Parafilariosis in African buffaloes (*Syncerus caffer*)

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ABSTRACT

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This is the first report on the occurrence of *Parafilaria bassoni* in the African buffalo (*Syncerus caffer*). Previously this parasite has been recorded only in springbok (*Antidorcas marsupialis*) in Namibia. Haemorrhagic perforations (bleeding points), the usual lesions seen in infected animals, were caused by gravid female parasites ovipositing embryonated eggs. These lesions occurred mainly on the dorsal and lateral sides of buffaloes. Complications of these lesions developed in a small number of buffaloes because of secondary bacterial infection [subcutaneous abscesses (3/178)] and as a consequence of a localized Type 1 hypersensitivity [large cutaneous ulcers (7/178)]. Red-billed oxpeckers (*Buphagus erythrorynchus*) appeared to play an important role in the epidemiology of this parasite as well as in the pathogenesis of the lesions. They reduced the likelihood of spread by ingesting blood containing embryonated eggs, and caused the development of large ulcers by feeding on superficial necrotic skin. From the results of an ELISA test it was determind that *P. bassoni*-infected buffaloes occur throughout the Kruger National Park complex, with a seroprevalence of approximately 34 %.

Keywords: African buffalo, *Antidorcas marsupialis*, *Buphagus erythrorynchus*, eosinophilic arteritis, Kruger National Park, *Parafilaria bassoni*, red-billed oxpeckers, springbok, *Syncerus caffer*

INTRODUCTION

Parafilariosis is a condition commonly seen in cattle (Pienaar & Van den Heever 1964) and rarely in equids, in South Africa (Ortlepp 1962a); it has not been reported in other ruminants. During 1992, large ulcerated skin lesions on African buffaloes, *Syncerus caffer*, were reported by game rangers from the Sabi Sand Game Reserve (SSGR), a privately owned reserve adjacent to the Kruger National Park (KNP). The ulcers occurred mainly on the dorsal and dorsolateral aspects of the thorax of adult animals and resembled those caused by *Stephanofilaria* spp. in cattle and buffaloes (*Bos bubalis*) in India (Sharma Deorani 1965; Patnaik & Roy 1967). Their occurrence was strictly seasonal, lesions appearing in about November and disappearing towards the middle of March in the following year, thus suggesting insect transmission. Initially, the lesions are inconspicuous bleeding points arising from small cutaneous ulcers, similar to those caused by *Parafilaria bovicola* in cattle. Red-billed oxpeckers, *Buphagus erythrorhynchus*, were attracted to the bleeding points to feed on the exuding blood, simultaneously removing embryonated eggs.

The condition has long been suspected to occur in African buffaloes in the KNP because of the presence

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of non-specific lesions in their subcutis and its musculature, but parasites were never found (Young & Van den Heever 1969). Basson, McCully, Kruger, Van Niekerk, Young & De Vos (1970) necropsied 100 buffaloes in the KNP and found some of them to have an eosinophilic cellulitis and panniculitis, but did not recover any adult nematodes. They did, however, find sheathed microfilariae in the infra-orbital skin and suggested that these could be either *Stephanofilaria* spp. or *Parafilaria* spp. or both (Basson *et al.* 1970).

This paper is a report on the cause and pathogenesis of the lesions, as well as on the distribution and prevalence of parafilariosis in buffaloes in the greater KNP complex.

MATERIALS AND METHODS

Study area

The KNP is a large reserve, about 20 000 km² in extent. It is situated in the north-eastern corner of South Africa, adjoining Zimbabwe in the north and Mozambique in the east (Fig. 1). The vegetation is diverse and several types and subtypes of Lowveld, Mopane veld and Bushveld are recognized (Acocks 1988). The SSGR is a privately owned and managed reserve on the western border of the KNP (Fig.1). It is approximately 570 km² in size and forms part of the greater KNP complex. The vegetation is classified as Lowveld (Acocks 1988).

Animals

A buffalo herd in the SSGR was monitored from October 1993 to April 1994. One dominant female in the herd was identified and immobilized according the method described by Bengis (1993). A radio transmitter (Telonics, 932E Impala Avenue, Mesa, Arizona, USA) was fitted around her neck. The herd was tracked on the ground twice a week with the aid of a radio receiver (Yaesu 2M FT-290R 2, Yaesu C.P.O. Box 1500, Tokyo, Japan) and an H-antenna. When the herd was difficult to find, a light aircraft was used to track it.

Buffaloes showing lesions suggestive of parafilariosis were immobilized and a skin-biopsy specimen was taken. When the lesion was a bleeding point, an incision 20 mm in length was made in a ventral direction, starting 30 mm above the lesion. An index finger was used as a probe to locate the granuloma (Fig. 2a) caused by the nematode in the subcutis. On locating the granuloma, the incision was extended in that direction so as to include the granuloma in the biopsy specimen. Tissue blocks, 15 mm square and containing all the layers of the skin, were removed from the edge and centre of ulcerated lesions. In all instances the biopsy wounds were packed with a sulphonamide-containing ointment (Acrisulph, Kyron

Laboratories, 84 Main Reef Road, Benrose, 2094, South Africa) and sutured with vertical interrupted mattress stitches and no. 1 nylon. The biopsy specimens were fixed in 10 % buffered formalin. Tissue blocks of the specimens were processed routinely, embedded in paraffin wax, sectioned (4–6 μm thick) and stained with haematoxylin and eosin for light microscopy.

Buffaloes were examined from a vehicle, by binoculars, and all lesions were recorded on a silhouette diagram of a buffalo. The lesions of each buffalo were plotted on a separate diagram. The behaviour of oxpeckers towards lesions was monitored and categorized as follows: ingestion of crusts around bleeding points; the extent of beak penetration into a bleeding point; attention given to large cutaneous ulcers; the number of oxpeckers feeding on a single ulcer; the frequency at which buffaloes fended off the oxpeckers; the reaction of the oxpeckers to the behaviour of the buffaloes; and the way in which buffaloes attempted to evade the oxpeckers.

Parasites

At first, attempts were made to remove the worms surgically from the subcutis of immobilized buffaloes. Then two buffaloes were killed at an interval of three weeks. The animals were skinned and the worms dissected from the bright green granulomas in which they were present (Fig. 2b) Worms collected in this manner were fixed in cold, 70 % ethyl alcohol.

A third buffalo was killed during January 1994 and its skin cut into pieces of approximately 40 x 70 cm. These were placed in normal saline at 40 °C on expanded metal sheets in plastic trays and incubated for 24 h. The saline was replaced every 4 h; each volume of saline was sieved through a sieve (150 μm apertures). The residue was inspected visually and the worms removed and fixed in boiling 70 % ethyl alcohol. The remaining residue was washed into a container, fixed by heating to 60 °C and preserved by adding 10 % buffered formalin.

A total of 15 specimens consisting of fresh blood and blood crusts from bleeding points of 15 buffaloes were collected in glass tubes. Water was added and the mixture was left for a period of 12 h for haemolysis to occur. The resulting suspension was centrifuged for 4 min at 3 000 *g*. The supernatant was discarded and an unstained smear prepared from the residue. This preparation was examined under a standard microscope at 50x magnification.

Seroprevalence

Serum samples collected from 184 buffaloes from 11 localities throughout the KNP complex, together with nine positive and six negative controls, were submitted for diagnosis. Each locality represented one
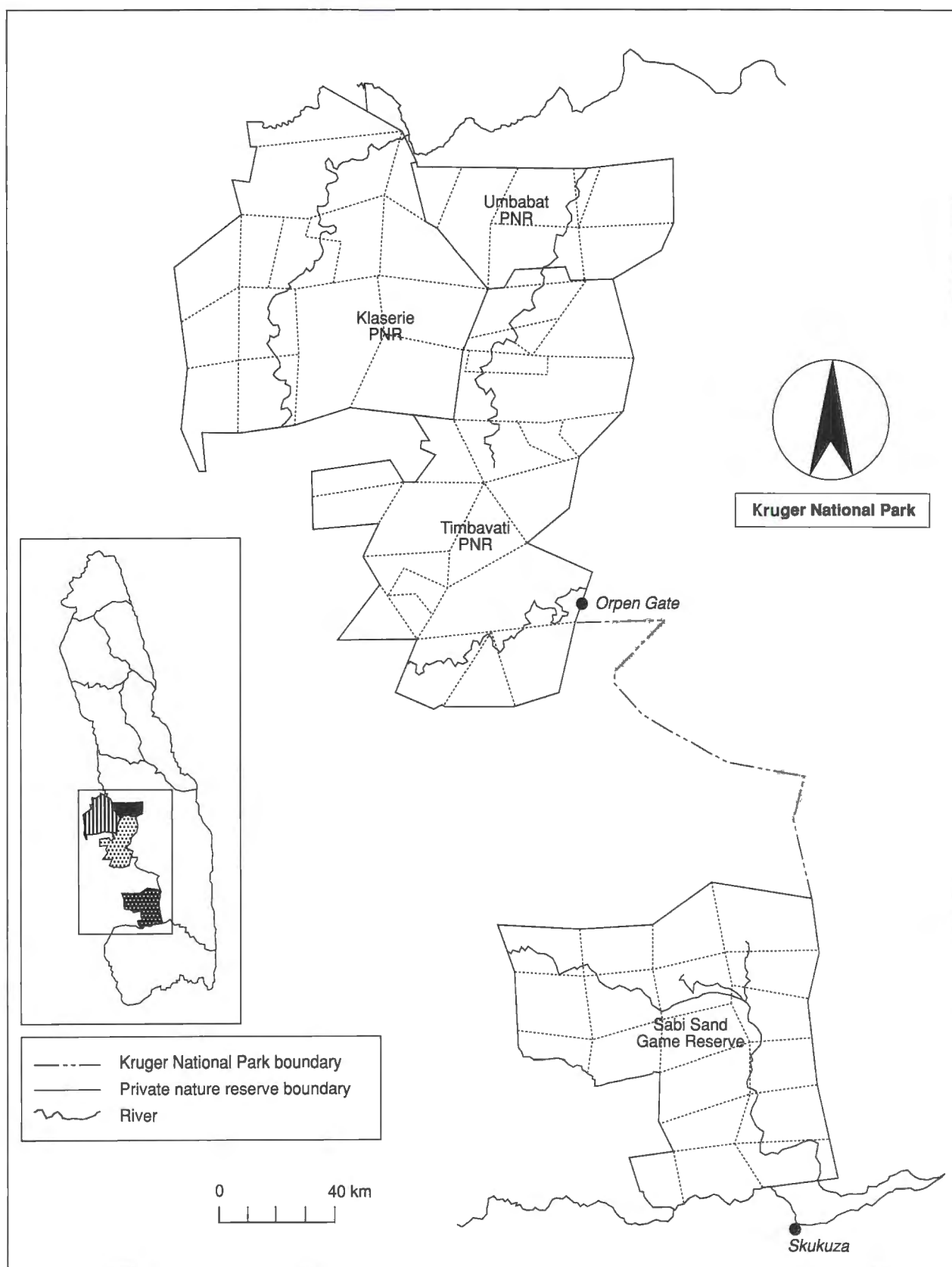
FIG. 1 Map of the Sabi Sand Game Reserve and other private nature reserves in relation to the Kruger National Park. Together they form the greater KNP complex

cluster and from these clusters samples were picked at random, with replacement. Positive control samples were obtained from animals in the SSGR and their status confirmed by the presence of the parasites as well as histopathology. Negative control samples were obtained from buffaloes born and bred in confinement. They were removed from the cows at about 3 months of age. These animals were regarded as negative as they had never shown bleeding points and had never had contact with free-ranging buffaloes. An ELISA test as described by Voller, Bidwell & Bartlett (1980) was employed. This test was developed for polypeptides of *P. bovicola* exoantigens with molecular masses of 41 and 36 Kda (Sundquist, Bech-Nielsen & Zakrisson 1989). Antigens were purified and characterized by chromatofocussing to eliminate cross-reactivity with antigens produced by concurrent nematode infections (Sundquist *et al.* 1989).

RESULTS

Animals

The lesions seen on the animals were categorized into three macroscopically discernible types that occurred at different times during summer. The primary lesion, the bleeding point or haemorrhagic perforation (Fig. 2c) was observed from the beginning of November to the beginning of February. Two secondary lesions developed subsequently: subcutaneous abscesses (Fig. 2d), which were seen from the middle of December to the middle of January; and large cutaneous ulcers (Fig. 2e) which appeared from about the middle of January and had healed by the middle of March, leaving a conspicuous scar (Fig. 2f).

The bleeding points were equally distributed on the left and right sides of the buffaloes (81 points on the left side and 84 on the right). The dorso-ventral distribution, however, was not uniform: 15,85% occurred dorsally, 58,53% laterally and 25,6% ventrally. The cranio-caudal distribution was even less uniform, with 83% of the lesions recorded cranial to the loins. Of these, 20,6% were seen on the neck, 23,6% on the shoulders, 39,4% on the ribs, 4,5% on the loins and 11,5% on the hindquarters. No lesions were encountered on the head or the tail.

Each bleeding point was located centrally in a poorly circumscribed swelling raised about 3–5 mm above the surface of the skin. These openings were about 1 mm in diameter and occluded by a coagulum of serum in which the heads of the female nematodes were embedded, though not visible externally. Intermittent haemorrhaging from the bleeding points occurred for up to 2 d from the time of their first appearing. The volume of blood seeping from these bleeding points could not be estimated as it was rapidly consumed by oxpeckers. On visual inspection of the subcutis, the female nematodes were found in their migration tracts which were enveloped in an elongated, brilliant green granuloma, measuring about 20 x 3 mm. The area immediately surrounding the granuloma appeared unaffected macroscopically (Fig. 2a).

Histologically, sections cut transversely through the migration tract, revealed a central core of necrotic eosinophils, cellular and fibrin. A pronounced granulomatous reaction consisting of epithelioid cells in palisade formation and a dense infiltrate of eosinophils, plasma cells and lymphocytes, together with oedema and fibroplasia, surrounded the necrotic core. Vascular lesions that occurred in the surrounding tissue included chronic proliferative phlebitis and a perivascular eosinophil and lymphocyte infiltration.

The subcutaneous abscesses were well-defined swellings, measuring up to 60 x 60 mm. The swellings contained circumscribed cavities filled with necrotic debris. The adjacent subcutis was pale green and slightly oedematous. Three out of 178 buffaloes had such abscesses as a complication.

Histologically, the walls of the abscesses consisted of granulation tissue, containing vast numbers of eosinophils. Fibrin and large numbers of erythrocytes, eosinophils and neutrophils accumulated in their centre. Vascular lesions in the surrounding tissue included eosinophilic arteritis, eccentric endarterial fibrosis, fibrinoid necrosis of the vessel wall, proliferative endarteritis and recanalization of thrombi.

Large cutaneous ulcers developed, mainly on the dorso-lateral area immediately behind the shoulders, but were also to be seen on the top of the hump of infected buffaloes. One ulcer was noted on the loin and two on the hindquarters. Ulcers were seen on seven of 178 buffaloes and only on adult animals. They varied in size from 50x40 mm to 300 x 200 mm, were well-circumscribed, had ragged, elevated edges and an irregular base. Even the largest lesions developed rapidly, the entire process taking about 10 d. During the early phase of development, intermittent haemorrhage occurred from the ulcerated areas, but this was caused mostly by oxpeckers feeding on the wound. The adjacent subcutis had a yellowish-green tinge, with signs of haemorrhage and oedema. Pruritus was never seen.

Microscopically, there was a sudden transition from normal to ulcerated skin. A marked acanthosis occurred at the junction, which progressed to full-thickness necrosis with ulceration. The ulcerated surfaces were covered by a scab consisting of fibrin containing numerous eosinophils. Below the scab a prominent layer of immature granulation tissue containing masses of eosinophils was seen. The dermis was relatively unaffected, with the exception of marked perivascular infiltrates of eosinophils. The inner layer

FIG. 2a   A brilliant green subcutaneous granuloma containing a gravid female filariid
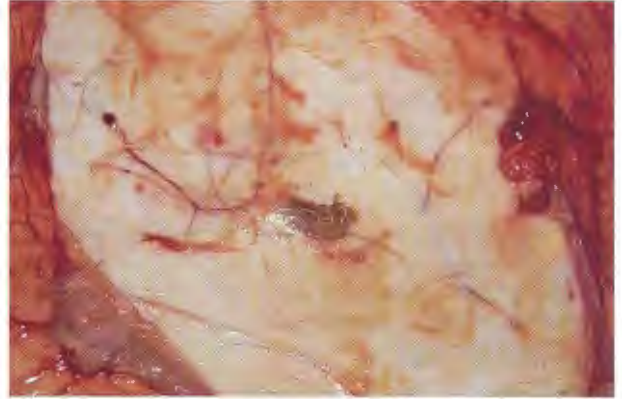

FIG. 2b   An exposed female filariid

FIG. 2c   A typical bleeding point

FIG. 2d   A subcutaneous abscess

FIG. 2e   A large cutaneous ulcer

FIG. 2f   A conspicuous scar that remains visible for a long time

of the dermis and the subjacent subcutaneous interface showed severe oedema and marked vascular involvement and contained numerous eosinophils. Mostly arteries were involved, a marked eosinophilic vasculitis, segmental medial hyperplasia and extensive recanalization of thrombi being the most common lesions present. Marked endothelial proliferation occurred in the affected vessels and some showed mild villous endarteritis. These lesions were accompanied by pronounced endothelial hyperplasia and subendothelial oedema, as well as eccentric, chronic, eosinophilic endophlebitis and early villous

endophlebitis. In addition to the eosinophils, the perivascular reaction also contained plasma cells and a few lymphocytes. In the central area of the lesion eosinophilic granulomas occurred deeper in the dermis. These exhibited the Splendore-Hoepplé phenomenon, the eosinophilic mass containing necrotic nematode fragments.

All the cutaneous ulcers healed spontaneously towards the beginning of March, leaving conspicuous scars characterized by greyish-white alopecic areas sometimes with marked hyperkeratosis that remained visible for years.

Microscopically, the regenerated epidermis covering the large ulcers, contained only a few hair follicles, sebaceous glands and dilated sweat glands. Excessive keratinization was a constant feature. The outer dermis showed few changes, while the inner dermis consisted of maturing connective tissue. Vascular lesions were advanced and consisted of marked, eccentric, medial and endothelial hyperplasia and hypertrophy. Vascular occlusion and angiogenesis in close association with affected blood vessels were also seen.

Oxpeckers were attracted to bleeding points and ulcers. Wet streaks and dry crusts were rapidly ingested with scissor-like movements of their beaks. Occasionally, they inserted the full length of their beaks into a perforation to feed on the contents. Up to 12 oxpeckers were seen feeding simultaneously on one large ulcer. They also fed off biopsy wounds within minutes of the buffalo being revived after having been immobilized. The attacks were so vigorous that wound dehiscence occurred within 12 h. During daylight hours buffaloes continually fended off the oxpeckers. On occasion they were seen to use their horns up to seven times per minute to chase the birds off. White areas of alopecia developed on the buffaloes' shoulders owing to the trauma caused by the impact of their horns. The oxpeckers reacted aggressively when buffaloes tried to fend them off. They made growling sounds, puffed themselves up and assumed a threatening posture. The animals also tried to protect the ulcers from the oxpeckers by positioning their bodies close to shrubs so that the wounds would not be visible or accessible. Temporary relief from harassment was also obtained by wallowing in mud.

Parasites

Surgical removal of the nematodes from the subcutis of immobilized buffaloes was relatively unsuccessful and only fragments of female worms were recovered, while entire female worms were recovered only by dissection of the subcutaneous granulomas. Incubation of pieces of skin in saline was more successful and one entire male, two entire females and two entire early fourth-stage larvae were recovered. A total of 23 nematodes were collected by use of the various techniques. A considerable number of these nematodes were already dead at the time of collection.

The nematodes were identified as *Parafilaria bassoni* because of the similarity of the morphological characteristics with the species described by Ortlepp (1962b) from the orbital connective tissue of a springbok (*Antidorcas marsupialis*).

Embryonated eggs were found in all the blood crusts and wet blood streaks that were examined, but no free microfilariae. No attempt was made to count the number of eggs, but from one to seven eggs were noticed on each slide.

Seroprevalence

All six the negative controls tested negative and eight of the nine sera from buffaloes with parafilariosis, tested positive. These trends indicate a specificity of 100 % and a sensitivity of 89 %. Twenty-five of 71 (35,21 %) male buffaloes tested positive and 37 of 113 (32,74 %) females, a total of 62 of 184 (33,7 %) animals examined. The youngest seropositive buffaloes were 2 years old. Twenty-three (12,5 %) suspicious readings were recorded and 99 (53,8 %) buffaloes tested negative.

DISCUSSION

Parafilariosis has been recorded in various Asian buffalo species (Srivastava & Dutt 1959; Patnaik & Pande 1963; Sahai, Singh & Varma 1973; Chauhan, Arora & Ahluwalia 1974) but never in African buffaloes. This is the first description of parafilariosis in African buffaloes. The lesions in buffaloes and the life cycle of the parasite resemble those seen in cattle parasitized by *P. bovicola*. Most typical lesions (bleeding points) heal without complications while some develop into abscesses or large distinct cutaneous ulcers. Red-billed oxpeckers appear to play an important role in the epidemiology of the parasite by feeding on blood containing embryonated eggs, and in the pathogenesis of lesions by removing superficial necrotic skin while feeding on large cutaneous ulcers, enlarging these ulcers. Infected buffaloes occur throughout the entire KNP complex and the prevalence of the infection is approximately 34 %.

The external appearance of bleeding points seen on buffaloes is similar to that seen on cattle infected with *P. bovicola*, with the difference that oxpeckers may have removed most of the oozing blood on buffaloes. No difference was seen between the histopathology of the granulomatous reaction surrounding nematodes or the polymorphonuclear cell composition seen in buffaloes and that reported in cattle (Pienaar & Van den Heever 1964). Patnaik & Pande (1963) attributed complications of lesions caused by *Parafilaria* in Asian

buffaloes to contamination with bacteria when wallowing, and myiasis. Abscesses that developed from bleeding points caused the skin to slough, leaving an ulcer (Srivastava & Dutt 1959). In our study, none of the abscesses developed into ulcers and it appears that abscessation is an uncommon complication in African buffaloes. Concurrent myiasis was never observed and wound-breeding blowflies were not observed to feed on these lesions. The known vectors of *Parafilaria* are dung breeders and they do not deposit eggs or larvae in open wounds. However, myiasis was observed during the study period in wounds caused by lions on the withers of two buffaloes.

The histopathologic changes in the large ulcers and underlying tissues suggest that the pathogenesis of the lesion is a localized Type 1 hypersensitivity reaction of which the severity is enhanced by an excessive anamnestic response. At the subcutaneous site of dermal penetration the female, while ovipositing, becomes enveloped in an eosinophilic parasitic granuloma. The fact that the female is relatively stationary leads to the continuous local deposition of eosinophils involved with immune reactions directed against metabolic products secreted by the nematode. It appears that female parasites do not survive ovipositing, as the enveloping immune reaction develops into an impermeable mass which restricts and kills her, as evidenced by the number of dead and decaying worms that were recovered. Female worms may also be killed by oxpeckers when they feed on the bleeding points.

Oxpeckers are usually associated with the larger mammals in the KNP where they play a beneficiary role by removing especially ticks. Once they have settled on a buffalo, they move to an area where blood is present on the skin. They have been incriminated as aggressively and actively attacking and enlarging open cutaneous wounds (McLachlan & Liversidge 1982). In this study it appeared that they were causing haemorrhage by removing necrotic debris and damaging granulation tissue that developed in the ulcer, and then feeding on the blood. The carnivorous behaviour of this otherwise mutualistic companion of the buffalo must reduce the possibility of vectors becoming infected, which in turn could ultimately reduce the prevalence of *P. bassoni* in buffaloes in the complex.

The continuous presence of feeding oxpeckers annoyed infected buffaloes during the day, their action clearly inflicting pain, and they made it difficult for the buffaloes to feed or ruminate. In addition, since the majority of large ulcers occurred on the dorsal and dorso-lateral aspects of the buffaloes, we presume that it was more convenient for the birds to enlarge existing wounds. The ulcers in different buffaloes began to heal simultaneously during March, despite the fact that the birds continued feeding on them.

The reproductive phase of *P. bassoni* appears to be shorter than that of *P. bovicola*. Nevill (1984) found that in cattle the first bleeding points appeared in June and disappeared the following May, with a peak during October and November. In buffalo, the first bleeding points appeared only in November and persisted to the end of February. The synchronous healing of the large ulcers during March, when the hypersensitivity reaction diminishes in the absence of living and/or dead adult nematodes, supports this assumption of a short reproductive phase.

Adult *P. bassoni* has so far been recovered only from the orbital connective tissue of springbok in Namibia (Ortlepp 1962b). Although *P. bovicola*, which cause similar lesions in cattle, occur primarily in the subcutis, Chauhan *et al.* (1974) and Chauhan, Arora, Agrawal & Ahluwalia (1976) recovered a juvenile male *P. bovicola* from the anterior chamber of the eye of an Asian buffalo, and Ortlepp (1962a), a gravid female *P. multipapillosa* from the posterior chamber of the eye of a horse. Nevill (1980) successfully infected cattle with infective third-stage larvae of *P. bovicola* per conjunctiva. Adult *P. bassoni* occurred in the orbital connective tissue of all five springbok examined (Ortlepp 1962b). He assumed that this site was not abnormal for this parasite (Ortlepp 1962b). In view of the above findings it seems that the eye is a normal site of entry of infective larvae of this genus. Unfortunately the eyes of buffaloes killed during this study were not examined for the presence of nematodes.

The factors governing the predilection sites of *P. bovicola* during oviposition in cattle are undetermined (Nevill 1984) but the dorsal and lateral aspects of the body seem to be preferred. The same tendency was observed with *P. bassoni* in buffaloes, 74,4% of bleeding points occurring in these regions. Since haematophagous or partially haematophagous flies are the intermediate hosts of all the *Parafilaria* spp. this may be to ensure that the vectors, which usually feed around the face, are attracted to blood containing embryonated eggs. It may also be that the higher up on the body the bleeding points occur, the longer the blood streak will be, thus providing a larger feeding area and greater volume of blood for the vectors. In cattle only 7,8% of lesions occur on the ventral aspects (Nevill 1980) as opposed to 25,6% in buffaloes. This suggests that in buffaloes a wider spectrum of vectors may be involved, some of which may be attracted to the shaded areas of the body.

The presence of embryonated eggs in fresh and crusted blood collected from primary lesions provides an easy method to immediately confirm a preliminary diagnosis of parafilariosis. However, the collection of specimens was complicated by the following factors: it took considerable time to immobilize and sample a buffalo after a fresh bleeding point had been seen and clotting may have set in; oxpeckers were quick to attend to the fresh bleeding spot and remove crusts;

buffaloes do not have a thick hair coat and it was often necessary to virtually scrape off remnants of crusts left behind by the oxpeckers; and the buffaloes were often covered with mud, which was of necessity included in the sample.

According to Sundquist *et al.* (1989) the 4l and 36 Kda antigens are specific for *P. bovicola*, and the ELISA test performed on bovine material showed a 95% specificity and a 92% sensitivity. Infected cattle were identified even before bleeding points appeared. The results obtained with the sera of the buffaloes suggest that the test is genus-specific. Cross-reactivity with antigens of other nematode genera (Neppert 1974) was not observed in any of the studies described by Sundquist, Zarkrisson, Bech-Nnielsen & Bianco (1988) and Sundquist *et al.* (1989).

Four to five months are required to develop a positive titre in cattle (Sundquist *et al.* 1989). It is not known for how long a positive titre persists in buffaloes but cattle have to be re-infected annually for the continuity of the life cycle from one season to the other, thus to maintain a positive titre (Sundquist *et al.* 1989). Suspicious reactions in buffaloes could either reflect recent infections or animals losing their positive titre because of not being re-infected. The absence of serologically positive buffaloes younger than 2 years of age can be ascribed to the fact that the majority of buffalo calves in the KNP complex are born between January and April (Pienaar 1969). During this period the number of bleeding points declines to such an extent that the possibility of newly-born calves being infected is probably very low. One can therefore assume that they are not infected shortly after birth but only during the following summer season.

The presence of this parasite throughout the KNP complex suggests that it must have been present for a long time and may even be endemic.

ACKNOWLEDGEMENTS

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