OBSERVATIONS ON BESNOITIA CYSTS IN THE CARDIO-VASCULAR SYSTEM OF SOME WILD ANTELOPES AND DOMESTIC CATTLE

R. M. McCULLY(1), P. A. BASSON(2), J. W. VAN NIEKERK(3), and R. D. BIGALKE(4)

INTRODUCTION

During the course of a survey of antelopes in the Kruger National Park for zoonoses, specifically schistosomiasis and gedoelstial myiasis, small, white, round objects measuring slightly less than half a millimetre in diameter were found attached to the endocardium and to the intima of various blood vessels of blue wildebeest [Connochaetes taurinus (Burchell, 1823)]. These objects proved upon subsequent microscopic examination to be cysts of a Besnoitia sp. This finding led to the examination of the cardiovascular system of cattle having chronic besnoitiosis and to a more extensive survey of antelopes.

This report is about these Besnoitia cysts and those subsequently found in corresponding sites in other blue wildebeest, impala [Aepyceros melampus (Lichtenstein, 1812)], a kudu [Tragelaphus strepsiceros (Pallas, 1766)] and domestic cattle. These antelopes were killed either for foot-and-mouth disease surveys or specifically for Besnoitia studies.

Since many aspects of this disease in domestic animals have received extensive attention from other investigators, no attempt is made to give a comprehensive account of it. Instead the reader is referred to the original report of the disease by Besnoit & Robin (1912), to the first report of the disease in South Africa by Hofmeyr (1945) and to the comprehensive work of Pols (1960). The latter is the most complete account of the knowledge of this disease at present, containing an excellent review of the literature and having an exhaustive bibliography. A report by Schulz (1960) emphasizes the features of the gross and microscopic pathology of the disease as it affects domestic cattle. A preliminary note on our observations of Besnoitia cysts in antelopes has been published (Basson, van Niekerk, McCully & Bigalke, 1965), and to our knowledge, this was the first report of such in the literature. The present report deals primarily with the involvement of the cardiovascular system in besnoitiosis. It should be of interest to investigators concerned with the epizootiology and the pathogenesis of the disease. The incidental finding of pentastomes in the cardiovascular system of antelopes is also reported.

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BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM

MATERIALS AND METHODS

Data and materials for study were collected between August, 1964 and September, 1965. Twenty-one blue wildebeest, seventy-four impala, eight kudu and five domestic cattle were examined. The antelopes were shot at random for foot-and-mouth disease inspection, for a besnoitiosis check, or for meat rations for Bantu labourers. Two cattle which had severe, chronic besnoitiosis were obtained from the Rustenburg district of the Transvaal. The other three cattle which showed no skin lesions were obtained from the Zoutpansberg district of the Transvaal.

The antelopes were examined for besnoitiosis as indicated in Table 1.

<table>
<thead>
<tr>
<th>Antelope</th>
<th>Vein Check</th>
<th>Autopsy</th>
<th>Total Examined</th>
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<tr>
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<td>16</td>
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<td>19</td>
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<td>Impala</td>
<td>56</td>
<td>18</td>
<td>74</td>
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<td>Kudu</td>
<td>3</td>
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<td><strong>GRAND TOTALS</strong></td>
<td><strong>64</strong></td>
<td><strong>39</strong></td>
<td><strong>103</strong></td>
<td><strong>53</strong></td>
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Those listed under "vein check" were examined in the field by opening either the jugular veins, the superficial veins of the limbs or a combination of these. Most of those listed under "autopsy" were first vein-checked and found to be positive by the presence of cysts, but are not included under "vein check". At autopsy particular attention was given to the cardiovascular system. After the identification of the organisms, those tissues known to be the location of cysts in chronic bovine besnoitiosis were carefully examined. Equally thorough autopsies were performed on two cattle having the chronic disease.

At autopsy, tissues and parasitic specimens were collected from the antelopes and cattle for further study. Cysts, which were collected from antelopes for the purpose of biological studies, were counted as they were removed from the vessels and other tissues. These data are shown in Table 2.

Tissues collected varied from one animal to the next in regard to number and the organs from which blocks were cut. In one or more sets of tissues, the following were preserved in 10 per cent formalin: brain, semilunar ganglion, hypophysis, skin, lung, nasal mucosa, meninges, periorbital tissues, eye, heart, thyroid, muscle, gastrointestinal tract, the parenchymatous organs and various other tissues including specific blood vessels, e.g. jugular veins.

Tissue sections three microns thick for histopathological examination were prepared in a routine manner using paraffin embedding, a sliding microtome and the hematoxylin and eosin staining technique.

COURSE AND RESULTS OF STUDY

Initially in the examination of the cardiovascular system of the first blue wildebeest for Schistosoma spp. and Gedoelstia larvae, numerous small, flat, elongated organisms (3 to 4 mm) were discovered in the lumen of the thoracic portion of the posterior vena cava. Identical organisms were found in the hepatic and other veins, beneath Glisson's capsule in the liver, in the chambers of the heart, the pulmonary
TABLE 2.—Besnoitia Cyst Counts in Antelopes

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<thead>
<tr>
<th></th>
<th>Impala</th>
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<td>Larynx and trachea</td>
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<td>153</td>
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<tr>
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<tr>
<td>Vv./Hind limbs</td>
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<td>109</td>
<td>77</td>
<td>189</td>
<td>244</td>
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± Positive, but few cysts
+ Positive, cysts common
++ Numerous cysts
— Negative

artery and the aorta. They were initially thought possibly to be specimens of unpaired 
Schistosoma spp., but on microscopic examination tentatively identified as pentastome 
nymphae. This was later confirmed by the parasitology section at Onderstepoort (Kruger, 1965). With the nymphae a few small, round, white, glistening objects measuring up to almost 0·5 mm in diameter were seen, first on the endocardium of the heart and the intima of the anterior vena cava, but subsequently in other vessels. They were especially numerous in the jugular veins where aggregates were accumulated on the cusps of the valves (Fig. 1). It was thought at the time that these white objects were nodules of lymphoid tissue as had been observed in some of the hepatic and portal veins of the hippopotami with bilharziasis in the Kruger National Park (McCully, van Niekerk & Kruger, 1965.) Microscopic examination of sections, however, revealed that they were Besnoitia cysts (Fig. 2).

The examination of the cardiovascular system of eight other blue wildebeest on the same trip yielded similar findings in regard to pentastome nymphae and Besnoitia cysts. Schistosoma spp. were not found in this group of animals but larvae of Gedoelstia spp. were present (Basson, 1966).
Following the identification of the Besnoitia cysts, it became apparent that a thorough examination of the cardiovascular system of cattle with natural besnoitiosis was imperative. Case reports of five such animals are to be seen below. Throughout this report, unless otherwise stated, “cysts” refers to Besnoitia cysts.

Case Reports of Bovine Besnoitiosis

History

Case A: This was an Afrikaner steer, approximately two years old and in rather poor condition. It had chronic besnoitiosis with extensive skin involvement, but the duration of the disease was unknown. The animal was killed by electrocution for post mortem examination.

Case B: A second, naturally occurring chronic case of besnoitiosis in a Friesian-cross steer, approximately three year old also showed extensive skin lesions. The duration of the infection was also unknown but the disease appeared less severe than case A. The steer was electrocuted for post mortem examination.

Cases C, D and E: Three adult Simmenthaler cows with an inapparent form of chronic besnoitiosis without any skin lesions and detected only by close examination of the bulbar conjunctiva for cysts were examined after slaughter at the Pretoria abattoir.

Macroscopic Findings

Case A: Natural body openings.—Several small cysts were present in the bulbar conjunctiva. Signs of anaemia were absent.

Integumentum.—The skin was thickened, wrinkled, hyperkeratotic and there was alopecia in the regions of the axillae, shoulders, flanks, sides of the thorax, lower portions of the limbs and generally over the head and neck.

Initial incision.—In the subcutis, fine, white “grains” approximately 0.25—0.5 mm in diameter were numerous. These proved histologically to be cysts. Serous atrophy of the subcutaneous fat indicated the state of emaciation. The blood appeared to be of normal colour and viscosity and there was no evidence of anaemia.

Body cavities.—The thoracic and abdominal cavities showed no recent changes of significance and a few fibrous adhesions of both the lungs and liver to the diaphragm appeared to be unrelated to besnoitiosis. Cysts were not seen in the pleura or peritoneum.

Respiratory system.—The nasal mucosa was also studded with numerous cysts of the same size as those seen in the subcutis. Similar cysts were present in the mucosa of the paranasal sinuses, the pharynx, the larynx and trachea. Other than the fibrous adhesions to the diaphragm, the lungs did not appear abnormal.

Cardiovascular system.—The pericardial sac contained 100 ml of clear, straw-coloured fluid. A few foci, suspicious for cysts, were seen in the endocardium of the right ventricle. All the veins of the lower half or two-thirds of the legs contained cysts (Fig. 3) which gave the intima a granular appearance (Fig. 4). A few areas of extremely fine granularity were seen in the jugular veins. The other large veins, the left atrium and left ventricle and the arteries did not show any significant change.
Musculoskeletal system.—Some of the muscles of the lower part of the legs had a rather whitish, mottled appearance. Small, white foci suspected of being cysts were observed intramuscularly. Others were observed in the fascia and the loose areolar tissue between muscles, in joint capsules, tendons and in the periosteum of the bones of the lower part of the legs.

Nervous system.—There were no cysts recognized in the meninges, brain or the spinal cord. There were suspicious white foci in the epineurium of some of the larger peripheral nerves, including the sciatic.

Lymph nodes.—The regional lymph nodes of the front and hind legs, head and neck were somewhat enlarged and moist on cut surface.

Other viscera.—Examination of the liver, spleen, adrenals, kidneys and urinary bladder revealed nothing unusual. Apart from a mild infestation of nodular worms, the condition of the gastro-intestinal tract was thought to be within normal limits.

Case B: Natural body openings.—A few cysts were visible in the conjunctiva. There was no sign of anaemia.

Integumentum.—The skin of the flanks and the lower legs were slightly wrinkled and there was mild hyperkeratosis with some alopecia. The skin of the face, particularly that above the muzzle, showed the most advanced changes and state of alopecia. Skin lesions in general were of a much milder nature than in the previous case.

Initial incision.—The subcutis of the lower abdomen and the lower half of some of the limbs was thickened by a yellow, gelatinous oedema. The blood appeared normal in colour and consistency and there was no indication of anaemia. There were fewer cysts in the subcutis than in the previous animal.

Body cavities.—The two large body cavities appeared to be normal. Cysts were not seen in the pleura or peritoneum.

Respiratory system.—Numerous cysts were present in the nasal mucosa and lining of the paranasal sinuses, pharynx and larynx, but few were present lower in the respiratory tract with only isolated cysts visible in the bronchi and lungs. Purulent foci about 2·0 mm in diameter were locally disseminated in the nasal mucosa.

Cardiovascular system.—The small veins of the limbs, tail and head were heavily parasitized, with the cysts being the most numerous in the more distal segments of these veins. The saphenous, cephalic and the facial veins and their tributaries were severely affected. The cysts were easily seen by the unaided eye and some of the veins had a rather uniformly granular appearance because of the many cysts. Thickened areas in the wall due to cysts and the accompanying phlebitis were prominent in some segments of various veins (Fig. 5), but thrombosis was not a feature. There were remarkable concentrations of these parasitic cysts in the veins at bends in their course and inlets of tributaries. The valves were a very common site for cysts. Isolated cysts were observed in the small arteries of the limbs. None were observed in the largest veins and arteries of the body or on the endocardium. Many segments were taken from named blood vessels and preserved separately.

Skeletal system.—Suspected cysts were noticed in the periosteum of the metatarsal and metacarpal bones.

Nervous system.—A Pandy test on a sample of cerebrospinal fluid collected immediately after death was negative. The brain, spinal cord and meninges appeared normal and no cysts were recognized.
BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM

*Lymphatic system.*—Particular attention was given to the large lymphatics. The thoracic duct and cisterna chyli were located, opened and carefully examined, but were found free of cysts. Lymph nodes were swollen and hyperplastic in appearance.

*Abdominal viscera.*—There was a mild splenomegaly. The liver, kidneys, adrenal and urinary bladder appeared normal. Except for a mild parasitism due to nodular worms, the gastro-intestinal tract and the mesenterium were normal.

*Cases C, D and E.* In one or more of these cows, cysts were observed in the following sites: bulbar conjunctiva, veins of all limbs, veins of the head and neck and a few in the nasal mucosa. Even on close examination skin lesions could not be located macroscopically in any of this group.

Microscopic Findings

*Case A: Cardiovascular system.*—Groups of free *Besnoitia* were present in the lumina of various blood vessels. As many as a hundred adhered together as if agglutination had occurred. A section from the upper respiratory tract, specifically, contained numerous vessels with groups of free parasites. At a point of bifurcation one vessel contained “agglutinated” organisms with part of the group in the main trunk and extensions from it into the tributaries. Nearby in the lumen the remains of a ruptured cyst were still partially attached to the intima. Small arteries as well as veins contained free organisms in their lumen. The lumina of other channels, presumably lymphatics, contained similar groups of organisms.

Cysts were located in either the intima or the media, or the adventitia or combinations of these layers of many arteries and veins (Fig. 7). Some present beneath the endothelium either bulged into the lumen or caused no bulging of the endothelium, extending into the media instead. In a section of one vein, more than eighty cysts were present in the intima and media of one relatively short segment. Subendothelial groups of about 10 to 20 cysts in close contact with one another resulted in elevations protruding into the lumen of the vein (Fig. 6). Some cysts extending into the lumen had sessile attachments (Fig. 15), but others were attached by narrow pedicles of connective tissue. Both types appeared to be covered by endothelium. The valves of veins were frequently the location of cysts. Some were attached rather superficially while others were more firmly attached to the valve cusps. In response to the cysts, the endothelium was sometimes hyperplastic (Fig. 9), but thrombosis was not observed. Occasionally sclerosis of the intima in the immediate vicinity of the cysts was observed. The initial subendothelial proliferation appeared to be fibroblasts maturing to fibrocytes with the production of collagen. While some cysts extended from the endothelium to or into the media, others were predominantly or entirely within the media (Fig. 7), where there was a tendency toward sclerosis in their immediate vicinity. Cysts in the veins with a rather thick, muscular media, often caused the muscular portion to bulge toward the adventitia. In some small muscular arteries, cysts occupied the entire thickness of the media at some point of the circumference (Fig. 8). The elastica interna was either somewhat indistinct at that point or there was no evidence of its presence at all. Some cysts were entirely in the adventitia (Fig. 6 and 7). A deeply eosinophilic substance was occasionally seen between the connective tissue strands of the adventitia.

Cysts were present in some thin-walled channels with large lumina lined by endothelial cells. These were believed to be lymphatics. No cysts were found in the heart of this animal and except for a minimal number of inflammatory foci of non-specific nature, there were no significant changes.
Integumentum.—Specimens from obviously affected areas of skin were taken and sections consequently contained many cysts in the dermis. Most of them were in the stratum papillare, the portion containing the adnexa and, more important, having the richest vascular network (Fig. 14). There were no cysts in the epidermis, but the plane of section through dermal papillae containing cysts sometimes made it appear as though they were in the epidermis. Serial sections showed clearly that these were in the outer tips of dermal papillae (Fig. 10). In the more dense portion of the dermis, the stratum reticulare, cysts were occasionally present, but seldom as numerous as in the more vascular stratum papillare or even the subcutis (Fig. 14). The vascularity of the stratum reticulare varied from section to section, and even in one section. With the increase in the number of vessels, there was an apparent increase in the number of cysts.

There was little response to the presence of the cysts in some areas, while in others the reaction was intense. The host response varied from the formation of a thick zone of hyalinized collagen to more cellular reactions. Some areas of reaction were composed of small round cells with confluent cytoplasm and in some instances there were spindle-shaped cells. Around some of the cysts there was a granulomatous reaction with a zone of epithelioid and multinucleated giant cells, which in turn was surrounded by a zone of small round cells and spindle-shaped cells. The granulomatous reaction was seen in response to either dead or alive-appearing cysts (Fig. 11). The cutaneous blood vessels were affected to the same extent and subject to similar variances as described above for affected blood vessels. There were a few cysts in the perineurium of some of the cutaneous nerves.

Respiratory system.—The stroma of the mucosa of the nasal septum (Fig. 13) and the turbinates frequently contained cysts, but in this site the host response was mild. The blood vessels and the lymphatics were affected rather extensively in some sections. One section near the external nares contained some bundles of skeletal muscle with numerous cysts. In contrast to that of the mucosa, there was a marked cellular response. Cysts were numerous in the larynx and trachea, also with a relatively mild host response. A few rather small, degenerated cysts were present in alveolar septa (Fig. 12) and some of them were surrounded by a granuloma, but otherwise the lung was normal. There was no specific evidence to explain the fibrous adhesions between the pleura and the diaphragm.

Musculoskeletal system.—The skeletal muscles, tendons and other fibrous tissues of the limbs contained many cysts. These and other sections of skeletal muscle showed a severe cellular host response. It was more severe in muscle than anywhere else. When either apparently alive or degenerated cysts were present anywhere in skeletal muscle, there was often an intense cellular response, primarily granulomatous with an inner zone of epithelioid and giant cells surrounded by innumerable mononuclear and spindle-shaped cells (Fig. 16, 17, 21).

The periosteum of the metatarsal and metacarpal bones was heavily parasitized and in a few instances the cysts were entrapped in newly formed periostial bone. Synovial sheaths and tendons contained cysts. In the tendons it appeared as though the sites of cysts were determined by the distribution of the meagre supply of blood vessels and nerves. In some instances the continuity of small blood vessels was apparently interrupted by the development of the cysts.

Other tissues.—The parietal lymph nodes showed lymphoid hyperplasia with very active germinal follicles and densely cellular medullary cords. Numerous reticuloendothelial cells were present in the medullary sinuses. There were many polymorphonuclear leukocytes in one prescapular node showing lymphadenitis.
Cysts were present in the connective tissue and muscles of the tongue and pharynx, but were not observed elsewhere in the digestive tract. Cysts were not present in the avascular cornea, but in one eye the cornea was mildly vascularized and contained a few cysts (Fig. 22). Cysts were present in the sclera, also more numerous in the more vascular episclera (Fig. 25), especially at the limbus (Fig. 23), and a few were present in the lamina fusca (Fig. 27). Cysts were frequent in association with the blood vessels between the sclera and conjunctiva (Fig. 24). There were many in the iris (Fig. 29) and a few in the ciliary body (Fig. 28). At the limbus there was a round cell infiltrate, but otherwise there was little host response. The choroid and the retina appeared to be free of cysts. In small blood vessels of the sclera, there were a number of bulbous aneurysmic-like dilatations with multiple large nuclei in the wall (Fig. 26). Although organisms were not evident, these were interpreted as representing cysts. In the lamina propria of the penis there were small numbers of cysts.

The following tissues were free of the cysts or related lesions: thyroid, liver, adrenal, kidney, pancreas, meninges, spinal cord, brain, semilunar ganglia and hypophysis.

Case B: Cardiovascular system.—The following veins were found microscopically to be affected similarly to those of case A: intercostal, metacarpal, cephalic, popliteal, saphenous, superficial mammary, superior labial, dorsal nasal, labial nasal and the facial. Intercostal arteries contained cysts also.

Sections of the following vessels did not show cysts: aorta, pulmonary artery, pulmonary vein, anterior vena cava, radial artery, coccygeal vein, metacarpal artery, posterior vena cava, external spermatic veins, vessels of spermatic cord and mesenteric veins. Serial sectioning might have revealed cysts in some of these.

Integumentum.—There was a tendency to take specimens from just the obviously affected areas of skin, so this study does not justify remarks on the topographical distribution of the cysts. The stratum papillare, as in case A, was the most severely affected portion both in terms of the number of cysts and the host response. Cysts were more numerous adjacent to adnexal structures than elsewhere. As the number of cysts varied from one section of skin to another, so did the host response vary. In areas with many cysts the focal reactions to individual cysts sometimes became confluent with adjacent reactions giving the appearance of a diffuse or generalized response. There were a few cysts in the confinement of the perineurium of some cutaneous nerves (Fig. 18).

Respiratory system.—A section of muzzle and external naris contained many cysts. There were many in the stratum papillare of the dermis, especially the dermal papillae, beneath the smooth, hairless, stratified squamous epithelium of the muzzle. The cysts encroached on the sudoriferous glands and other adnexal structures in the area. The cellular reactions outside the hyalinized cyst wall varied from mild to severe. The severe reactions were of two types, one composed primarily of small round cells and the other a granulomatous reaction of epithelioid cells. The naris portion of the section, as indicated by the mucous glands, also contained numerous cysts to which there was a similar response. There were numerous eosinophiles in the mucous membrane.

There were also many eosinophiles on the epithelium over the nasal septum as well as in the numerous mucous glands and ducts, the openings of which were often obstructed. Dilatation due to the accumulation of mucus, eosinophiles and possibly other leukocytes caused the purulent, macroscopically visible foci. This material was sometimes inspissated. Cysts were so numerous in the underlying propria, that the reactions to individual ones had become confluent so that the result appeared
as a diffuse rhinitis. There were other cysts, however, to which there was little host response. Others were surrounded by an inner zone of epithelioid and multinucleated giant cells which, in turn, was surrounded by a thick zone composed primarily of small round cells, but with some spindle-shaped cells having confluent cytoplasm. There were also ruptured cysts filled by inflammatory cells. The tissue in their immediate vicinity was densely infiltrated by both mononuclear and polymorphonuclear leukocytes (Fig. 20). Eosinophiles, plasma cells and Russell-Fuchs bodies were common and the presence interstitially of a finely granular and fibrillar substance indicated oedema.

The nasal turbinates were parasitized to the same degree as the mucosa of the nasal septum, but the host reaction was more intensive. Some focal reactions were different from any mentioned yet. There was a central accumulation of eosinophiles surrounded by a zone of epithelioid and multinucleated giant cells. An outer zone consisted of more eosinophiles and other leukocytes which tended to blend in with the rather diffuse reaction between the cysts.

The granulomatous reaction was more severe around dead cysts, some of which were calcified. The reactions around ruptured cysts were primarily granulomatous, but some were of a more acute type. A few blood vessels of the nasal septum and turbinates contained cysts. The mucosa of the paranasal sinuses contained a few cysts with a variable host response. Cysts were numerous in the stroma underlying the epithelium of the larynx with relatively little reaction to their presence. A cyst was observed in the perichondrium of the laryngeal cartilage with matrix deposited in contact with the cyst wall. Only a few cysts which provoked little reaction were observed in the trachea. Neither cysts nor related changes were observed in the lung sections.

Musculoskeletal system.—The muscles of the lower part of the legs contained many cysts and the host response was similar to that present in the previous animal, except for mild Zenker's necrosis in the vicinity of some cysts.

Other tissues.—In the eye, cysts were found in sites corresponding to those in case A. In all lymph nodes examined there was lymphoid hyperplasia, but no evidence of cysts. The prefemoral and prercural nodes contained numerous plasma cells.

Sections of the following tissues were found to be free of cysts: liver, kidney, spleen, brain, meninges, gastro-intestinal tract, sciatic nerve, ligamentum nuchae, heart and the flexor tendons of the metacarpus.

Cases C, D and E: Only tissues from cases C and D were examined histologically. Cysts were observed in all layers of some veins and arteries. A few were present in the dermis and in a peripheral nerve. Reactions to the cysts were essentially the same as in the preceding cases.

The Findings in Antelopes

From a total of twenty-one blue wildebeest, sixteen (76 per cent) had pentastome nymphae and nineteen (90 per cent) were positive for cysts. Of eight kudu, three were positive for the presence of pentastome nymphae (37.5 per cent) and one for the cysts (12.5 per cent). Thirty-three of seventy-four impala (45 per cent) had cysts, but none was positive for the presence of pentastomes. None of the antelopes with cysts showed clinical besnoitiosis with skin lesions as seen in cattle.
Macroscopic Findings

Blue Wildebeest.—A thorough macroscopic examination of blue wildebeest at the veterinary laboratory in the Kruger National Park revealed cysts in the following veins: jugular, azygos, posterior and anterior vena cava, superficial mammary, axillary, iliac, inguinal, femoral, others of the front and hind limbs, intercostal, auricular, coeeygeal, cutaneous, coronary, sternal and the superficial ones of the head. The attachments of the cysts to the intima of the jugular vein were sometimes encircled by slightly elevated zones (Fig. 35). On the endocardium of the right atrium and ventricle, on the leaflets of tricuspid valves and the pulmonary valves, and in the pulmonary artery, the bulbar conjunctiva, nasal mucosa, lung, joint capsules, synovial sheaths, periosteum, tendons and rarely the pleura, cysts were also found. Some were present in the vessels of the subcutis, especially over the cartilage of the ears.

With the exception of the pulmonary artery, cysts were not observed in the arteries of the body and in the chambers of the left heart. Cysts were not observed in the mesenteric, renal, vesicular, portal or hepatic veins and in the dermis.

Impala.—Cysts were usually found in the following sites: jugular veins, veins of both front and hind limbs, veins of the head, blood vessels and lymphatics of the skin and subcutis (Fig. 40), tricuspid valves, pulmonary valves, pulmonary artery, areolar connective tissue between ligaments and tendons of the limbs, periosteum, pampiniform plexus, cremaster muscle and the nasal mucosa. Many cysts were found in lymph nodes as small white spots in the capsule (Fig. 42) and on the cut surface in the cortex and medulla. Cysts were rare in the following: bulbar conjunctiva, peripheral nerves, myocardium, renal vein, spleen, renal cortex, mesenterium and mesenteric vessels, pleura and loose connective tissue around the adrenals and kidneys. The thoracic ducts of two impala specifically examined were negative for cysts.

In some of the vessels the cysts were irregular in shape and larger than adjacent ones (Fig. 37). Still others appeared to be pedunculated simulating small golf balls sitting on tees (Fig. 36).

Kudu.—Cysts were found in the jugular veins of one out of eight kudu.

Microscopic findings

Blue Wildebeest.—In the group of blue wildebeest initially examined, cysts were found attached to the intima of the jugular veins, especially over the valves. Some were attached by rather delicate fibrous strands (Fig. 2), while others had a more sessile attachment. One cyst was found in the wall of a branch of a pulmonary vessel (Fig. 33). Additional cysts were observed microscopically at other sites in blue wildebeest subsequently examined. These included tendon (Fig. 32), a vein of the pampiniform plexus (Fig. 30) and a vein on the posterior aspect of the globe of the eye, probably the ophthalmic vein (Fig. 34). There was little cellular response at any of these sites. In one of the jugular veins examined, there was quite an appreciable cellular response to one of the cysts. Around the base of the cysts, embedded in the intima, there were numerous cells with confluent cytoplasm and ovoid or elongated nuclei, which were round on cross section (Fig. 31). Somewhat similar cells were found between the media and adventitia of the vein. The other feature of interest was that at this point in the vessel wall the media was vascularized as though the inflammatory reaction in the intima around the cyst was supplied with blood by these small vessels. Many cysts attached to the intima and protruding into the lumen were either covered by endothelium alone, or additionally by some underlying fibrocytes. In the capsule of one lymph node a mineralized cyst with the remnants of its hyalinized wall was seen protruding into the lumen of a vessel.
Impala.—In the material from impala most of the cysts had caused no visible host response, but some had provoked a granulomatous reaction in various veins. Such reactions were seen around viable cysts (Fig. 46) as well as those appearing dead. In one cyst the necrotic contents were mineralized. The cyst was surrounded by a zone of epithelioid and multinucleated giant cells which, in turn, was surrounded by a fibrous capsule infiltrated with small round cells and covered by endothelium (Fig. 47). Around the viable cysts the granulomas were less mature, composed primarily of epithelioid cells without a fibrous capsule. In the intima of one of the veins of the pampiniform plexus there was practically nothing left except a fibrous capsule and a granulomatous reaction shaped like a cyst. The central portion of the reaction was filled with giant cells containing necrotic debris in their cytoplasm (Fig. 48).

There were several spermatic granulomas in an epididymis of one impala. The relationship of these to besnoitiosis has not yet been established, but there were bodies resembling individual free Besnoitia. There was interstitial epididymitis and localized vasculitis (Fig. 49). It appeared as though the necrosis of the epithelium of adjacent tubules resulted in the confluence of their lumina, which, with the accumulation of sperm and the resultant reaction, formed the spermatic granulomas.

There was little reaction to the few cysts seen in the dermis (Fig. 45) or in the walls of the vessels of the skin (Fig. 44). Some lymph nodes contained either viable appearing or non-viable cysts and sometimes both. They were most commonly present in the capsule or in association with the trabeculae (Fig. 43). Some, however, were in the cortex (Fig. 41) surrounded by lymphoid cells with quite a few eosinophiles intermingled. Some of the cysts were mineralized and remnants of the hyalinized cyst walls had often provoked a foreign body type of granulomatous response.

Kudu.—Cysts from the kudu were studied microscopically in squash preparations, but not in paraffin-embedded tissues.

DISCUSSION

The significance of the presence of Besnoitia cysts in the cardiovascular system of wild ruminants of Africa cannot be fully evaluated at present. The regularity with which they can be found, however, suggests that this infection is common among blue wildebeest and, in certain localities, impala. Finding the cysts in game served as a stimulus to study the cardiovascular system of cattle. Unfortunately naturally occurring acute cases of besnoitiosis showing anasarca were not available for study during this period. From the study of the three inapparent cases which were detected only by close examination of the bulbar conjunctiva, it is known that cysts occur in the blood vessels of this type of case as well.

In both antelopes and cattle, the ease with which the cysts are seen in the lymphatics and blood vessels with the unaided eye suggests that they were overlooked in the past, probably because the cardiovascular system was not adequately examined. This is somewhat surprising in view of the fact that in the original report of this disease Besnoit & Robin (1912) mentioned and illustrated (their Fig. 6) the microscopical detection of cysts within the walls of certain blood vessels. Amongst other things they discussed the possibility of rupture of the cysts with subsequent distribution of the organisms within the bloodstream and suggested that this could explain the generalization of the lesions over the cutaneous surface. Smith & Jones (1957) called attention to the presence of cysts in the walls of small blood vessels of cattle and horses with “globidiosis”. Schulz (1960) pointed out that the cysts
seem to have an affinity for areolar connective tissue and specifically mentioned that of the vascular system. He listed arteritis as having been seen, but made no specific reference to cysts within the vessel walls or lumina thereof. Pols (1960) made no mention of the cysts within blood vessels but presented information regarding the extracellular parasites in the blood one or two days after the height of the febrile reaction. He concluded that they were rare in the circulating blood and lymphatic tissue. In some of the blood smears studied, he found intracellular parasites in monocytes and occasionally in neutrophiles. It was further noticed that intracellular proliferating forms were in the cytoplasm of the host cells unencapsulated by any structure, but in chronic cases no evidence was found “that the cysts ruptured to liberate their contents on the surface of the skin or discharged them into the host’s circulation”. Vsevolodov (1961) described and illustrated the presence of the cysts in the lumina of lymphatics and a small vein.

The macroscopical detection or distribution of Besnoitia cysts in the cardiovascular system of domestic cattle, to our knowledge, has not been reported previously. Mention of the incidence and distribution of the cysts in the cardiovascular system of antelopes, however, was made in our preliminary report of this study (1965).

The general pattern of distribution of the cysts in the cardiovascular system of both antelope and cattle seemed to be more peripheral in nature and similar in several respects. The predilection sites were evidently the veins of all the legs, head and neck. A significant difference, however, between the topographical distribution in antelopes and cattle occurred in the jugular veins and the superficial veins of the head. In antelopes, particularly the blue wildebeest, the jugular veins were one of the most common sites, but this was not the case with cattle. On the other hand, in cattle the superficial veins of the head, especially those of the sides of the face, were heavily involved, but the corresponding veins were only mildly affected in antelopes.

In cattle, veins of the neck contained either a small number or no cysts and those of the thoracic and abdominal viscera contained none. Apart from the presence of numerous cysts in the jugular veins of antelopes, a few were often found in the anterior vena cava, the chambers of the right heart, the pulmonary artery and rarely the coronary veins. In one impala, cysts were quite numerous in the mesenteric veins.

Also in cattle the severity of the involvement of the facial veins at inlets of tributaries or at bends in the course of the veins, suggests that a slowing of the blood flow or stasis favours a site as a point of predilection. In support of this view is the fact that in lower segments of the facial vein, where flow was enhanced by gravity and the size of the lumen, there were few, if any, cysts (Fig. 6, 7). The presence of cysts on the valves in the heart and cusps in the jugular and other veins seems to lend further support to this view.

In addition to the macroscopically easily observable cysts, others were detected microscopically in all layers of both veins and small muscular arteries of cattle.

A point also appearing to be of significance is that, while it is true that Besnoitia cysts seem to have a predilection for connective tissues such as tendons and areolar tissue, they are most often near and sometimes apparently within small blood vessels. This was so obvious that it appeared as though the position of cysts depended upon the presence of the blood vessels and thus the number was proportional to the vascularity of such peripheral tissues. Apparent proof of this seemed to lie in the fact that in the dermis cysts were most numerous in the more vascular portion, the stratum papillare, and less common in the less vascular stratum reticulare. Further support for this view is the absence of cysts in avascular tissues such as mature cartilage,
the epidermis and the cornea. In one of the cases in cattle the cornea of one eye was vascularized and contained cysts. One may suggest that vascularization followed involvement by cysts, but at present it seems unlikely that they will occur in the cornea in the absence of blood vessels. Other sites seem to point to an affinity for small blood vessels peripherally. These include the vascular periosteum and perichondrium, the interstitial vessels of skeletal muscle and dense connective tissue, and the more vascular portion of the sclera. Conversely, vessels of the same size in visceras and connective tissue of the body cavities were either infrequently and less severely parasitized or free of cysts.

An important aspect of these observations is the possible bearing the presence of cysts in the cardiovascular system may have on the transmission of besnoitiosis. Some speculation along these lines is therefore justified. It has been shown that chronically infected bovines with numerous cysts in the skin may serve as a source of infection for mechanical transmission of the disease by tsetse flies, tabanids and stable flies (Bigalke, 1960, 1964). Although analogous chronic cases have apparently not been observed in antelopes, the possibility that they do occur cannot be excluded. Conceivably, such cases could more easily fall prey to lions and other large carnivores. Should chronic cases with skin lesions occur, mechanical transmission by insects would have to be considered. On the basis of the evidence obtained to date, however, it seems unlikely that this mode of transmission is operative in antelopes where cysts occur in many of the blood vessels and lymphatics, but are distinctly rare in the skin. Yet an apparently well-established cycle of transmission occurs, particularly in blue wildebeest. Perhaps a more logical assumption would be that in both cattle and antelopes a parasitaemia occurs at some stage of the disease, which permits blood-sucking arthropods to transmit organisms to susceptible animals, either mechanically or biologically.

Studies by Cuillé, Chelle and Berlureau (1936) and Pols (1954, 1960) have shown that transient parasitaemia occurs during the early febrile stage of the disease in cattle. It is not unreasonable to assume that this also applies to besnoitiosis of antelopes. Transient parasitaemia, however, would also not offer a completely satisfactory explanation for all problems in connection with transmission in antelopes.

Should some cysts in the blood vessels rupture and thus discharge their contents into the bloodstream, a source of viable organisms for transmission by a bloodsucking vector would be present. Besnoit & Robin (1912) suggested that there might be a periodic release of organisms. Cuillé et al. (1936), Cuillé & Chelle (1937) and Bigalke (1965), however, could find no evidence of parasitaemia in chronically infected cattle on subinoculating blood into susceptible animals. The finding of "agglutinated" organisms within the lumina of some blood vessels of case A in this investigation cannot be regarded as unequivocal evidence of a parasitaemia prior to death, because of the high probability of the release of the organisms from cysts in the process of cutting the fresh tissues. The hypothesis of a periodic release of organisms into the bloodstream, nevertheless, remains very attractive. Otherwise the cysts in the blood vessels of the antelopes are redundant, which seems highly unlikely.

Cysts were common in the lymphatics and blood vessels of the skin and subcutis of impala and in subcutaneous blood vessels of cattle. By carefully removing the surrounding fat and connective tissue from parietal lymph nodes, viz. prescapular of impala, many cysts were observed in the capsule and within the cortex on cut surface. This was confirmed microscopically. Although cysts were not seen in the lymph nodes of the bovine cases reported here, we have observed cysts in sections of lymph nodes from other chronic bovine cases which we had the opportunity of seeing.
Neuman & Nobel (1960) observed granulomatous changes in response to cysts in lymph nodes of a bovine case.

Leitao (1949) reported chronic lesions in the peritoneum and pleura in besnoitiosis of cattle. These were referred to as "gravel". Neither of the present bovine cases showed such lesions, but a few cysts were found in the pleura of a blue wildebeest.

Cysts in the bulbar conjunctivae have served as a useful sign for detecting otherwise inapparent bovine cases (Bigalke & Naude, 1962). The cysts present in the eyes of two of these cattle are apparently the first ones to be reported in the deeper structures of the eye of a ruminant. Unfortunately, eyes from acute cases were not available for study. One can imagine, however, that at an earlier stage the organism would cause an acute ophthalmitis. This would probably be accompanied by oedema, increased lacrimation, and photophobia.

The host responses to the cysts were lucidly described by Besnoit & Robin (1914) and other authors (Smith & Jones, 1957; Schulz, 1960). Schulz emphasized that the degenerating and/or ruptured cysts resulted in necrotizing inflammatory or mild granulomatous reactions in their vicinity. He observed that the intact cysts, though persisting for the lifetime of the host, elicit slight or no chemotactic response. The observations of the reactions of the host in the bovine cases reported here generally agree with his, with some exceptions, e.g. in skeletal muscle and in nasal mucosa. In the skeletal muscle of these cases the host response was by far the most consistent and intense reaction seen in any of the tissues. In contrast to the observations of Schulz, there were frequently leucocytic and granulomatous responses to apparently viable cysts as well as to the non-viable and degenerated ones. These observations are consistent with those of Besnoit & Robin (1914), who speculated that the leucocytic action could bring about premature death of the organisms.

Apart from the features common to the disease in cattle and in antelopes, a few significant dissimilarities were also observed. One of the most prominent differences concerns the position of the cysts in relation to the three histologic layers of the blood vessels. In cattle, all three layers contained cysts often accompanied by either subacute or chronic inflammation. In the antelope they were virtually always attached to the intima and reaction to them was usually absent with a very few exceptions. When reactions were present, however, those in the impala were more marked than in the blue wildebeest. Common sites of cysts, in the clinically apparent, chronic besnoitiosis cases of cattle, viz. dermis, subcutis, nasal cavity and bulbar conjunctiva, were seldom found to be the location of cysts in antelopes, particularly blue wildebeest. In the clinically inapparent cases of cattle, however, the distribution of the cysts more closely paralleled that in the antelopes.

The above factors seem to point to the host-parasite relationship being more in favour of the host in the antelope than in cattle. It is even more favourable in blue wildebeest than in impala, primarily because of the following: the more severe host response, the more frequent appearance of cysts in the subcutis and lymph nodes and the occasional occurrence of cysts in the myocardium, renal cortex, perinasal and periadrenal connective tissue and mesenteric vessels of impala.

In conclusion attention is drawn to further work which is in progress in order to determine the susceptibility of bovines and laboratory animals to these organisms and their relationship to B. besnoiti.
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SUMMARY

Following a previous preliminary note on besnoitiosis in South African impala, blue wildebeest and kudu, more details are reported on this disease in game. Particular attention is given to the distribution of the cysts and the associated macro- and histopathological changes. A comparative study with besnoitiosis in five bovines is outlined.

Some of the more salient findings of the investigation are the following: the apparent absence of clinical signs of the disease in the antelopes examined; the almost exclusive confinement of the cysts to the cardiovascular system of antelopes; the marked incidence of cysts in the subcutaneous lymphatics of impala and in the peripheral veins of the limbs of cattle and antelopes, the head of cattle and the jugular veins of antelopes.

The significance of Besnoitia cysts within the vessels is discussed in relation to the pathogenesis and transmission of the disease.

An incidental finding recorded is that of the nymphae of a specifically unidentified pentastome within the cardiovascular system and liver of blue wildebeest and kudu.

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REFERENCES


**BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM**


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**PLATE I**

**Fig. 1.**—Blue wildebeest. Small, round, white, glistening objects attached to intima of the jugular vein were concentrated at the valves.

**Fig. 2.**—Blue wildebeest. Photomicrograph of the objects in Fig. 1 which proved to be *Besnoitia* cysts. Notice attachment to valve.

**Fig. 3.**—Bovine. The common digital vein of a front limb opened to expose the numerous cysts. Notice valve (arrow).

**Fig. 4.**—Bovine. A closer view of the preceding vein (Fig. 3) shows the granular appearance of the intima. Notice the cysts concentrated on the V-shaped valve cusp (arrow).

**Fig. 5.**—Bovine. The cysts and accompanying phlebitis caused a thickening of the tributaries of the facial vein.

**Fig. 6.**—Bovine. Groups of cysts in the intima resulted in bulges into the lumen.
BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM

PLATE 2

Fig. 7.—Bovine. Though usually more numerous in the intima, cysts were present in all three layers of this vessel. Notice reaction in adventitia.

Fig. 8.—Bovine. In this small artery, the entire thickness of the media at one point was occupied by a cyst.

Fig. 9.—Bovine. Hyperplasia of the endothelial cells of the intima was often present.

Fig. 10.—Bovine. Cysts were often present in the outer tips of the dermal papillae.

Fig. 11.—Bovine. The stratum papillare of the skin showing an inflammatory response to viable-appearing cysts.

Fig. 12.—Bovine. Degenerated cyst in alveolar septum of the lung.
PLATE 3

Fig. 13.—Bovine. Cysts were numerous in the nasal mucosa.

Fig. 14.—Bovine. Cysts were numerous in the more vascular subcutis and stratum papillare of the dermis, being rarer in the stratum reticulare.

Fig. 15.—Bovine. This cyst had a sessile attachment to the intima.
BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM

PLATE 4

Fig. 16.—Bovine, skeletal muscle. Granulomatous response to a cyst containing organisms showing early degenerative changes.

Fig. 17.—Bovine, skeletal muscle. Host response to degenerative cysts.

Fig. 18.—Bovine. Cysts and accompanying reaction in the perineurium of a cutaneous nerve.

Fig. 19.—Bovine. Three viable cysts within the intima of a small vein.

Fig. 20.—Bovine. Around degenerated cysts in the nasal mucosa there were epithelioid and giant cells among many eosinophiles.

Fig. 21.—Bovine, skeletal muscle. Host response surrounding a cyst which shows liquefaction of the organisms.
BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM

PLATE 5

Fig. 22.—Bovine eye. Cysts within vascularized cornea.

Fig. 23.—Bovine. Cysts were numerous at the conjunctival limbus and accompanied by a round cell infiltrate.

Fig. 24.—Bovine. Cysts along the course of blood vessels between the sclera and conjunctiva.

Fig. 25.—Bovine. Cysts in the sclera were more numerous in the outer vascular episclera.

Fig. 26.—Bovine. An aneurysmic-like dilatation of a small blood vessel (arrow) in the sclera, interpreted as a cyst containing multiple large nuclei. Notice other cysts in course of the other vessel.

Fig. 27.—Bovine. The lamina fusca showing a few cysts.

Fig. 28.—Bovine. Cyst present in the ciliary body.

Fig. 29.—Bovine. Multiple cysts in the iris.
PLATE 6

Fig. 30. --Blue wildebeest. Cyst in a vein of the pampiniform plexus.

Fig. 31. --Blue wildebeest. Host response at the base of one of the parasitic cysts in the intima of a vein.

Fig. 32. --Blue wildebeest. Cyst in a tendon. Notice small blood vessel nearby.

Fig. 33. --Blue wildebeest. A cyst in a branch of a pulmonary vessel. Notice also the larvae of lungworm to the right.

Fig. 34. --Blue wildebeest. A blood vessel on the posterior aspect of the eye containing a cyst in its wall.

Fig. 35. --Blue wildebeest. Jugular vein with attachment of the cysts encircled by a slightly elevated zone.
**PLATE 7**

**FIG. 36.**—Impala. Some of the cysts on the surface of the intima of the pulmonary artery looked like small golf balls sitting on tees.

**FIG. 37.**—Impala. A large irregular-shaped object (arrow), compared to two cysts, is the result of the host response to another cyst.

**FIG. 38, 39.**—Impala. Close views of cysts in lymphatics of the skin.

**FIG. 40.**—Impala. The inner surface of the skin showing cysts in two lymphatic channels and one vein (arrow).

**FIG. 41.**—Impala. Cyst in the cortex of a lymph node.

**FIG. 42.**—Impala. Prescapular lymph node with cysts visible as white foci on the surface.

**FIG. 43.**—Impala. Photomicrograph of a cyst in the capsule of a lymph node. Notice round cell reaction.
BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM

PLATE 8

FIG. 44.—Impala. Cyst in the wall of one of the cutaneous veins.

FIG. 45.—Impala. Cyst in the stratum papillare of the dermis.

FIG. 46.—Impala. Granulomatous reaction around cyst with viable-appearing organisms.

FIG. 47.—Impala, blood vessel. Mineralized cyst surrounded by a granulomatous response.

FIG. 48.—Impala. Granulomatous response to remains of a cyst in a vein of the pampiniform plexus.

FIG. 49.—Impala, epididymis. Infiltration of leucocytes in the interstitium.