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### ABSTRACT

P. A. BASSON, R. M. McCULLY, S. P. KRUGER, J. W. VAN NIEKERK, E. YOUNG & V. DE VOS. Parasitic and other diseases of the African buffalo in the Kruger National Park. Onderstepoort J. vet. Res. 37 (1), 11-28 (1970).

One hundred randomly-collected buffaloes [Syneerus caffer (Sparrman, 1779)] were autopsied, and tissues and parasites were collected for histopathological studies and identification. Blood smears were prepared from 92 animals, of which 97 per cent proved to be positive for piroplasms of an unidentified *Theileria* sp. Schizonts were found in the lymph nodes, liver, kidneys and myocardium of three young buffaloes. Granulomatous mycotic lymphadenitis of the peripheral lymph nodes occurred in 15 per cent of the animals and 86 per cent contained large sarcosporidial cysts in the oesophageal, pharyngeal and laryngeal musculature. Neoplasia, a squamous cell carcinoma, was encountered in only one animal. The most significant metazoan diseases were pentastomiasis (69 per cent), bilharziasis (62 per cent), cysticer-cosis (29 per cent), filariasis (16 per cent), hydatidosis (5 per cent) and mange caused by an unidentified *Psoroptes* sp. Thy nymphs of *Linguatula serrata* Frölich, 1779 were recovered mostly from the hepatic veins, sometimes from the right heart chambers and rarely from the liver substance, lymph nodes and pulmonary artery. *Schistosoma mattheei* Veglia & Le Roux, 1929 was mainly responsible for the bilharzial seions. However, and the hydrogenergy found to be inforted with hepaticities. A third theory is used lesions. However, none of the buffaloes were found to be infected with besnoitiosis. As this disease is very common in antelopes in the same area this finding suggests that the buffalo is not susceptible to it. A list of the intestinal and other helminths encountered is given.

### INTRODUCTION

In the interests of good nature conservation, reduction in the numbers of certain game species in the Kruger National Park has become a necessity during the past ten years. This provided an opportunity of studying some of their parasites and diseases. The present investigation was made on 100 buffaloes [Syncerus caffer (Sparrman, 1779)] killed mainly during the winter and spring of 1966 in the southern part of the Park (Pienaar, 1969; Young & Van den Heever, 1969).

#### MATERIALS AND METHODS

Randomly-selected buffaloes were immobilized with succinylcholine chloride administered by means of a Van Rooyen crossbow and dart-syringe and were subsequently exsanguinated. The age of each animal, with the exception of four solitary bulls from riverine areas (SB 1 to 4) was determined by using a method based on the findings of Marsboom (1950), Longhurst (1958) and de Vos (1969, unpublished data) that the tooth replacement in the buffalo takes place about 1 to 3 months later than in cattle. A macroscopic examination was made of 97 buffaloes and specimens from all the organs and tissues except the head, bones and genitalia were collected in 10 per cent buffered formalin for light microscopy. The heads and genitalia were saved for biological studies but specimens from a few cases were obtained for histopathological studies. Blood smears were prepared from 92 animals, stained with Giemsa and examined microscopically for parasites. Internal and some external parasites from the skin, eyes, liver, muscles, blood vessels and gastro-intestinal tract were collected in 10 per cent formalin for identification purposes. In addition to this group of 97 buffaloes, three other animals were later examined and their genitalia collected in 10 per cent formalin. The formalin-fixed tissues were prepared in a routine manner for light microscopy. The haematoxylin and eosin (HE) method

of staining was routinely used, but special stains such as periodic acid-Schiff (PAS) (Pearse, 1961), Gomori's methenamine silver impregnation (GMS) (Anon., 1960), Gram, Ziehl-Neelsen (ZN) (Cruickshank, 1962) and Gridley's method for fungi (Anon., 1960) were also employed when indicated.

#### RESULTS

The most significant findings in the main group of 97 buffaloes are shown in Table 1; the helminths and other parasites are listed in Table 2.

### Theileriosis

A total of 97 per cent of the blood smears from the 92 buffaloes that were examined contained small piroplasms in some of the erythrocytes. Small numbers only were present in the majority of animals (77 per cent) but they were more frequent in 23 per cent and fairly numerous in 6.5 per cent of the buffaloes. In the animals under two years old their incidence was significantly higher than in the older buffaloes. A few suspected schizonts, but none which could be identified with certainty, were seen in the blood smears. Three out of twelve young buffaloes under two years old showed clinical and histopathological evidence of theileriosis. Two of them, cases 32 and 49, were calves with live weights of 87 and 89.8 kg respectively. Case 32 had very enlarged lymph nodes. These proved to have both lymphoid and reticulo-endothelial (RE) hyperplasia and contained numerous plasma cells, some eosinophils and several Koch's bodies. Both macro- and microschizonts were recognized [Plate 8 (35 and 36)]. The splenic corpuscles were large and hyperplastic. Foci of mild round cell infiltrates or vessels with perivascular cuffing were found in the subendocardium, myocardium, lungs and kidneys. A small number of myocardial and skeletal muscle fibres revealed Zenker's degeneration. Case 49 had a very marked splenomegaly and prominently enlarged peripheral and visceral lymph nodes. The histo-

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pathological findings corresponded to those of the pre-vious case, but RE hyperplasia and lymphoid hyperplasia in association with numerous Koch's bodies were very extensive. Koch's bodies were most numerous in the peripheral lymph nodes but could not be identified in the spleen or mesenteric lymph nodes. The iliac lymph nodes contained only a small number of schizonts. The round cell reaction in the myocardium, kidneys and liver was more prominent than in the previous case. In the liver it was present both peripherally and centrilobularly and even within the intima of some of the veins. Both the renal cortex and medulla were affected similarly and mild mononuclear infiltrates were also seen subepicardially. Koch's bodies were recognized in the myocardium, but not in the liver and kid-neys. The third buffalo, Case 90, was a young heifer which weighed 249 kg. Enlarged lymph nodes were visible externally. This, however, was a relatively mild case, lymphoid hyperplasia being less prominent and a relatively small number of schizonts being noticeable in the prescapular lymph node and within a lymphocytic focus in the myocardium.

# Bilharziasis

Infestation by schistosomes was diagnosed either by demonstrating the parasites in the mesenteric or portal veins or by the recognition of characteristic microscopic lesions (vide infra). Schistosoma mattheei Veglia & Le Roux, 1929 was specifically identified as the cause of most of these cases of bilharziasis. One female with ova similar to those of S. haematobium (Bilharz, 1852) Weinland 1858 was also encountered. The measurements of the ova in the latter specimen were as follows: mean length 0.0998 mm, standard deviation 0.0031 and coefficient of variation 3.1; these fall well into the range of variation for S. haematobium (Kruger, 1969, unpublished observations). In general, the average number of intrauterine ova in the female parasites was relatively large and corresponded with the mean of parasites found in cattle.

Macroscopic findings: Schistosomes were most frequently found in the old riverine buffaloes, seldom in the others. In some of the more heavily infested animals conjoined pairs of S. mattheei were easily seen in mesenteric vessels by holding the mesentery up against the light. The most commonly observed lesion in the mesenteric and intestinal veins consisted of a thickened portion of vein surrounded by a slightly greenishyellow zone of inflammation and some petechiae or vibices. These lesions were usually situated at the mesenteric attachment and involved the intestinal as well as the mesenteric portion of the vein. In one case there were some very striking lesions involving the majority of the mesenteric veins, which were frequently varicose and contained large thrombi [Plate 1 (1 and 2)]. When the veins were opened it could be seen that the light reddish thrombi practically filled the lumens. Incisions through some of them revealed laminations and cross sections of enmeshed schistosomes.

Macroscopic lesions in the liver which proved to be indicative of bilharziasis were found in branches of the intrahepatic portal veins. The lesions were characterized by either a marked thickening and prominence of the vessel wall, with a corresponding narrowing of the lumen, or roundish areas of lymphoid tissue in the course of the vessel. Both of these findings were referred to as "edge" lesions. They were best seen by making a series of roughly parallel incisions around the sharp edges of the liver lobes. The cuts were made at approximately 1 cm intervals to a depth of 3 to 4 cm. The urinary bladders of more than 50 per cent of the buffaloes were examined, but none contained lesions.

*Microscopic findings:* The lesions observed grossly in the mesenteric and intestinal veins proved to be a phlebitis and periphlebitis with a predominance of eosinophils and round cells. The infiltrate frequently extended into the surrounding mesentery and wall of the intestine. The large number of eosinophils readily accounted for the greenish colour observed macroscopically. Polypoid intimal proliferations were present, presumably in response to the presence of adult schistosomes. In their piths as well as on the surface there were many eosinophils.

In the animal which had many thrombi in the vari-cosed mesenteric and intestinal veins, there was a direct association between thrombosis and dead adult schistosomes. Other thrombi were present in veins which had extensive lesions of proliferative endophlebitis. The organization of some of the thrombi superficially resembled the changes seen in proliferative endophlebitis but lacked the numerous eosinophils of the latter. Thrombosed vessels with dead schistosomes sometimes had granulomatous reactions around the remnants of these parasites. The walls of some of the veins were sometimes markedly altered. Apparently in response to the lesions in the lumen and the wall itself, there were irregular prominences of hypertrophic and hyperplastic smooth muscle. This was most prominent in the liver of the animal with numerous mesenteric thrombi. Emboli were not recognized as a cause of the thickening of the vessel walls.

Besides the vascular lesions in the wall of the intestine, the mucosa and submucosa were frequently heavily infiltrated with eosinophils and small round cells and schistosome ova were found enclosed in granulomatous reactions. Some granulomas contained only remnants of ova, while others contained none. Similar granulomatous reactions caused by ova were seen in the mesenteric lymph nodes and livers of a few buffaloes. The number of ova in both organs, however, was invariably small.

Lesions of the intrahepatic branches of the portal vein were commonly seen. They consisted of two types, and occasionally of combinations of the two. Live adult schistosomes provoked a proliferative endophlebitis similar to that present in the mesenteric and intestinal veins [Plate 2 (8) and 3 (9)]. The polypoid intimal projections [Plate 3 (10 and 11)] were frequently heavily infiltrated and their surfaces covered with eosinophils. The lumen was greatly diminished by the space-occupying proliferations. Some of the projections contained smooth muscle [Plate 3 (10 and 11)]. At sites in small veins where dead schistosomes were enclosed, granulomatous reactions and very pronounced lymphoid proliferations were present. The degree of involvement of these vessels ranged from a proliferation in the intima to filling of the lumen and complete destruction of the wall of the vein. Lymphoid nodules far larger than the pre-existing vein at that site were frequently seen [Plate 1 (3)]. In smaller nodules there were sometimes remnants of smooth muscle from the media. Some of the nodules were well organized with distinct follicles [Plate 2 (6 and 7)]. Dead schistosome remnants were sometimes observed in the midst of these lymphoid nodules [Plate 2 (6)], either in the presence or absence of epithelioid cells, giant cells and fibroblasts [Plate 1 (4) and 2 (5)]. Some schistosomes were calcified and some of the nodules contained haematin.

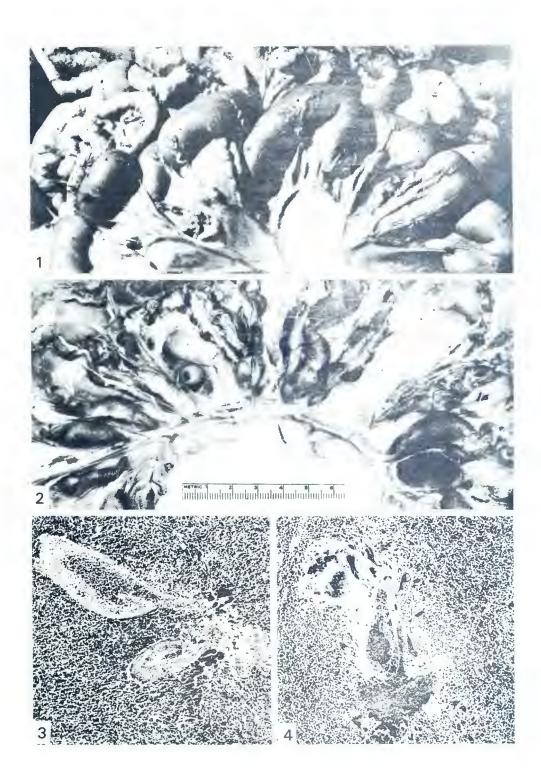


PLATE 1 Bilharziasis. 1. An exceptionally severe case of bilharziasis with pronounced phlebitis and thrombosis of the intestinal and mesenteric veins. 2. Formalin-fixed portion of the mesenterium and adjacent intestine of the same case at a higher magnification showing the marked vascular lesions; one thrombus on the lower right being incised. 3. Liver: Disintegrating schistosome surrounded by a marked round cell reaction. HE.  $\times$  75. 4. Liver: Disintegrating schistosome surrounded by a granulomatous reaction. Many giant cells are present and haematin pigment is noticeable towards the left and within the parasite. HE.  $\times$  75.

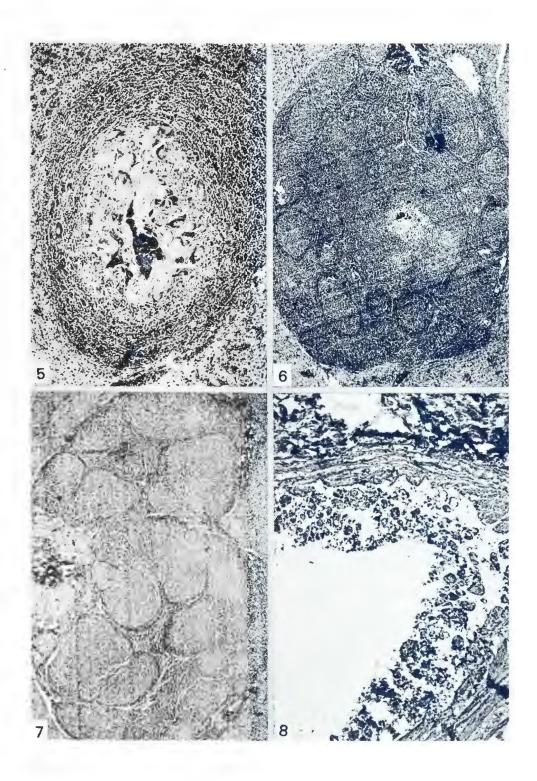


PLATE 2 Bilharziasis. 5. Liver: Advanced granulomatous reaction around an almost completely disintegrated dead schistosome. A collar of round cells and inner zone of giant cells are prominent. HE.  $\times$  75. 6. Liver: A lymph nodule has developed in and around this granulomatous lesion and a small remnant of a schistosome containing haematin is still present in the centre, HE.  $\times$  30. 7. Liver: A complete lymph nodule without any remnants of a parasite. HE.  $\times$  30. 8. Liver: Intrahepatic segment of a portal vein showing villous endophlebitis. HE.  $\times$  75.

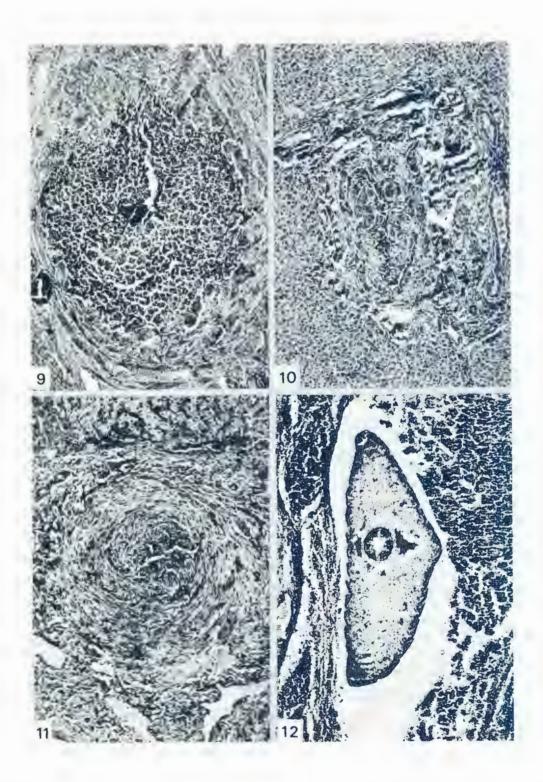


PLATE 3 Bilharziasis (9–11) and Pentastomiasis (12). 9. Liver: Intrahepatic branch of the portal vein with very marked chronic villous endophlebitis. HE.  $\times$  50. 10. Liver: Vein in a portal triad with chronic polypoid and some villous phlebitis. Smooth muscle proliferation is evident. HE.  $\times$  70. 11. Liver: Vein in a portal triad showing marked smooth muscle hyperplasia, fibroplasia and both polypoid and some villous phlebitis. HE.  $\times$  75. 12. Lymph node: A pentastome nymph within the medulla. No host response was present. HE.  $\times$  75.

# Filariasis

Sixteen per cent of the buffaloes had nodules containing Onchocerca synceri Sandground, 1938 in the subcutis or subcutaneous muscles. These nodules were more frequently encountered in the thoracic, sternal and abdominal regions. One was located in an eyelid. Two bulls had a few nodules in the prepuce, epididymis and testes [Plate 6 (26)]. Aggregates of extratubular sperm without any reactions were present in the testes and epididymis of one animal.

The nodules proved to be parasitic granulomas which contained both live and dead helminths, some of the latter being calcified [Plate 7 (27)]. An eosinophilic substance which was present immediately adjacent to the parasites, was surrounded in turn by epithelioid and giant cells, eosinophils and fibrovascular tissue. Microfilariae were noticed in the surrounding area of eosinophilic cellulitis.

Eosinophilic lymphadenitis was encountered in 46 per cent of the buffaloes. These reactions were either mild or pronounced and were present in the capsule, trabeculae and sinuses. Microfilariae, however, were observed in the lymph nodes of only three animals. One buffalo had unidentifiable necrotic helminths in the dermis. These parasites had denticulate cuticles and were surrounded by a granulomatous reaction [Plate 7 (28)]. A specimen of skin from another buffalo had a helminth in cross section within the epidermis [Plate 7 (29)]. Specimens from the infraorbital skin of three buffaloes and one unidentified skin specimen contained microfilariae in the dermal papillae [Plate 7 (30)]. Each was enveloped in a very loose cuticle which contained several adjacent pairs of crescent-shaped elevations [Plate 7 (31 and 32)]. These parasites were associated with foci of micro-abscessation in both epidermis and dermis, hyperkeratosis, mild, mixed cell perivasculitis and hypertrophy of the endothelial cells of the small vessels, particularly in the stratum papillare. Areas of eosinophilic dermatitis accompanied by alopecia, hyperkeratosis and acanthosis in other areas such as the flanks [Plate 8 (38)], were very common but microfilariae were never encountered in them. In the dermis of the affected parts the dermal papillae and stratum papillare were mainly involved and showed areas of eosinophilic or mixed cell perivasculitis or juxtavasculitis. Plasma cells were sometimes abundant.

# Pentastomiasis

The majority of buffaloes (69 per cent) harboured nymphs of Linguatula serrata Frölich, 1779 in their blood streams, but only one young buffalo out of 12 under two years of age was positive, the incidence being significantly higher beyond this age. These parasites were located in the hepatic veins, sometimes in the right heart chambers and rarely in the pulmonary artery. They were never seen in other arteries. In heavily parasitized buffaloes, the pentastomes were also noticed beneath Glisson's capsule in areas which appeared to be small cysts. They were identified microscopically in the livers of seven buffaloes (10 per cent). Some of them were present within veins and others within the parenchyme, surrounded by a narrow haemorrhagic and degenerative zone [Plate 4 (13)]. However, more advanced lesions were encountered in three buffaloes. In one case, shed cuticles and live parasites apparently within the portal veins had provoked a peripheral round cell reaction and intimal proliferation [Plate 4 (14)]. Other cuticles and parasites seemed to be extravascular and within cystlike cavities. Another case contained edge lesions which were indistinguishable macroscopically

from the typical edge lesions of bilharziasis. Microscopically they contained dead, partially calcified nymphs and cuticles surrounded by granulomatous reactions [Plate 4 (15 and 16)]. The lymphocytic hyperplasia, so characteristic of bilharziasis, was also present. The lesions, however, were larger than those of bilharziasis and the necrotic foci also more extensive. The unequivocal differentiating feature was the cuticular spines of the necrotic nymphs. The third case contained one nymph surrounded by a granulomatous response. Pentastome nymphs were encountered also in the medulla of two mesenteric lymph nodes of two different buffaloes [Plate 3 (12)]. No accompanying lesions, however, were present. A shed cuticle was found adjacent to one of these parasites.

# Cysticercosis and hydatidosis

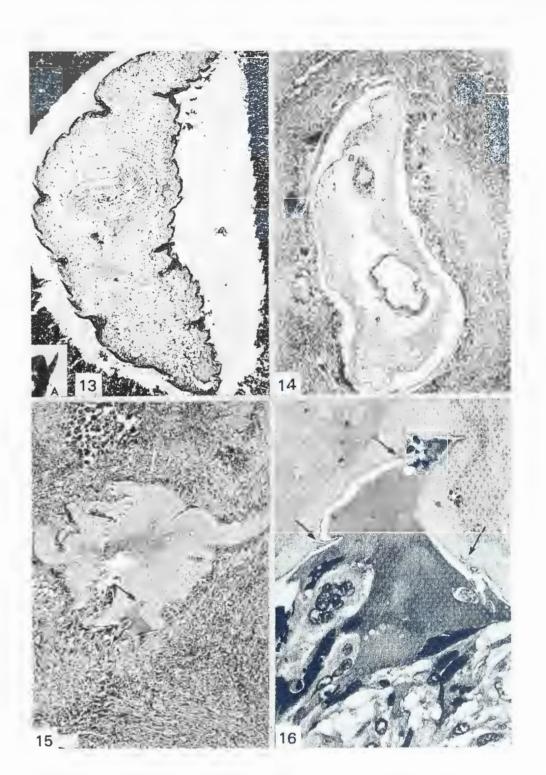
The incidence of cysticercosis and hydatidosis was 29 and 5 per cent respectively. Marie Collins (Veterinary Research Institute, Onderstepoort, unpublished observations, 1969) identified cysticerci of *Taenia regis* Baer, 1924, while Verster (1969) recorded *Taenia gonyamai* Ortlepp, 1938. The cysticerci were mainly found in the skeletal muscles and myocardium, but more frequently in the latter. Exceptional cases had odd cysticerci in the submucosa of the small intestine, udder and lymph nodes. Some of the cysticerci were disintegrated, calcified and surrounded by a granulomatous reaction. Hydatids were found in the lungs and livers of six cases.

### Non-specific lymphadenitis

Neutrophilic or mixed polymorphonuclear reactions of various intensities, but more frequently of a rather mild nature were seen in various lymph nodes of 23 per cent of the buffaloes. These lesions could not be definitely related to any cause and were suspected of representing a response to trauma, filariasis or, possibly, the early stages of mycotic infections.

# Granulomatous mycotic lymphadenitis

Granulomatous lymphadenitis of the peripheral lymph nodes occurred in 15 per cent of the buffaloes and fungi were demonstrated in all except two of these cases. The incidence was significantly higher in the older animals; it was absent in those under two years and most frequent in the aged group (36 per cent). The lesions were usually localized and were yellowish in colour macroscopically. Some, however, were not detected at autopsy. Forty per cent of the affected lymph nodes were prescapulars, 13.5 per cent prefemorals, 6.5 per cent supramammary nodes and 40 per cent were, unfortunately, not topographically identified. Microscopically, the mycotic granulomas somewhat resembled an actinomycotic lesion [Plate 5 (17)]. They contained a central core of organisms consisting of groups of semicircular and even somewhat crescent-shaped spores [Plate 5 (19)]. They stained faintly and partially basophilically with Giemsa and with HE, argyrophilically with GMS and were weakly Gram-positive, very mildly PASpositive, and negative with ZN and Gridley's method. With GMS a few short, fairly thick uninucleated, unicellular mycelia were detectable in some of the lesions, features which would possibly classify them under either Ascomycetes or Fungi imperfecti. However, in the supramammary lymph nodes mycelia were numerous and the spores resembled those found in the other nodes [Plate 5 (20 and 21)]. The organisms in most cases were surrounded by eosinophilic clubs, a subsequent zone of purulent reaction, a layer of epithelioid, giant and mononuclear cells and an outer more fibrous zone with



**PLATE 4** Pentastomiasis. 13. Liver: Pentastome nymph with its spinulated cuticle surrounded by a narrow haemorrhagic zone. One enlarged spine ( $\times$  500) is illustrated on the left (A). HE.  $\times$  75. 14. Liver: Another nymph and remnants of a shed cuticle within a vein surrounded by a prominent granulomatous response. Several giant cells are present. HE.  $\times$  45. 15. Liver: A dead disintegrating nymph surrounded by a prominent granulomatous response. The area indicated by the arrow is illustrated at a higher magnification in 16. HE.  $\times$  75. 16. Liver: Area from 15 at higher magnification showing the cuticular spines (arrows) and giant cell reaction. HE.  $\times$  500.

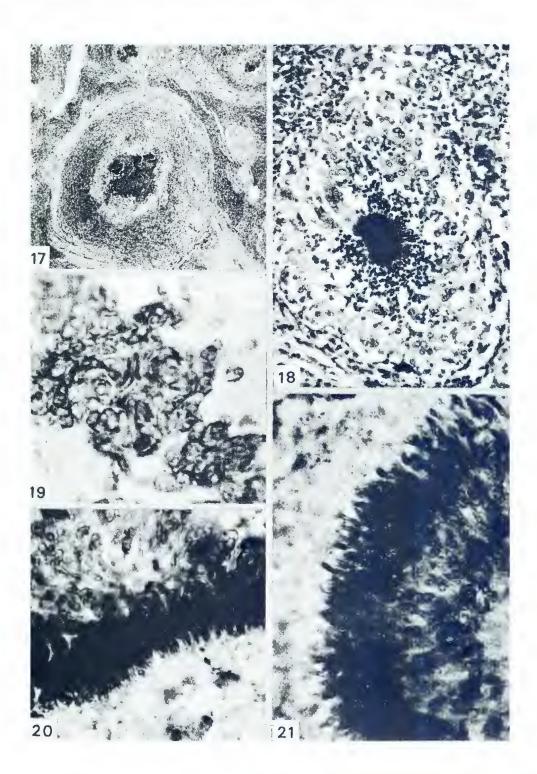


PLATE 5 Mycotic lymphadenitis. 17. One large and two small granulomas in a peripheral lymph node. HE.  $\times$  30. 18. A very small granuloma showing in the centre the dark area containing the fungi immediately surrounded by radially arranged clubs, a zone of neutrophil reaction and an outer periphery of epithelioid and giant cells. HE.  $\times$  200. 19. The fungus from a peripheral lymph node demonstrated by a special technique. Parts of septate mycelia are noticeable at the top and centre. Peculiarly shaped spores are also present. GMS.  $\times$  1200. 20 & 21. Fungus in the supramammary lymph node demonstrated by the same technique. Mycelia are more prominent than in the previous case. GMS.  $\times$  1200.

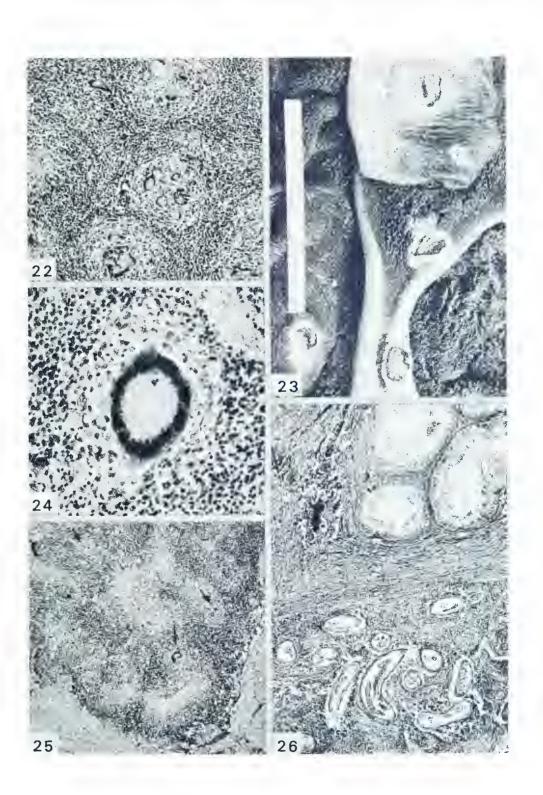


PLATE 6 22. Lymph node: Granulomatous reaction with numerous giant cells. No acid-fast bacteria were demonstrable. HE.  $\times$  75. 23. Focal disseminated rumenitis. 24. Lymph node of the same case illustrated in 22, showing a very striking multinuclear giant cell. HE.  $\times$  200. 25. Section of the rumen illustrated in 23. Granulomatous reaction with several giant cells (arrows) in the deeper portion of the ulcer. HE.  $\times$  30. 26. Epididymis with adjacent *Onchocerca* lesion. HE.  $\times$  30.

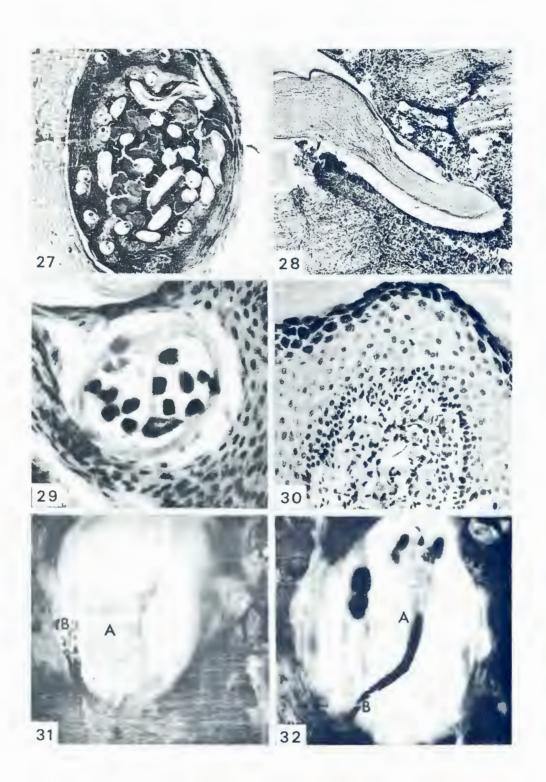


PLATE 7 Filariasis. 27. Subcutaneous *Onchocerca* lesion. HE.  $\times$  20. 28. Infraorbital skin: An unidentified dead helminth with a finely denticulated cuticle in the stratum reticulare. The granulomatous host response is prominent at the lower right. HE.  $\times$  75. 29. Infraorbital skin: An unidentified helminth in the epidermis. HE.  $\times$  340. 30. Infraorbital skin: Cuticulated microfilariae (vide 31 & 32) in an inflamed dermal papilla. HE.  $\times$  200. 31 & 32. Infraorbital skin: Sheathed (A) microfilariae (B) probably representing larvated ova. HE.  $\times$  1200.

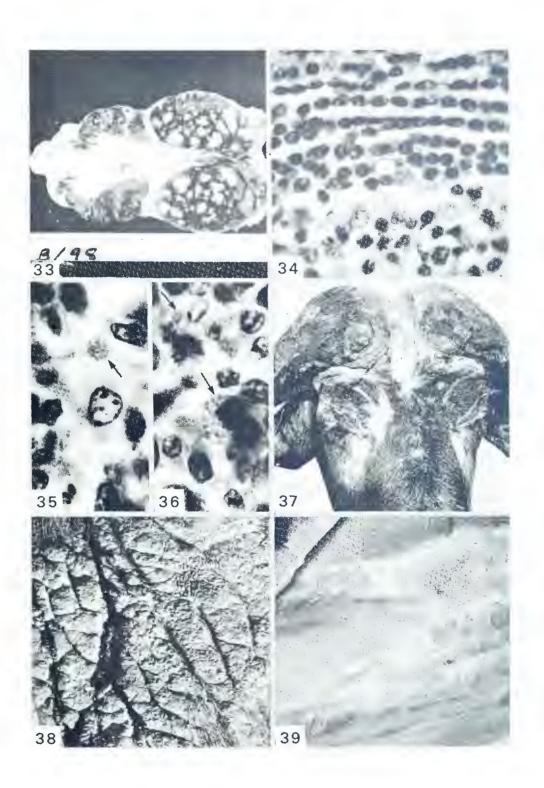


PLATE 8 33. Hyperplastic lymph node. 34. Hyperplastic lymph node showing the regimentation of lymphocytes around a lymph follicle. HE.  $\times$  200, 35 & 36. Lymph node sections containing micro- and macro-schizonts (arrows) HE.  $\times$  1200. 37. Buffalo head showing alopecic areas around the horns and above the eyes caused by mites and bilateral infraorbital filarid lesions. 38. Hyperkeratotic skin lesion of the flanks which was fairly commonly encountered. 39. Thoracic cavity: Chronic parietal pleuritis.

PARASITIC AND OTHER DISEASES OF	AFRICAN BUFFALO IN THE KRUGER NATIONAL PARK
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	Posi- tive				1 yr.	d					1-2 yr.	yr.				2-3 yr.	JT.				ŝ	3-4 yr.	÷						4	4-5 yr.						ц.	5-6 yr.		
Serial No. of cases		58	33	92	32	48	49	94	43	90	45	100	0 10	95	24	1 73	66	61	89	72	52	29	27	38	26	76	28	65	68	17	35	86	57	64	31	12	93	11	39
Theileriosis: Piroplasmas in bloodsmears . Schizonts in sections	97 3	×	×	×	××	×	××	×	×	××	×	×	0	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	X	×	×	×	×	0	×	0	×
Bilharziasis	62			×	×				×	×		-	-	1	-		×		×	×			×				×	×		×	×		×	-	×	-	×	X	×
Onchocerciasis.	16								×	×		×		×	-		×		×							×		×			-	1	×		×		×		-
Filarial lymphadenitis.	3						×						-								×									-	-					-			-
Filarial dermatitis	4												-	-			×														×						×		
Eosinophilic lymphadenitis	46				×		×		×		×	×		×		×	×		×			×	×		×				×		×	×		×	×	×		×	-
Pentastomiasis	69											×	-	×	×	×	×		×	×	×	×	×			×	×			×	×		×	×	-	×	×	×	×
Demodicidosis	10								×		×															×				-		×			-				
Cysticercosis	29								×				-	×			×				×					×			×		×			×			×		-
Hydatidosis	9		×												-																								
Granulomatous lymphadenitis .	15												-			×					×					×				-					-	-			
Lymphadenitis	23														-					×	×			×						×			×						×
Inflammation of serous membrane	79	×	×	×	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×		×		×	×	×	×	×	×	×	×	X	X	×	×	×	×
Pneumonia	4													-																									
Neoplasms	1																														-								
Sarcosporidiosis	86								×		×	×	×		×	×	×	×	×	×	×		×	×	×	×	×	X.	×	×	×	×	×	X	×		×	-	×
				-									-																		-			-					-

TABLE 1 Buffaloes: Some of the most significant findings in 97 cases

	% Posi- tive						Yc	gune	adul	Young adults (6–8 yt.)	-8 yf.	$\hat{}$											4	Adul	Adults (8–15 yt.)	-15 y	rt.)					
Serial No. of cases		36	80	13	ŝ	83	47	59	88	30	71	78	84	25	42	40	7	00	74	82	46	22	96	67	20	41	76	69	34	55	19	81 63
Theileriosis: Piroplasmas in bloodsmears Schizonts in sections	97 3	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×		×	×	×
Bilharziasis	62		×	×	×	×	×		×			×	×	-	×	×			×	×	×	×	×	×		×	×	×	×		×	×
Onchocerciasis	16						1								×										-							
Filarial lymphadenitis	3									-							×															
Filarial dermatitis	4																															
Eosinophilic lymphadenitis	46	×			×		×		×			×					×			×	×	×						×		×		×
Pentastomiasis	69	×				×	×	×		×	×	×	×	×	×	X	×	×	×		-		×				×	×	×			×
Demodicidosis	10					×	×	×		×						×				×					-							
Cysticercosis	29			×		×		×								×		×	×		×								×		×	
Hydatidosis	6			×						×							×		×													
Granulomatous lymphadenitis	15									×						×				×			×					×				
Lymphadenitis	23					×	×				×		×	×					×					×	×		×				×	
Inflammation of serous membranes	97	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Pneumonia	4						×														×					×						
Neoplasms	-																															
Sarcosporidiosis	86	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

TABLE 1 (contd.)

TABLE 1 (contd.)

					Ψ	ults (	Adults (8–15 yr.)	yr.)								V	Aged (15–20 yr)	(15-2	20 yr)	-					Adult or aged*	+- +
Serial No. of cases	85	91	87	51	77	21	62	15	75	23	6	60	37	16	98	54	53	9	62	66	56	50 4	4	1.8.2	S.B. 3	4 .8.S
Theileriosis: Piroplasmas in bloodsmears Schizonts in sections	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	0	0	0 0
Bilharziasis	×	×		×	×		×		×		×	×			×	×	×	×	×	×	×	×	×	X	X	×
Onchocerciasis				×															×			×	×		×	
Filarial lymphadenitis																										
Filarial dermatitis	X																	-								
Eosinophilic lymphadenitis			×		×						×	×	×	×				×			×	×	×	×	×	× ×
Pentastomiasis	×	×	×	×	×				×		×	×			×	×	×	×			×	X		X	×	
Demodicidosis																		×								
Cysticercosis	×	×	×	×								×	×				×				×		-		~	×
Hydatidosis																	×									
Granulomatous lymphadenitis		×									×			×		×			×			×		×		
Lymphadenitis		×															×			×		×				×
Inflammation of serous membranes	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	X
Pneumonia														×												
Neoplasms																×										
Sarcosporidiosis	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

many mononuclear cells [Plate 5 (18)]. Eosinophilic clubs and giant cells were absent in a small number of cases. The number of plasma cells was usually increased and globular lymphocytes were noticed in one case. The udder of the cow with the affected supramammary lymph nodes was not affected and mycotic lesions elsewhere in the bodies of the other affected buffaloes were absent. Unfortunately, specimens for the cultivation and specific identification of the fungus or fungi could not be collected.

The lesions in the two cases in which fungi were absent were somewhat different. Purulent reactions were absent and the giant cells were distributed throughout the affected lymph nodes [Plate 6 (22 and 24)]. It looked suspiciously like mycobacteriosis but no acidfast organisms could be demonstrated. Only one lymph node was affected in each animal, the one being a prefemoral node and the other an unidentified one. The case in which the prefemoral lymph node was affected contained several fairly large ulcers in the rumen [Plate 6 (23)]. Microscopically these ulcers revealed exactly the same type of giant cell and mononuclear response as were found in the lymph nodes [Plate 6(25)]. This reaction penetrated deeply into the muscular layers. As with the lymph nodes, no specific organisms or parasites, or more specifically fungi, acid-fast organisms, metazoa or foreign bodies, could be demonstrated.

# Inflammation of the serous membranes

All the animals including the calves had very mild to fairly marked and fairly diffuse subacute or chronic proliferative, villous, inflammatory reactions in the parietal peritoneum and pleura [Plate 8 (39)]. Both the

 TABLE 2
 Parasites encountered in the buffaloes

epicardium and pericardium were also frequently affected. The epicardium was more frequently involved, the reaction being usually confined to the base around the large vessels. However, adhesions to the pericardium were never noticed. But for exceptional localized adhesions to the lungs, adhesions were not encountered elsewhere. Microscopically the lesions appeared to be either chronic or subacute. Small numbers of mononuclear cells, consisting mainly of plasmocytes and lymphocytes, were sometimes seen. Plasmocytes were occasionally predominant. Very small numbers of eosinophils were noticed in a few cases.

### Pneumonia

One buffelo suffered from an extensive subacute bilateral bronchopneumonia of the apical and cardiac lobes. Peribronchial fibrosis and hyperplasia of the bronchial and bronchiolar epithelium were evident. Three other cases showed localized areas of pneumonia, one case being of an eosinophilic nature with the involvement of small arteries, another granulomatous with large syncytial giant cells and the last case fibrinous and eosinophilic. No indications of aetiological agents were found but the cosinophilic reaction in two buffaloes is thought to be due to helminth migrations.

### Neoplasms

Only one neoplasm was encountered. This was a squamous cell carcinoma situated mainly in the vagina, but the vestibulum and vulva were also partially involved. Its surface was ulcerated and it appeared to be a very invasive and rapidly growing type. The central core of epidermoid cells, some of which were keratin-

Parasite	Habitat	Incidence	Infestation
Paramphistomum microbothrium	Rumen	Fairly common	Mild
Cotylophoron cotylophorum	Rumen	Fairly common	Mild
Haemonchus contortus	Abomasum	Common	Mild
Haemonchus bedfordi	Abomasum	Common	Mild
Parabronema skrjabini	Abomasum	Fairly common. Mainly in adults	Mild. Severe in riverine buffaloes
Cooperia fülleborni	Small intestine	Rare	Very mild
Trichostrong ylus axei	Small intestine	Rare	Very mild
Avittelina centripunctata	Small intestine	Rare	Very mild
Agriostomum gorgonis	Small and large intestines	Fairly common	Very mild
Trichuris globulosa	Large intestine	Very rare	Very mild
Oesophagostomum radiatum (Rudolphi, 1803)	Large intestine	Fairly common	Very mild
Schistosoma mattheei	Mesenteric veins	Common in riverine buffaloes	Mild to severe
Schistosoma haematobium	Mesenteric veins	Exceptional	Only one parasite
Linguatula serrata	Mainly in hepatic veins	Common	Mild to severe
Frölich, 1779 Thelazia rhodesii	Conjunctival sac	Rare	Mild
Thelazia lacrymalis	Conjunctival sac	Rare	Mild
Onchocerca synceri	Subcutis	Fairly common	Mild
Taenia regis Baer, 1924 and Taenia gon- yamai Ortlepp, 1938	Muscles	Common	Mild to fairly severe
Hydatids	Liver, Lungs	Rare	Mild

ized, contained intercellular bridges. The peripheral cells were less differentiated and formed finger-like papillae which extended into the underlying tissue. An accompanying inflammatory reaction was present.

### Sarcosporidiosis

Only the parasitization of the oesophagus, pharyngeal and laryngeal musculature by the large type of sarcosporidial cysts is included in this survey. This was done purely on macroscopical evidence. However, those sarcosporidia which are usually only microscopically visible in the myocardium and skeletal muscles were very common. Neither of these types provoked any host response. The young buffaloes under a year old were not parasitized by either of these two forms.

### Miscellaneous findings

Mites, classified as unidentified *Psoroptes* sp., were found around the bases of the horns. This parasite was discovered at an advanced stage of the survey and no account of its incidence can therefore be given. The incidence is believed, however, to be fairly high among the adult and aged groups. The affected areas were light grey, scaly, alopecic and somewhat hyperkeratotic [Plate 8 (37)]. Mild cases of demodectic mange were encountered in 10 per cent of the animals. Some of the parasites were dead and surrounded by a granulomatous response. Other skin lesions were evidently due to trauma.

Many of the buffaloes had hyperplastic lymph nodes [Plate 8 (33)]. In some of these the enlarged lymph follicles had a peculiar arrangement of lymphocytes which were lined up in several almost circular rows around the germinal centres. This phenomenon is referred to as regimentation of lymphocytes [Plate 8 (34)]. These enlarged lymph nodes could not always be related to specific diseases, but subclinical theileriosis is thought to be one of the diseases responsible for their enlargement.

Eosinophilic intracytoplasmic oval or roundish bodies resembling inclusions were found in the neurones of all the buffaloes from which gasserian and spinal ganglia were collected. Their significance is not yet understood.

One case each of subacute villous tendo-vaginitis and suspected very mild myocardial glycogenosis were found. Eosinophilic cellulitis and panniculitis of the subcutis were seen in a few buffaloes. Although somewhat resembling lesions of *Parafilaria bovicola* Tubangui, 1934 as described by Pienaar & Van den Heever (1964), these parasites could not be isolated or detected in sections.

# Negative findings

The buffaloes were specifically examined for several common parasitic and other disease entities with negative results. These included nasal and paranasal myiasis by Oestridae, lung-worms, cordophilosis, fascioliasis, setariasis, foot and mouth disease and besnoitiosis.

#### DISCUSSION

The present survey in a 100 buffaloes was conducted mainly during the dry season and confined to the southern part of the Kruger National Park. Consequently the findings should not be regarded as representative for the entire Park throughout the year. Significant summer diseases could well have been missed. Some interesting information was, however, gathered.

One of the most important diseases appeared to be bilharziasis, and the lesions noted resembled those seen in cattle rather than those in sheep. In the latter, pigmentation of the liver and lung, caused by haematin released from the parasite's digestive tract, is a prominent feature which is not seen in the ox or buffalo. This phenomenon may, however, be related to the size of the host and the level of infestation. The presence of small numbers only of ova in the livers of the buffaloes, even in those with heavy infestations, also corresponds to the findings in cattle with bilharziasis. McCully & Kruger (1969) observed that even in heavy infestations there were fewer ova in the bovine livers relative to the numbers found in sheep. The average number of intrauterine ova in the female parasite from the buffalo was relatively large and corresponded well with the mean in bovine parasites. In contrast, the numbers of ova recovered from parasites in sheep, donkeys and baboons were significantly lower (Kruger, 1969, unpublished observations). These findings seem to indicate that cattle and buffaloes are probably better natural hosts than the other animals mentioned. Bilharzial endophlebitis, resulting from the influence of live, adult schistosomes, was observed in man by Letulle (according to Dew, 1923), in cattle and sheep (McCully, 1966; McCully & Kruger, 1969) and in the hippopotamus (McCully, van Niekerk & Kruger, 1967). This lesion, which seems to be very characteristic, is also present in buffaloes. Similarly, the lymphoid nodules formed at the sites from which the dead schistosomes were removed by the host reaction have also been observed previously in sheep and cattle (McCully, 1966; McCully & Kruger, 1969).

The incidence and distribution of bilharziasis in the various age groups and the severity of the lesions caused by the parasite in the older age group is interesting. Some of the old bulls commonly leave the herd for one reason or another, frequently because they are expelled by younger, stronger animals or perhaps because they have been wounded and can no longer keep up with the herd. They either lead a solitary existence or remain in the company of one or two other old bulls. A number of the more severely affected animals in the older age group definitely was of this riverine type. These animals, which lack the protection afforded by the herd, frequent the rivers and reed-beds and are commonly referred to as "rietbuffels" in Afrikaans. Especially during warm summer days these old buffaloes often wallow in the shallow pools. Under these conditions they are frequently exposed to water and thus to cercariae. Such a severe challenge may cause a breakdown in their immunity to reinfestation, or their resistance may have been lowered as a result of general loss of condition and/or concurrent disease. Whatever the cause, these animals become heavily infested.

The small piroplasms and schizonts encountered in the blood smears and sections were not specifically identified, but are thought to be either *Theileria mutans* (Theiler, 1906) or *Theileria lawrencei* Neitz, 1955, or possibly both species. Buffaloes are known to be carriers of both parasites (Neitz, 1965) and *T. lawrencei* has been proved to cause corridor disease in cattle (Neitz, Canham & Kluge, 1955). The present finding of an extremely high incidence of piroplasms (97 per cent) in the blood smears of 92 buffaloes again emphasizes the significance of these animals as reservoirs of these parasites. Fatal cases of theileriosis in buffalo calves have been reported by Neitz (1965). During this study a fairly high incidence of schizonts was found in the tissue sections of the calves, which suggests that theileriosis may be one of the causes of mortality amongst them in the Kruger National Park. However, as sick animals so easily fall prey to predators, it is extremely difficult to assess the significance of any disease in an area such as this where so many predators occur. Only by carrying out experimental work will such problems be solved.

Many buffaloes had hyperplastic lymph nodes, some of which could have been caused by theileriosis. The frequent inflammatory reactions, being mainly eosinophilic or neutrophilic, were apparently caused by circulating or migratory parasites, such as microfilariae, or by fungi. Only a very small number of microfilariae was found in the affected lymph nodes, but the difficulty of detecting the parasites in a single section must be taken into consideration. Apart from O. synceri at least one other filarid is thought to occur in the buffalo in the Kruger National Park. This comment is based on the presence of both unidentified parasites in the skin [Plate 7 (28 and 29)] and on the strange sheathed microfilariae encountered in the infraorbital skin. Their morphological features, such as their denticulated cuticle and sheaths respectively, and their localization suggest that they could be either Stephanofilaria sp. or P. bovicola (Mönnig, 1950) or perhaps both. Skin lesions were also caused by horn mites, Demodex sp., ticks, trauma and possibly by the bloodsucking Lyperosia sp. which haunt the animals in large numbers and which are known to be the intermediate hosts of Parabronema skrjabini (Ivashkin, 1956).

Unidentified pentastome nymphs within the cardiovascular system and liver of the blue wildebeest [Connochaetes taurinus (Burchell, 1823)] and greater kudu [Tragelaphus strepsiceros (Pallas, 1766)] were reported previously by McCully, Basson, van Niekerk & Bigalke (1966). These subsequently proved to be similar to those in the buffalo identified as L. serrata (Kruger, cited by Young & van den Heever, 1969). Von Haffner, Sachs & Rack (1967) and Sachs & Sachs (1968) described several species of pentastomes, including L. serrata and a new species, L. multiannulata Von Haffner, Sachs & Rack, 1967, from various organs and tissues of a number of different antelopes in central Africa. Cystic lesions and haemorrhagic foci were reported. Although very common in the blood vessels of the buffalo of the Kruger National Park (69 per cent), nymphs of L. serrata provoked relatively few hepatic lesions in only a small number of the animals (10 per cent). Some of the parasites were enclosed in granulomatous reactions in the liver, sometimes with shed cuticles adjacent to them. In the lymph nodes, however, where larvae and lesions are commonly found in domestic animals (Jubb & Kennedy, 1963) the parasites were rare and no specific lesions were encountered. Two buffaloes had nymphs in their mesenteric lymph nodes, one with an adjacent shed cuticle, but did not show any accompanying host reactions.

The point of entry of the fungus causing the granulomatous lymphadenitis is, in view of the peripheral distribution of the lesion, thought to be via injuries in the skin. Fungi could readily enter wounds that are produced when these animals barge their way through thickets. The infection apparently then becomes successfully localized in the regional lymph nodes. There appeared to be some evidence that the responsible fungus or fungi belong to the Ascomycetes or Fungi imperfecti.

The chronic inflammatory reactions of the serous membranes are not fully understood at present.

The absence of *Besnoitia* cysts in such a large sample of buffaloes is significant in view of its high incidence in both blue wildebeests and impalas [Aepyceros melampus (Lichtenstein, 1812)] in this area (Basson, Van Niekerk, McCully & Bigalke, 1965). It suggests that this animal is not susceptible to besnoitiosis.

### SUMMARY

The most significant findings of a histopathological and parasitological study on a hundred random buffaloes of the Kruger National Park were the following:

1. Theileriosis, as revealed by blood smears from 92 animals, of which 97 per cent were positive, was very common. The incidence in animals under two years old was significantly higher and the pathological changes in three such cases are described. The parasite was not, however, specifically identified.

2. Nymphs of *L. serrata* occurred in 69 per cent of the animals, most frequently in the liver and hepatic veins, in which associated lesions were occasionally found.

3. Bilharzial lesions were found in 62 per cent of the buffaloes and were caused mainly by S. mattheei. One specimen of S. haematobium was also identified. The vascular lesions, granulomas and lymphoid hyperplasia in the livers were the most characteristic features, but some hepatic lesions closely resembled those seen in pentastomiasis.

4. Sixteen per cent of the buffalocs contained filarial lesions in the subcutis, skin and lymph nodes.

5. Mycotic and eosinophilic lymphadenitis of the lymph nodes was diagnosed in 15 and 46 per cent of the buffaloes respectively.

6. Cysticercosis (29 per cent), hydatidosis (5 per cent), oesophageal sarcosporidiosis (86 per cent), mange and neoplasia — a squamous cell carcinoma (1 per cent), were also encountered.

7. None of the animals examined revealed any lesions of besnoitiosis. As this disease is very common in the area, this finding evidently suggests that the buffalo is not susceptible to it.

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### REFERENCES

- ANON., 1960. Manual of Histologic and Special Staining Technics. Washington: Armed Forces Institute of Pathology.
- BASSON, P. A., VAN NIEKERK, J. W., MCCULLY, R. M. & BIGALKE, R. D., 1965. Besnoitiosis in South African antelopes: A preliminary note on the occurrence of *Besnoitia* cysts in the cardiovascular system. *Jl S. Afr. vet. med. Ass.*, 36, 578.
- CRUICKSHANK, R., 1962. Mackie and McCartney's Handbook of Bacteriology. 10th ed. London: E & S Livingstone Ltd.
- Dew, H. R., 1923. Observations on the pathology of schistosomiasis (S. haematobium and S. mansoni) in the human subject. J. Path. Bact., 26, 27-39.
- IVASHKIN, V. M., 1956. Elucidation of the life cycle of the nematode Parabronema skrjabini of ruminants. In Russian. Dokl. Akad. Nauk. SSSR., 107, 773-775.
- JUBB, K. V. F. & KENNEDY, P. C., 1963. Pathology of domestic animals. Vol. 1. New York and London: Academic Press.
- LONGHURST, W. M., 1958. Progress Report No. 3. Wildlife Research in Uganda.
- MARSBOOM, R., 1950. De Buffel en zijn economische betekenis voor Belgisch-Congo. Bull. Agrie. Congo belge, 51.

- McCully, R. M., 1966. Letsels in die portale vene van beeste en skape met bilharzia-behandelde en onbehandelde gevalle.
- Geneskunde, 8, 90–91. MCCULLY, R. M., BASSON, P. A., VAN NIEKERK, J. W. & BIGALKE, R. D., 1966. Observations on *Besnoitia* cysts in the cardiovascular system of some wild antelopes and domestic cattle. Onderstepoort J. vet. Res., 33, 245–276.
- MCCULLY, R. M. & KRUGER, S. P., 1969. Observations on bilharziasis of domestic ruminants in South Africa. Onderstepoort J. vet. Res., 36, 129-162.
- Res., 36, 129-162.
   MCCULLY, R. M., VAN NIEKERK, J. W. & KRUGER, S. P., 1967.
   Observations on the pathology of bilharziasis and other parasitic infestations of *Hippopotamus amphibius* Linnaeus, 1758, from the Kruger National Park. Onderstepoort J. vet. Res., 34, 563-618.
   MÖNNIG, H. O., 1950. Veterinary helminthology and entomology.
- 3rd ed. London: Baillière, Tindall and Cox. NETTZ, W. O., 1965. A checklist and hostlist of the zoonoses oc-
- curring in mammals and birds in South and South West Africa.
- Onderstepoort J. vet. Res., 32, 189-376.
   NEITZ, W. O., CANHAM, A. S. & KLUGF, E. B., 1955. Corridor disease: A fatal form of bovine theileriosis encountered in Zululand. Jl S. Afr. vet. med. Ass., 26, 79-87.

- PEARSE, A. G. EVERSON, 1961. Histochemistry, Theoretical and Applied. 2nd ed. London: J. & A. Churchill Ltd.
- PIENAAR, U. DE V., 1969. Observations on developmental biology, growth and some aspects of the population ecology of African buffalo (Syncerus caffer caffer Sparrman) in the Kruger National Park. Koedoe, 12 29–52.
- TIAN, ROLAN, LE D. DEN HEEVER, L. W., 1964. Parafilaria bovi-cola (Tubangui, 1934) in cattle in the Republic of South Africa. JI S. Afr. vet. med. Ass., 35, 181-184. SACHS, R. & SACHS, C., 1968 A survey of parasitic infestation of
- wild herbivores in the Serengeti region in Northern Tanzania and Lake Rukwa region in Southern Tanzania. Bull. epizoot. Dis. Afr., 16, 455-472.
- Verster, Anna, 1969. A taxonomic revision of the genus Taenia Linnaeus, 1758 s. str. Onderstepoort J. vet. Res., 36, 3-58.
   Von HAFFNER, K., SACHS, R., & RACK, G., 1967. Das Vorkommen von Stachellarven aus der Familie Linguatulidae (Pertastomida) in afrikanischen Huftieren und ihr Parasitismus. Z. ParasitKde,
- 29, 329–355. YOUNG, E. & VAN DEN HEEVER, L. W., 1969. The African buffalo as a source of food and by-products. *Jl S. Afr. vet. med. Ass.*, 40, 83-88.

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