milled and pelleted form. These pelleted screenings consisted of fine plant material, broken barley, malt culms, small barley kernels, ryegrass seed and variable amounts of ergot sclerotia. When clinical signs appeared on more farms, the feed company immediately stopped including the suspected pellets in their feed.

Severe, characteristic clinical signs appeared in cows on all affected farms over a period of 14 d, whereas it took 2–3 months for all signs to disappear completely. As far as is known, no mortalities could be attributed directly to this ergot-alkaloid intoxication, but many cows were obviously in severe distress during hot weather or after even mild exercise. All private veterinarians involved, observed serious reproductive problems in affected herds during subsequent months. Anoestrus, silent heat and abnormally low conception figures were reported. Many more cows than normal had to be culled because of infertility (Dr J.R. Adam).

The high-producing groups of cows were affected worst. They were fed a "complete ration", containing up to 30% of the pelleted barley screenings. Lower-producing groups were fed on smaller quantities of a concentrated dairy ration which also contained less of the barley screenings. They also consumed more hay and straw than the high-producing groups.

As most affected farms were large units, milking 200–800 cows on zero grazing, the "complete feed" was delivered in bulk. It was, therefore, impossible to obtain samples of feed that had been fed during the preceding weeks. We did, however, succeed in obtaining one unopened bag of suspected "complete feed" (produced by the same milling company on 10 March 1994) from a small dairy at Worcester. On this farm all of 28 cows in the high-producing group showed severe clinical signs, including a drop in average milk production from 32–12 ℓ/cow/d. Twenty-three cows in the low-producing group, ingesting only 5 kg of dairy meal (not complete feed) per day and grazing lucerne *ad libitum,* showed no clinical signs of hyperthermia. Milk production was unaffected in the region of 15 kg/cow/d.

Materials and methods

A sample of this "Worcester feed" was kept for analysis and the balance fed to two sheep at the RVL

Stellenbosch. Three samples of feed and screenings were analysed by ELISA and HPLC for ergot alkaloids. They consisted of the following:

Sample 1: Suspected complete dairy feed from Worcester farm

Sample 2: Suspected screenings, milled and pelleted, from the malt-producing plant received during the first 10 d of March

Sample 3: The identical complete feed as in sample 1, but without the suspected screenings

(Samples 2 and 3 were supplied by the feed company.)

Five blood samples from five cows showing signs of severe hyperthermia syndrome on the first farm visited in this major outbreak, were collected on 22 March 1994, in sterile Vac-U-Test tubes, with no additives. The serum samples were separated from the clotted blood and kept frozen at –20 °C until dispatched on ice to Irene Animal Production Institute for prolactin determination.

Results

The following ELISA results regarding the three feed samples extracted with 2:1 chloroform-methanol, were received from Ruakura Agricultural Centre:

Sample 1: 20,3 ppb lysergol equivalents

Sample 2: 17 ppb lysergol equivalents

Sample 3: 1,4 ppb lysergol equivalents

The actual levels of total ergot alkaloids might be considerably higher than these figures because of variations in the cross reactivity of the various alkaloids with the antibody.

The following HPLC results were received from Dr E.L. Piper, University of Fayetteville, Arkansas, USA (personal communication 1994)

Sample 1: Contained ergotamine and ergosine

Sample 2: (The barley screenings) contained the following alkaloids levels (in ppb): ergosine (168), ergotamine (175), ergocornine (147) and ergocryptine (148)

Sample 3: Contained no measurable alkaloids

He remarked that the presence of all of these compounds would certainly suggest ergot contamination of the screenings and said that he believed that there were enough ergopeptides present to cause the type of problems being experienced.

Neither ergovaline, ergonovine nor lysergic acid amide were found in any of the three samples. Because of the elaborate test procedures, the alkaloids were quantified only in Sample 2.

The five serum samples were tested for prolactin by a radio-immunoassay method supplied by the USDA Animal Hormone Program, Beltsville, Maryland, USA. The test was performed by Irene Animal Production Institute, Irene. The level of prolactin for the five samples in ng/mℓ were: 0,22, not detectable, 0,9, 0,68 and 0,16 (average 0,27 per cow).

According to the records of a well-managed dairy with 420 Ayrshire cows, 200 high-producing cows were fed on the suspected "complete feed". Of these, 177 showed clinical signs of hyperthermia syndrome. The maximum milk-production loss occurred during March and April, but on recovery, they did not reach the expected levels of production during that lactation period.

The total loss of milk production for the complete lactation of all affected cows on this farm was calculated at 180,000 ℓ. The average intercalving period for this herd for 1994, is 410 d. For 1993, it was 388 d, and for 1992, the figure was 384 d (C.A. McCarthy, personal communications 1994).

In a Friesian herd consisting of 390 adult cows, about 50% showed clinical signs of the hyperthermia syndrome. Of 79 cows that calved during December 1993 and January and February 1994, 55 were certified as being pregnant by 14 November 1994. Their average inseminations per conception were 3,09, and days open, 151. The average figures for the herd in 1993, were 2,03 and 107 d, respectively (Dr J.R. Adam, personal communication 1994).

EXPERIMENTAL REPRODUCTION OF HYPERTHERMIA IN DAIRY COWS

Materials and methods

Cattle trial

An experiment was carried out by Anglo American Farms, Werda, Paarl (C.A. McCarthy, personal communication 1994) and Meadow Feeds Paarl (C. Harrington, personal communication 1994) to evaluate the effect of the suspected complete dairy feed produced in early March 1994.

Two similar groups of six high-producing Ayrshire cows and three heifers in each group were selected. These two groups of cows were kept in two adjacent pens in the same shed from day 0 to day 22 (day 0 being 3 May 1994).

Both the test group A and the control group B were fed on the control ration from days 0–2. From day 2, however, the suspected dairy ration was fed to group A. Group B remained on the control feed. Each group received 80 kg of feed three times daily as well as 28 kg each of the same batch of lucerne hay once daily. The average days in lactation on day 2 were

31,8 for group A and 31,9 for group B, and the average daily milk production per cow was 26,2 kg for group A and 27,2 for group B.

Hanging gas heaters were installed in both pens and operated from days 15–22, when it was decided to terminate the experiment. The feeding of the suspected feed was ended on day 22 for humane and economic reasons, and because all parties involved were satisfied that group A had developed typical clinical signs of hyperthermia.

During the day, the temperature in both pens reached a maximum of 35 °C, but dropped to a minimum of 9 °C during one night. Blood samples from the two groups of cows in this trial were collected in Vac-U-Test tubes 1 h before milking on day 17, day 22 and day 36. Unfortunately, blood was not taken at the beginning of the feeding trial. The last sample was collected 2 weeks after the feeding of the suspect feed had ceased.

Sheep trial

Two year-old sheep were fed on the balance of the suspect feed (obtained from the Worcester farm) on 25 April to 5 May 1994. They were kept indoors and received no other feed. During this period, they ingested a total of 52 kg of the "complete dairy feed". Blood was collected from these two sheep in Vac-U-Test tubes on days 0, 4, 7, 11 and 23 (12 d after cessation of the suspect feed).

The serum samples from these experimental cattle and sheep were kept frozen at –20 °C and sent to Irene Animal Production Institute for prolactin determination.

Results

Cattle trial

On day 10, a marked increase in respiratory rate was obvious in two cows in group A. Signs of hyperthermia increased daily until, by day 22, all cows in group A were panting during the middle of the day. Three of these cows breathed with open mouths, protruding tongues and with saliva drooling.

Average feed consumption per cow per day was identical for the two groups, (29,88 kg). In group A, average daily milk production per cow decreased slightly from 26,2 ℓ on day 2 to 25,2 ℓ on day 22.

In the control group (group B), the average daily production per cow increased from 27,2 ℓ–32,6 ℓ over the same period.

Note that while the milk production increased significantly in the group-A cows over the 3-week period after withdrawal of the suspected feed, it was still lower than that of the control group (see Table 2).

The serum-prolactin levels for the experimental cattle are reflected in Table 3.

The very low prolactin levels in the group-A cows on days 17 and 22, are of diagnostic importance.

Sheep trial

No abnormal clinical signs were shown by these two sheep, but the prolactin levels dropped markedly (see Table 4).

Discussion

Clavicipitaceous endophytes (*Balansiae*) and the closely related genus, *Claviceps*, can produce different ergot alkaloids which induce similar clinical signs in animals. The chemical composition of the various alkaloids of the endophyte-infected grasses and the sclerotia of *C. purpurea*, vary according to the fungus species involved, the host species, the growing conditions and the geographic location (Bacon *et al.* 1986; Coppock, Mostrom, Simon, McKenna, Jacobson & Szlachta 1989).

The endophytes grow intercellularly in leaves, stems and seed of host-grass species, and their mycotoxins are usually found in all parts of the plant, although higher levels are found in the ripe seed and leaf sheath. Ergovaline is the predominant peptide alkaloid produced by the fescue endophyte *Acremonium coenophialum* (Bacon *et al.* 1986; Garner, Rottinghaus, Cornell & Testereci 1993). When grasses

TABLE 2 Average milk production/cow/d in ℓ for the two groups

Days of trial	Day 2	Day 22	Day 43
Group A	26,2	25,2	29,5
Group B	27,2	32,6	33,4

TABLE 3 Average prolactin levels per cow in ng/mℓ for the two groups in the cattle trial

Date of trial	Day 17	Day 22	Day 36
Group A	0,29	0,29	2,37
Group B	1,52	1,28	1,6

TABLE 4 Prolactin levels in sera of two sheep fed on ergot-contaminated cattle feed from 25 Apri to –5 May 1994, in ng/mℓ

Days of trial	Day 0	Day 4	Day 7	Day 11	Day 23
Sheep A	17,30	5,20	2,39	6,63	5,34
Sheep B	15,90	6,18	4,56	4,15	6,74

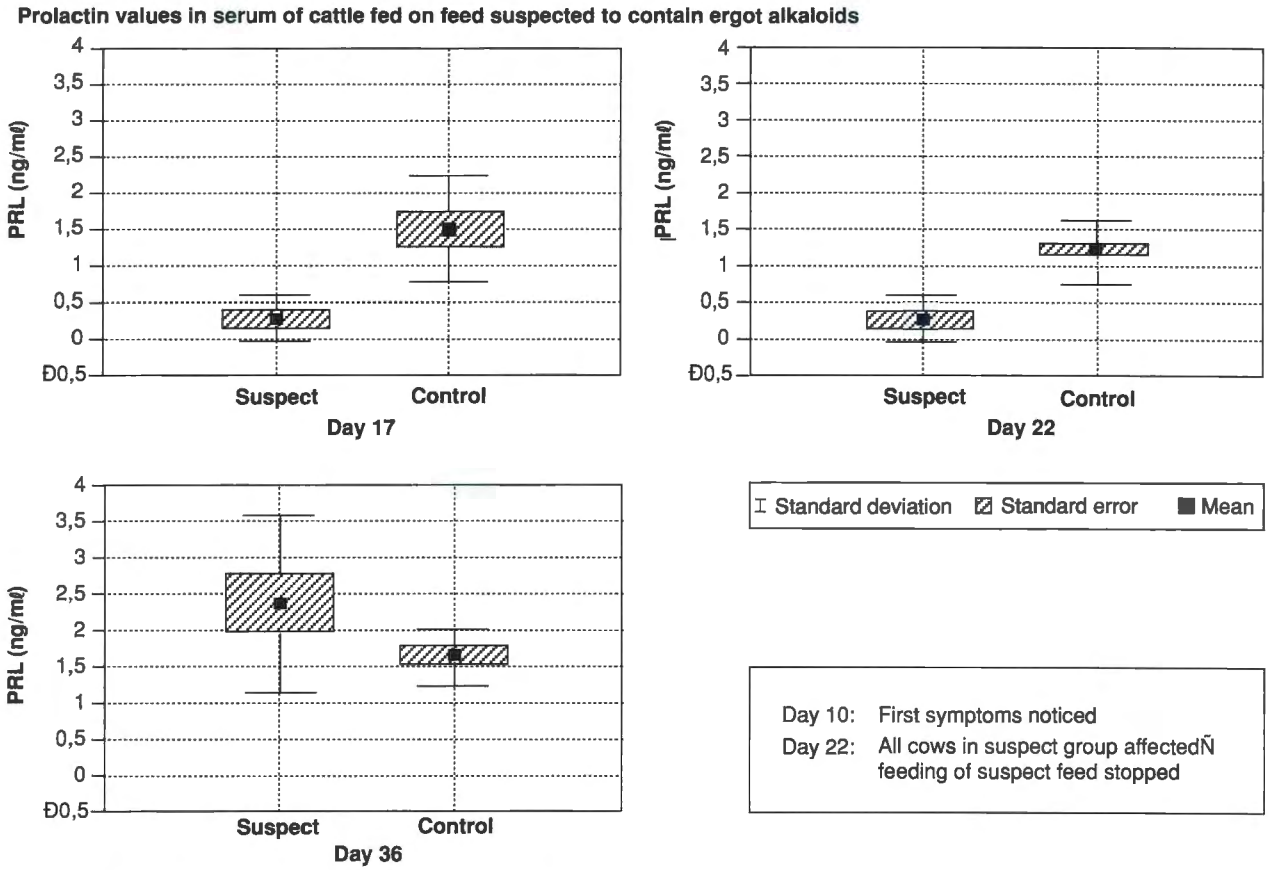

FIG. 5 Prolactin values of sera of cows in the experimental reproduction trial

are infected with *C. purpurea*, the ergopeptide alkaloids are present only in the sclerotia (which replace seeds in the ripe seedheads) and ergotamine is usually the most important ergopeptine alkaloid. (Rowan & Shaw 1987; Rottinghaus *et al.* 1993).

For this reason, endophyte-infected grasses can be toxic when ingested in the green or dry stage, but the toxic sclerotia of *C. purpurea* are found only on ripe seedheads. Animals can ingest toxic levels of ergot alkaloids from *C. purpurea* by ingesting ripe infected pasture grass, hay and silage, but grain screenings can be regarded as most dangerous, as the sclerotia are concentrated in screenings (Ross *et al.* 1989; Peet, McCarthy & Barbetti 1991; Rottinghaus *et al.* 1993; Scrivener & Brydon 1993).

Prolactin appears to fulfil functions essential to mammary secretory cell proliferation and differentiation during late pregnancy and is, therefore, together with cortisol and insulin, essential in order to obtain an active secretory response in the mammary gland. Prolactin is essential for maintenance of protein synthesis in lactating animals and is also required for the maintenance of the corpus luteum in female animals. (Cole & Cupps 1977). This, as well as the effect of the vasoconstriction and resultant hyperthermia, can be regarded as the cause of the decreased milk production and poor conception figures found in our outbreaks of ergot-alkaloid toxicosis and those described by Schmidt & Osborn (1993). Low serum-prolactin levels are regarded as extremely sensitive and reliable indications of the ingestion of ergot alkaloids (Cheeke *et al.* 1993).

In one sample of annual-ryegrass seed examined in the second and third Bredasdorp outbreaks of hyperthermia, high levels of lysergol equivalents and an HPLC profile resembling that observed with *C. purpurea* infestations, were found, but ergopeptides were not detected in ergot-free seed separated from this sample by G.A. Lane. Ergot sclerotia and *Acremonium*-like endophytes were also present in all the ryegrass screenings examined in the Bredasdorp outbreak.

Ergot alkaloids—mainly ergotamine, ergosine, ergocornine and ergocryptine—are almost certainly responsible for the major incident (outbreak 4). As the screenings had been milled, ergot sclerotia in the feed could not be identified by macroscopic examination. Low serum-prolactin levels found in cattle and sheep fed on the suspected feed in outbreak 4, supported the diagnosis of ergot-alkaloid toxicosis.

The dairy feed used at Malmesbury (outbreak 1) and the grain screenings fed in the outbreak of gangrene, were not analysed for ergot alkaloids, as, at the time, they were not suspected of being involved. In retrospect, there can be little doubt that, in the gangrenous outbreak, the barley screenings included in the calf meal, contained ergot alkaloids. It was discovered only years later, that the affected steers on farm A had been fed the calf meal prepared on farm B. In the Malmesbury outbreak, low concentrations of ergot sclerotia and endophytes were found in most oathay samples examined in 1993. Ergot alkaloids in the hay on its own, or together with possible alkaloids in the dairy feed, were most probably responsible for these Malmesbury outbreaks between 1988 and 1993.

Although ergot sclerotia and an *Acremonium*-like endophyte were both present in almost all the ryegrass-seed samples examined, we suspect the sclerotia from *C. purpurea* to be the major source of ergot alkaloids, because significant levels of ergovaline (the ergot alkaloid most commonly found in endophyte-infected grasses) were not present. However, high levels of ergotamine, ergosine, ergocornine and ergocryptine—the alkaloids most commonly found in *C. purpurea* sclerotia—were present. An *Acremonium*-like endophyte is known to occur in annual-ryegrass seed but, unlike *Acremonium lolii* infecting perennial ryegrass, it is not known to produce mycotoxins (Latch, Christensen & Hickson 1988).

These outbreaks in South Africa are identical to those described by Jessep *et al.* (1987) in Australia, except that in Australia the *Claviceps sclerotia* were associated with perennial-ryegrass seed. Scrivener & Bryden (1993) described an identical outbreak where cattle grazed annual ryegrass infected with *C. purpurea*. Peet *et al.* (1991) described outbreaks of hyperthermia and death in feedlot cattle, due to ingestion

FIG. 6 Suspected sclerotia of *Claviceps purpurea* on *Bromus diandrus* (left) and *Lolium rigidum* (right)

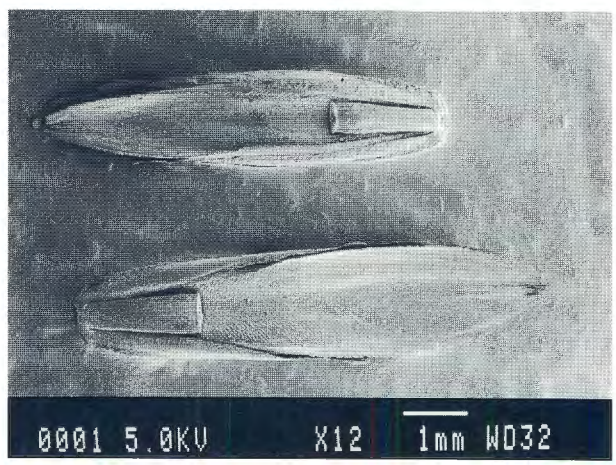

FIG. 8 Annual-ryegrass seed

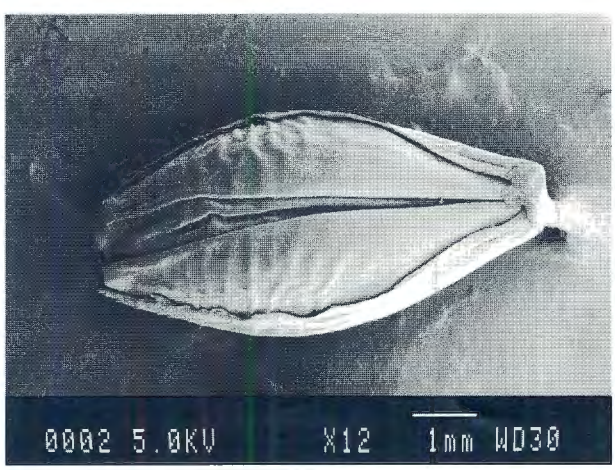

FIG. 7 Barley kernel

FIG. 9 Ergot sclerotia

of sclerotia of *C. purpurea*. The barley in the feed mix was contaminated with annual-ryegrass (*L. rigidum*) seed infested with *C. purpurea*.

No mention was made of endophyte infections in these outbreaks. No neurological signs, abortions or mortalities were reported in any of the South African outbreaks of the hyperthemia syndrome.

The vascular changes found in the lungs and hearts of the nine cows culled in the Malmesbury outbreak (1), are a possible reflection of chronic vasoconstriction. The inflammatory and emphysematous changes in the lungs may possibly be related to prolonged open-mouth breathing over periods of many weeks. The aetiology of the myocarditis cannot be explained, but may possibly have been related to either the long-term effects of intermittent vasoconstriction or the low levels of gossypol in the dairy feed (J.W. Nesbit, personal communication 1995).

Cattle seem to be more susceptible to ergot-alkaloid toxicosis than are sheep, but we are aware of at least one unconfirmed outbreak of poor growth and agalactia in a sheep stud associated with feeding on annual-ryegrass screenings and on grazing heavily contaminated with ergot sclerotia and endophytes. On investigation, none of the usual causes of these clinical signs could be identified.

We suspect that the shallow cultivation and/or monoculture grain production commonly practised in this winter rainfall region over the past decades, have favoured the enormous proliferation of annual ryegrass, and other weed grasses such as *Bromus diandrus*. The availability of host grasses and the shallow cultivation probably also favoured the infestation and reproduction of *C. purpurea*.

Lolium temulentum has presumably been present in the RSA for centuries, but in recent decades other annual-ryegrass species, e.g. *Lolium multiflorum* and *L. rigidum*, have been sown in pastures. In 1994, ergot sclerotia were found on annual ryegrass in many districts in the winter rainfall region, but sclerotia on *Bromus diandrus* were seen at only one location of only 1 m^2, growing among infected annual ryegrass on a farm in the Malmesbury district (see Fig. 6).

The possibility that the endophyte commonly found in annual ryegrass in this area is responsible for the production of toxins under particular circumstances (e.g. hot weather), should be investigated.

ACKNOWLEDGEMENTS

The Director of Animal Health is thanked for permission to publish this article.

We wish to thank Dr L.P. Bush, University of Kentucky, USA, Dr E.L. Piper, Department of Animal Science, University of Arkansas, USA, Dr Geoff A. Lane, AgResearch Grasslands, Palmerston North, New Zealand, for chemical analyses of feed and screenings, Prof. J.W. Nesbit, Department of Pathology, Faculty of Veterinary Science, University of Pretoria, for the histopathological examination of organs of culled cows, and Dr Margaret E. di Menna for mycological examinations.

We also wish to thank Dr J.W.S. Liebenberg, Malmesbury, for referring the Malmesbury outbreak (1) and Dr M. Wilson, Bredasdorp, for referring the outbreak of gangrenous necrosis.

We are most grateful for prolactin analyses done by the Production Physiology and Technology Division, Irene Animal Production Institute, Irene, and to the USDA Animal Hormone Program, USA, for donation of the antiserum and immonochemical-grade hormone.

We are indebted to Dr T.S. Kellerman and Prof. T.W. Naude for advice and support throughout the investigation and preparation of this manuscript.

Some reproduction results were kindly supplied by Dr J.R. Adam of Durbanville, and the detail of the cattle trial was made available by Mr C.A. McCarthy of Anglo American Farms, Werda, Paarl.

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