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DISSEMINATED PROTOTHECOSIS IN A DOG

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ABSTRACT

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This is a report on the clinical history and pathological lesions of a dog suffering from disseminated protothecosis due to *Protothecа zopfii*. Clinically, the dog was presented with bilateral conjunctivitis followed by blindness, deafness and posterior paresis. Pathological lesions were most severe in the eyes and consisted of subacute panophthalmitis with secondary posterior subcapsular cataract, posterior synechia, retinal detachment and microscopic evidence of glaucoma. The kidney, liver, brain, spleen and lungs were also affected. This is believed to be the first published account of protothecosis in mammals other than man in Africa. A review of the literature is included.

Résumé

LA PROTOTHÉCOSE DISSÉMINÉE CHEZ UN CHIEN

Les auteurs rapportent l'anamnèse et les lésions pathologiques d'un chien atteint de la protothécose disséminée, maladie due à Prototheca zopfii. *Les anamnestiques ont été: une conjonctivite bilatérale aboutissante à la cécité; la surdité et la parésie postérieure. Les lésions les plus graves au niveau des yeux ont été une panophthalmie subaiguë avec une cataracte capsulaire postérieure, une synéchie postérieure, un détachement de la rétine et des signes microscopiques d'un glaucome. Les reins, le foie, le cerveau, la rate et les poumons ont été également atteints. Les auteurs croient que ce compte rendu est le premier à signaler en Afrique la protothécose chez un mammifère autre que l'homme. Ce rapport comprend une revue de la bibliographie.*

INTRODUCTION

The purpose of this presentation is to document a case of disseminated protothecosis in a dog from Westville, Natal, Republic of South Africa. This is believed to be the first reported instance of proven tissue invasion by *Protothecа* in mammals other than man on the African continent.

Since the literature on this disease is recent and only fragmentary, a comprehensive review of what is available is thought expedient.

LITERATURE REVIEW

Protothecа is an acholoric genus of algae which was first isolated from tree sap by Kruger (according to Ashford, Ciferri & Dalmau, 1930), who described 2 species, viz., *Protothecа zopfii* Kruger, 1894 as the type species, and *P. moriformis* Kruger, 1894. He gave the generic characteristics as "fungi without mycelium and not reproducing by gemmation, with round, ovoid or elliptical sporangia in whose interior are successively formed septae, forming in their turn compartments which contain spores". He further stated that there was no connection with the Saccaromycales or Phycomycetes but they were like the algae, Protococcaceae. The genus was placed with the algae by Chodat in 1913 (according to Davies, Spencer & Wakelin, 1964). A new species, *P. portoricensis* (Ashford, Ciferri & Dalmau, 1930), and a variant *P. portoricensis* var. *trispors*, were isolated by Ashford *et al.* (1930). These isolates were from stools of two persons suffering from atypical and suspicious tropical sprue, respectively. In 1940, a further new species, *P. ciferri* Negroni & Blaisten, 1940, was isolated from a mange-like lesion of the skin of a potato. The organism produced local granulomas when injected into the testes of laboratory animals, and peritonitis when injected intravenously and intraperitoneally (Negroni & Blaisten, 1940). Other species include *P. wickerhamii* Tubaki & Soneda, 1959 which was isolated from tree flux (according to Fetter, Klintworth & Nielsen, 1971), *P. segbwema* Davies, Spencer & Wakelin, 1964, isolated from a human cutaneous lesion (Davies *et al.*, 1964), and *P. stagnora* Cooke, 1968 which was recovered from sewage treatment systems (according to Fetter *et al.*, 1971). The taxonomy of the genus was reviewed and several isolates of the above species were studied by Arnold & Ahearn (1972). They concluded that the genus *Protothecа* is distinct from *Chlorella* (the coloured algae) and lies between the algae and fungi. They considered *P. ciferri*, *P. portoricensis* and *P. segbwema* synonymous with *P. zopfii*, but identified a new species, *P. filamenta* Arnold & Ahearn, 1972, thereby reducing the recognized species to 5. Sudman & Kaplan (1973) studied *Protothecа* species by immunofluorescence and further substantiated the findings of Arnold & Ahearn (1972), but found little difference between *P. zopfii* and *P. moriformis*. They therefore limited the valid species to 4, viz., *P. filamenta, P. stagnora, P. wickerhamii* and *P. zopfii*.

The first proven and published account of spontaneous mammalian tissue invasion by the genus was reported by Davies *et al.* (1964). The patient was a rice farmer from Sierra Leone who had a lesion located on the medial surface and adjacent dorsum of his right foot, apparently of 5 years' duration before medical assistance was sought. Organisms were both cultured and seen in tissue sections. In the opinion of the authors the organism did not conform to any recognized species and a new name, *P. segbwema*, was given to it. A follow-up report by Davies & Wilkinson (1966) indicated that, despite treatment, the lesion was much more severe, and had spread to the sole of the foot and the femoral lymph nodes. Organisms were again both cultured and seen in histological sections.

In the U.S.A. in 1966, a cutaneous lesion of the thumb was reported by Gordon (according to Klintworth, Fetter & Nielsen, 1968), and these authors reported a 3rd human case which occurred as papulopustular cutaneous lesions on the lower leg. The

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patient, a white woman from North Carolina, U.S.A. was suffering from widespread metastatic breast carcinoma, diabetes mellitus and drug-induced Cushing's disease. Previously she had had oral candidiasis, two attacks of herpes zoster and repeated lower urinary tract infections with *Escherichia coli* and Aerobacter. The protothecal organism was identified as *P. wickerhamii* from its cultural characteristics. Three human cases were added to the literature by Tindall & Fetter (1971). Two of these were from Florida, U.S.A. and involved the olecranon bursa without any history of known skin injury; the other occurred in a physician from Mississippi, U.S.A., at the site of a minor surgical incision of the hand. The second recorded human case in Africa occurred as cutaneous lesions on the forehead and scalp of an African ditch digger from Benoni, Transvaal, Republic of South Africa (Mars, Rabson, Rippey & Ajello, 1971). The organism was seen in and cultured from skin biopsies and identified as *P. wickerhamii*. Material from this case was also examined by electron microscopy (Silk, 1973). Nosanchuk & Greenberg (1973) described another human infection of the olecranon bursa, again with no history of known skin penetration, in a white man in South Vietnam. They also mentioned 2 additional unpublished human cases affecting the olecranon bursa.

The first presumed natural tissue invasion in mammals other than man was a case of mastitis in a cow from which *P. zopfii* was isolated by Lerch (1952). In 1955, a single case of protothecal bovine mastitis was reported in England from which *P. portoricensis* was isolated (Ainsworth & Austwick, 1955). A *Prototheca* sp. mixed with *Staphylococcus aureus* and *Streptococcus agalactiae* was isolated from a case of bovine mastitis in South Africa in 1968 (Giesecke, Nel & Van den Heever, 1968).

Binford observed organisms, believed to be *Prototheca,* which were causing gastrointestinal lesions (according to Klintworth *et al.,* 1968) in a cow, and an epizootic of protothecal bovine mastitis occurred in a herd of 93 cows in Ohio, U.S.A. (Frank, Ferguson, Cross & Redman, 1969). Thirty cows were slaughtered because of their failure to recover after treatment and because of decreased milk production. Organisms identified as *P. segbwema* were cultured from the milk prior to slaughter and from the quarters, supramammary lymph nodes and uterus of 1 of the 4 cows examined after slaughter.

Three bovine cases which involved lymph nodes were reported by Migaki, Garner & Imes (1969). Besides lesions which were detected during routine meat inspection in federally inspected abattoirs, numerous organisms were seen histologically and were circumstantially identified as *P. segbwema* because of their morphological similarity to that species as described by Davies *et al.* (1964).

Two instances of protothecosis have been recorded in wildlife. The first, which occurred in a deer in Germany, was the first published case of proven tissue invasion by *Prototheca* in animals (Frese & Gedek, 1968). The organisms which were seen microscopically in gross lesions in the skin of the extremities, adjacent bone, regional lymph nodes, lower lip and nose were cultured and identified as *P. zopfii*. The other was considered to be a probable case of protothecosis by Sileo & Palmer (1973), since organisms, morphologically similar to *Prototheca,* were present in lesions in a frozen pelt of a beaver which had been trapped in Canada. Four nodules, 1–3 cm in diameter, pea-green in colour and well encapsulated, were described in this case.

Protothecal infection in a dog was first briefly mentioned by Saunders (1968) in his chapter on eye pathology and he described it as causing a purulent panophthalmitis. A 2nd case involving the eyeball of a dog was recorded at the Armed Forces Institute of Pathology, Washington, D.C., U.S.A. (Migaki *et al.,* 1969).

Van Kruiningen, Garner & Schiefer (1969) reported a generalized protothecal infection in a 9-year-old Boxer female suffering from chronic bilateral iritis, polydipsia, polyuria and bloody diarrhoea, with gross lesions in the eyes, myocardium and kidneys. Histologically, lesions with organisms were observed in the eyes, brain, liver, kidney and para-adrenal tissue, but little cellular response was present. Identification was based on organism morphology in tissue sections. Povey, Austwick, Pearson & Smith (1969) described another generalized infection which occurred in a 3½-year-old Springer Spaniel. Clinically, the infection was characterized by bloody faeces and posterior paresis. Later, vomiting, polydipsia, haematuria and proteinuria developed, and the dog died. Gross examination revealed haemorrhagic necrotic enteritis of the terminal colon and rectum, necrosis of 2 papillae of 1 kidney, diffuse pale areas in the myocardium and slight thickening of the right atrioventricular valve. Organisms morphologically identical to *Prototheca* were seen in sections of these tissues. Necrosis accompanied the organisms, but little cellular response was noted. Another case was reported in a 3-year-old Springer Spaniel which died after an 8½-month illness which was clinically evidenced as bloody diarrhoea and diagnosed as chronic colitis (Van Kruiningen, 1970). Mild inflammation of the small intestine and severe colitis with ulcerations were noted by the prosector. Unfortunately, he submitted only portions of the small intestine, caecum, colon and regional lymph nodes for microscopic examination. *Prototheca* was seen in the jejunum, ileum, caecum and colon, the severity of tissue changes being in increasing order. Most of the mucosa of the colon had been lost. Regional lymph nodes contained organisms with the largest numbers in the colic node. The only cellular inflammatory response was a few large mononuclear cells and focal increases in lymphocytes and plasma cells. Carlton & Austin (1973) reported bilateral ocular protothecosis in a 5½-year-old male Collie. The dog was blind when first examined and there was no history of previous disease. Only the eyes were examined histologically and the changes seen in them, viz., retinal detachment, posterior synechia, haemorrhage, atrophy of the iris, oedema and haemorrhage of the ciliary bodies and formation of a cyclitic membrane, were severe and chronic in nature. There were masses of organisms morphologically identified as *Prototheca* but little cellular inflammatory response.

The first recorded instance of a cutaneous infection by *Prototheca* in a dog was by Sudman, Majka & Kaplan (1973). This occurred in a 2-year-old Miniature Schnauzer which had legions on both ears and chronic otitis externa, a small granuloma on the dorsum of 1 foot and a severe nasal exudate. Biopsies and smears were taken and shortly thereafter the dog was killed and necropsied.

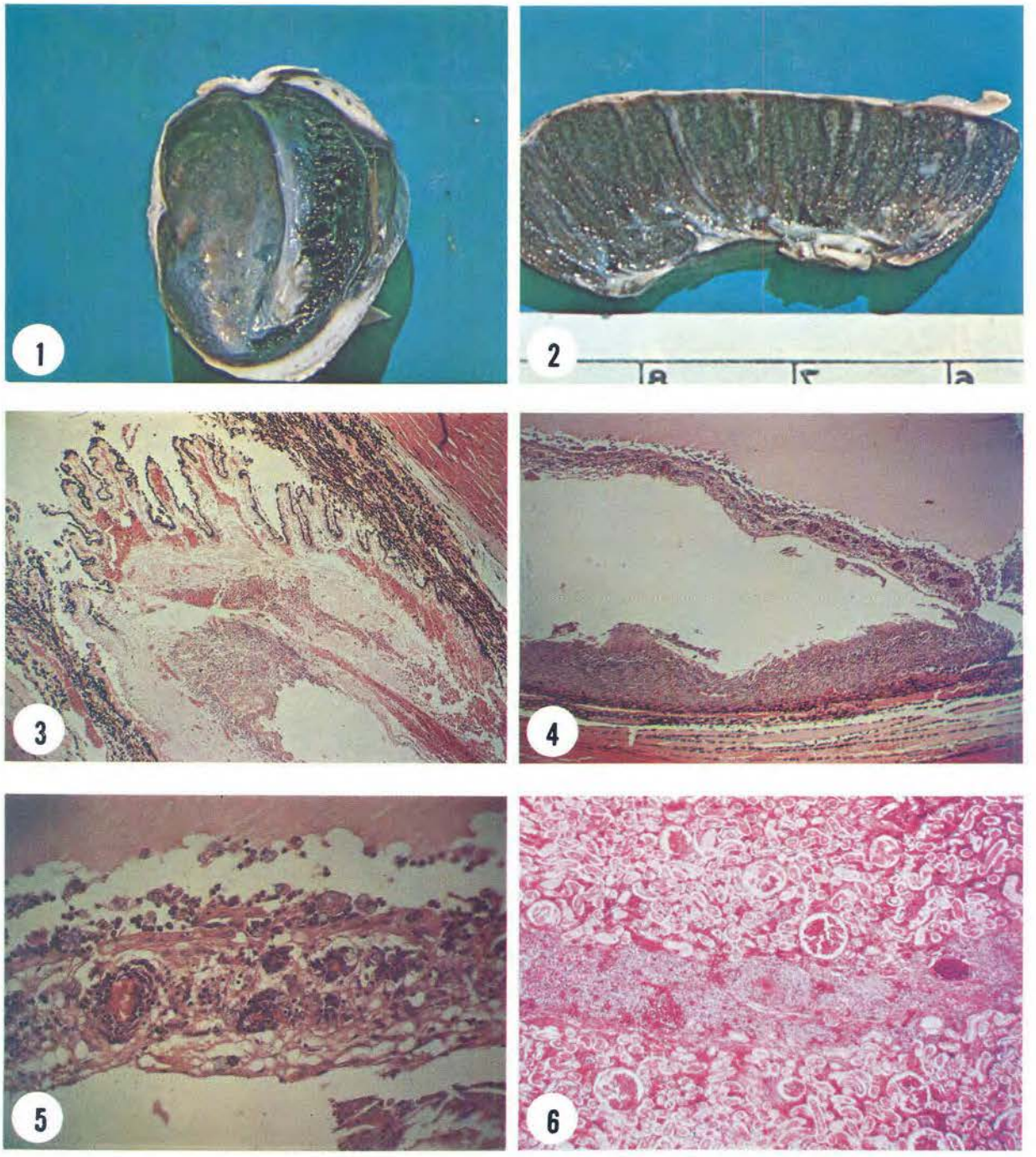

FIG. 1 Eye calotte. Tan-coloured foci of organism growth on the retina and 1 focus in the region of the iris and ciliary apparatus
FIG. 2 Kidney. Cortical streaks and necrotic foci caused by *Prototheca*
FIG. 3 A basophilic staining colony of *Prototheca* and severe oedema and congestion of ciliary processes. HE × 40
FIG. 4 Eye. Cellular cuffing of retinal vessels. Retinal detachment by scores of organisms. Severe pink-staining oedema of the pigmented portion of the choroid. HE × 40
FIG. 5 Retina. Plasma cell cuffing of retinal vessels and severe degeneration of retinal layers. Organisms and plasma cells between the vitreous body and retina. HE × 200
FIG. 6 Kidney. Small subacute infarct giving the kidney cortex the streaked appearance noted on gross examination. HE × 40

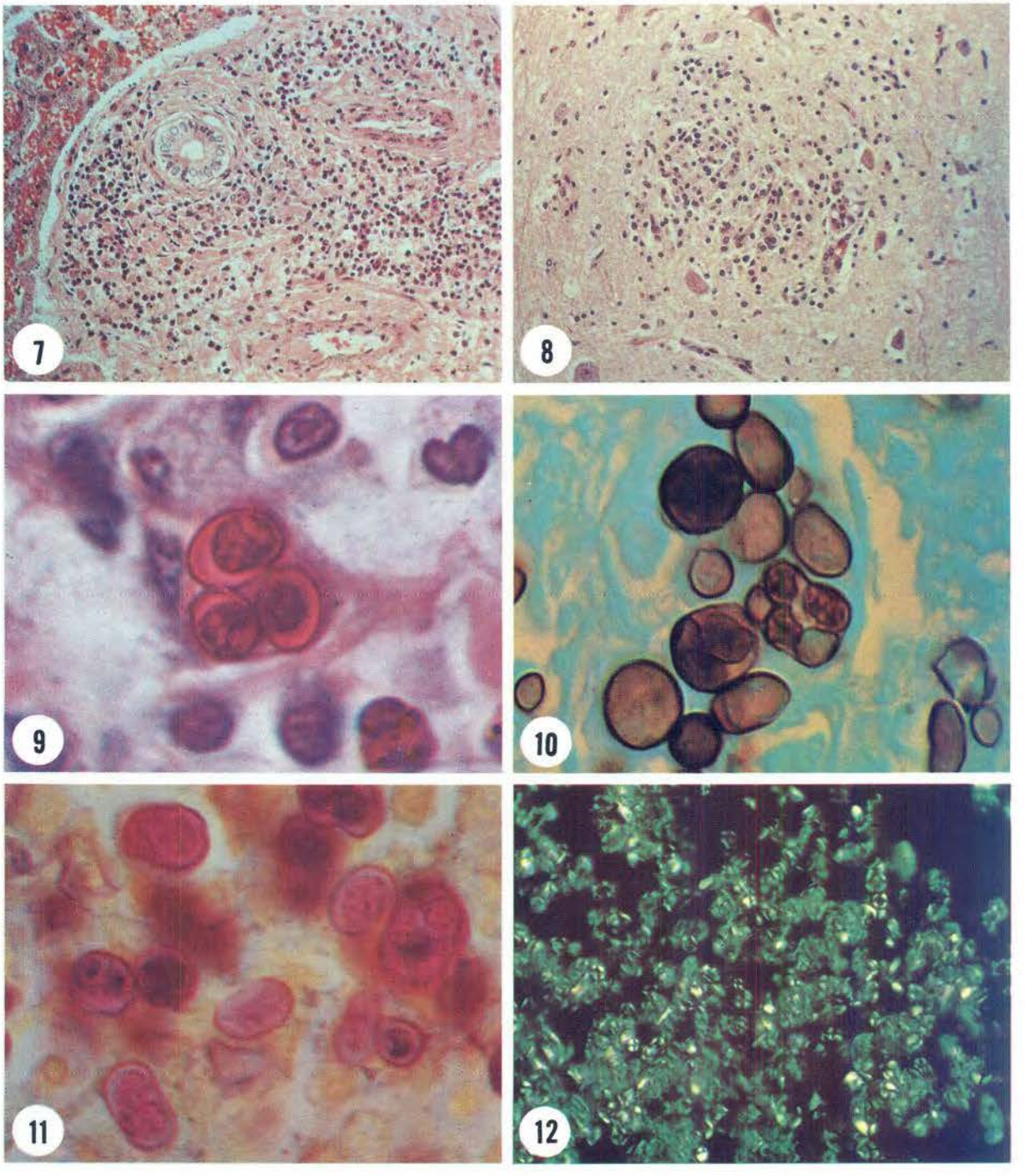

FIG. 7 Liver. Portal triad with fibrosis, cellular infiltration and organisms. HE × 100
FIG. 8 Brain. Typical microgranuloma found in all brain sections. HE × 200
FIG. 9 *Prototheca*. Three daughter cells visible. Walls and cytoplasm strongly PAS positive. PAS × 400
FIG. 10 *Prototheca*. Several organisms varying from unicellular to 1 containing 5 daughter cells. Walls heavily stained by silver. Cytoplasm of some cells more lightly stained. GMS × 1 000
FIG. 11 *Prototheca*. Walls stained by Schiff reaction. Cytoplasm of some organisms also stained. Gridley fungus stain × 1 000
FIG. 12 Thick, unstained, deparaffinized section viewed with polarized light. Note Maltese cross appearance of organism walls. × 400

Gross lesions which extended into the external auditory canals were seen on the ears. There was a small focal lesion on the dorsal thorax and the nasal mucosa was thickened and the turbinates haemorrhagic. *P. wickerhamii* was identified culturally and by the fluorescent-antibody (FA) technique described by Sudman & Kaplan (1973) on both fresh smears and formalin-fixed tissue. Microscopically, organisms were seen in the gross lesion, cervical lymph nodes and alveolar spaces of the lung. Examination of the brain as well as parenchymatous organs did not reveal any organisms and again the cellular response was not prominent. Moderate numbers of plasma cells were seen in the ear lesions, but inflammatory cell numbers were negligible in other tissues.

The most recent report of canine protothecosis was in a spayed female Collie with haemorrhagic diarrhoea and blindness (Buyukmihci, Rubin & De Paoli, 1975). This constituted the 5th case of canine protothecal eye infection in print. She was observed clinically for 19 days and then killed. Gross lesions were seen on the visceral and parietal peritoneum, in the myocardium, kidneys, liver, spleen, mesenteric lymph nodes and intestinal wall. Histologically, the reaction was described as granulomatous but there were, however, relatively few inflammatory cells.

History and Clinical Signs

A 6-year-old spayed Dobermann from Westville, Natal, Republic of South Africa, suffering from bilateral conjunctivitis, was presented for examination on 29 December 1973. After the condition had been treated with a neomycin/hydrocortisone eye ointment, the patient was seen again on 8 January 1974 when a marked hyphema was found in the left eye. The owner also commented that the dog seemed lame in her hind quarters. The eyes and hind limbs were examined under general anaesthesia and the pelvis was screened radiographically, but no abnormalities of the musculo-skeletal system could be detected. Treatment with eye ointment was continued and in addition a 5-day-course of a proteolytic enzyme was prescribed. On 17 January 1974, the dog seemed deaf, and on re-examination on 26 January 1974 when no improvement was noticed, ampicillin was prescribed. She was seen again 2 days later but still showed no improvement. A tentative diagnosis of a space-occupying intracranial lesion was then made. On 3 February 1974, she was blind and deaf, and seemed to have lost her sense of smell. Although she also had posterior paresis, she remained alert and had a good appetite. The dog was killed on 6 February and necropsied.

Materials and Methods

Portions of brain, kidney, liver, spleen and lungs were collected and placed in 10% formalin. One eye was incised, examined and placed in formalin. The other eye was placed in formalin intact and, following fixation, was further processed by being placed in 60% ethyl alcohol for a few days to facilitate incising and partially to restore its original colouration. The calottes were then removed and their internal structures examined. After a gross examination, the calottes and the central portion were embedded in paraffin wax for sectioning and the central portion was cut to the optic nerve with the microtome. Other tissues were embedded as usual and sectioned at 4–6 microns. Additional sections from all tissues were cut at 10–15 microns. Staining was by routine haematoxylin-eosin (HE), the periodic acid Schiff reaction (PAS), Gridley's fungus stain, Gomori's methenamine silver stain (GMS), Mayer's mucicarmine stain and Southgate's modification of Mayer's mucicarmine stain (Gurr, 1958). The thick sections were deparaffinized and coverslipped but left unstained for examination by polarized light. A portion of kidney fixed in 10% formalin and embedded in paraffin was submitted to the Mycology Division of the Department of Health, Education and Welfare, Center for Disease Control, Atlanta, Georgia, U.S.A. for species identification by the fluorescent antibody (FA) technique.

Results

Gross pathology

The eye which was incised and examined prior to formalin fixation had granular-like foci on the retina. Incision and examination of the formalin-fixed eye revealed that the vitreous body was white, hardened, and surrounded by a thin layer of brown material. The retina was detached and covered with a brown, granular-like material (Fig. 1), sometimes focal in nature and up to 0,5 mm in height and 2,5 mm in diameter. One tan focus 1,5 × 4,0 mm was seen in the region of the iris and ciliary apparatus (Fig. 1). White streaks were seen in the kidneys, extending from the capsule to the medulla (Fig. 2). There were also tan foci 0,5–1,5 mm in diameter in the cortex, especially at the corticomedullary junction.

Histopathological findings

The most severe lesions were in the eyes, and the corneas appeared normal. Microscopic description is limited primarily to the eye incised after fixation unless otherwise stated.

The conjunctival inflammation consisted of congestion and a mild diffuse infiltration of the lamina propria by plasma cells, melanin-bearing cells, a few lymphocytes and neutrophils. There were microscopic haemorrhages and congestion, and also mild inflammation of the sclera which was focal in nature and again comprised chiefly plasma cells with a few lymphocytes. The anterior chamber contained red blood cells (hyphema) and a proteinaceous exudate. Fontana's spaces were compressed and blocked by haemosiderin-bearing macrophages, plasma cells and red blood cells. Oedema was present and also severe congestion of the iris and focal aggregates of plasma cells which became more diffuse at the outer periphery extending posteriorly into the ciliary muscle. Occasional nodular thickenings made up of melanin-bearing cells were also seen. The posterior chamber contained a proteinaceous exudate and erythrocytes. Posterior synechia had also occurred. Oedema and congestion of the ciliary processes were very severe but cellular infiltrate was observed only in the ciliary muscles. A cyclitic membrane, composed of fibrin and proteinaceous fluid, had formed and contained a few large and small mononuclear cells, red blood cells and a large colony of organisms (Fig. 3) which were lightly interspersed by large and small mononuclear cells, neutrophils and nuclear debris. The choroid was congested and the suprachoroid portion, containing a protein-rich exudate, widely separating the melanocytes, was severely oedematous (Fig. 4). There was also slight haemorrhage and, in addition to the above, the choroid of the other eye also had a severe diffuse plasma cell infiltrate and a few organisms as revealed by special stains.

The retina was severely oedematous, causing disruption of the normal architecture. Plasma cell vascular cuffing was severe in certain areas (Fig. 5) and plasma cells were scattered throughout the various retinal layers. Below the inner nuclear layer and extending to the choroid, all retinal architecture was lost and the retina itself was detached at this point. This was due to scores of organisms and inflammatory products and cells (Fig. 4). In the first eye described, there was a fairly diffuse layer of giant cells, many of which contained acicular clefts and a very few contained organisms. Epithelioid cells and focal accumulations of plasma cells were also present. The large colonies of organisms had only a few plasma cells in their midst, but in the other eye plasma cells around organisms were striking in number and extended into the choroid.

There was a posterior subcapsular cataract, characterized by proliferation, swelling and vesiculation ("bladder cell" formation) of the subcapsular epithelium.

The vitreous body had numerous organisms on its periphery, but invading organisms and plasma cells were seen in only one microscopic field and these occurred in a typical single file manner.

Next in order of severity were the lesions in the *kidneys*. These were found primarily in the streaks and tan-coloured foci in the cortex seen on gross observation. In addition microscopic foci were scattered throughout the medulla. The cortical streaks were subacute infarcts (Fig. 6) which were infiltrated by plasma cells. Numerous organisms were also present and they extended into the kidney capsule overlying the infarcts. One glomerulus was packed with organisms, and Bowman's space contained proteinaceous exudate, a mixture of inflammatory cells and organisms. The Bowman's capsule was surrounded by inflammatory cells, chiefly plasma cells. Other lesions scattered throughout the cortex and medulla consisted of central foci of necrosis, organisms, plasma cells and usually haemorrhage. Organisms in the kidney were predominantly those with basophilic staining qualities and, when seen within tubules, often resembled the large hyperchromatic tubular nuclei associated with tubular regeneration.

The *liver* was congested and lesions were found in the portal triads (Fig. 7). There was a mild inflammatory cell infiltration consisting of plasma cells, neutrophils, lymphocytes and occasional epithelioid cells. Organisms were present but few in number. There also appeared to be an increased amount of mature collagenous tissue.

Brain sections included cerebral cortex, cerebellum and pons. Microgranulomas containing a few plasma cells, microglial cells and organisms were found in all the sections (Fig. 8). Vessels were sometimes seen in association with granulomas and perivascular cuffing by plasma cells was present.

The *spleen* exhibited no particular lesions, except for a few megakaryocytes, a small amount of haemosiderin and foci of small mononuclear cells, mainly lymphocytes, around splenic arteries. However, the PAS reaction revealed organisms in the lymphocytic aggregates and occasional solitary fragments of organism capsules and rarely an intact organism in the remainder of the spleen. These were both free and phagocytized.

The *lungs* were severely congested and a few organisms were found in alveolar septae in specially stained sections.

Description and Identification of the Organism

Organisms appeared as either unicellular, packeted or compartmental structures containing several spores or daughter cells or empty shrivelled wrinkled capsules. The capsules were lightly basophilic and refractile while there was a variation in the tinctorial qualities of the cytoplasm and nuclear material. Most unicellular organisms had pronounced basophilic, foamy cytoplasm with a distinct border and a small and deeply basophilic nucleus, the combination resembling a host cell nucleus with a prominent nucleolus. Some unicellular organisms had eosinophilic cytoplasm the nuclei of which were somewhat indistinct and more eosinophilic to amphophilic. In others the cytoplasmic granules were very slightly eosinophilic but highly refractile, and no nucleus could be seen. Many organisms, particularly multicellular ones, which were especially plentiful in the eyes, showed little or no evidence of intracellular material. The cell walls were strongly stained by the PAS reaction, GMS and Gridley's fungus stains (Fig. 9, 10, 11). Unstained thick sections viewed with polarized light showed that the walls were highly birefringent, with a Maltese cross appearance (Fig. 12). The cell walls were not stained by Mayer's mucicarmine, but Southgate's modified mucicarmine stained the capsule a light rose colour.

The cytoplasm in many cells was strongly PAS positive (Fig. 9, 11) and, when stained by GMS, varied from light- to very dark-brown (Fig. 10). Organisms which stained in this manner were invariably the same as those which had deeply basophilic cytoplasm and nuclei when seen in HE stained sections. Morphologically and histochemically the organisms were identified as a *Prototheca* sp.

Identification of species

The organism was stained by the FA conjugate specific for *P. zopfii* but not by conjugates for other *Prototheca* spp.

Discussion

The number of documented cases of protothecosis is small and the sources and reasons for infection are still conjectural, being based on circumstantial evidence. Some patterns appear to be emerging, however, and there appears to be sites of predilection for entrance and further multiplication. Since the first reported case of protothecosis in man was that of a rice farmer (Davies *et al.*, 1964) cutaneous lesions have been associated with continuous exposure to non-potable water or moist conditions. The woman from North Carolina with leg lesions shaved her legs with water from a tank overgrown by scum, the physician from Mississippi repeatedly exposed the infected surgical incision on his hand when cleaning tropical fish aquaria (Tindall & Fetter, 1971), while the African from South Africa with forehead and scalp lesions was a ditch digger (Mars *et al.*, 1971), and, therefore, continuously exposed to moist earth.

In cattle, 4 reports have dealt with mastitis (Lerch, 1952; Ainsworth & Austwick, 1955; Giesecke *et al.*, 1968; Frank *et al.*, 1969) and, although no suggestions were made that water was the source of infection the possibility should not be overlooked. It is not unusual for cattle to wade into water above their udders while drinking and cooling.

Water exposure undoubtedly occurred in the suspected *Prototheca* infection found in a beaver pelt by Sileo & Palmer (1973).

In the case under review, the owner of the dog revealed that his house was situated in a valley with a stream passing through part of the property and often causing marshy conditions. The fact that the dog had a habit of drinking water from dripping taps and any puddle in her vicinity may have been an indication of polydipsia.

Clinical signs in 4 other dogs suggest the route of infection was alimentary. These had bloody faeces or intractable diarrhoea (Povey *et al.*, 1969; Van Kruiningen *et al.*, 1969; Van Kruiningen, 1970; Buyukmihci *et al.*, 1975). Binford saw gastrointestinal lesions containing *Prototheca* in a cow (according to Klintworth *et al.*, 1968). One of the bovine cases described by Migaki *et al.* (1969) had a severe protothecal peritonitis and they suggested leakage of gastrointestinal contents into the peritoneal cavity as the source of infection. A case of chlorellosis (coloured algae) in a lamb had a distribution of lesions which were indicative of gastrointestinal entrance and portal dissemination (Cordy, 1973). Although the present case had no history of gastroenteritis, the drinking habits and lesions in the portal triads suggested alimentary infection to the liver via the portal system. Haematogenous dissemination following portal entrance was evidenced by organisms in the choroid and retina of the eye, vascular oriented microgranulomas in the brain, embolic nephritis and organisms filtering through the spleen and lungs.

Van Kruiningen *et al.* (1969) described their dog as having a gait suggestive of arthritis in one hind leg, and the dog reported by Povey *et al.* (1969) seemingly dragged its hind limbs. In the clinical course of this case, the dog progressed from an apparent lameness of the hind quarters to complete posterior paralysis in less than a month. Unfortunately, neither spinal cord, regional nerves nor vessels were examined in any of these dogs. Negative radiological findings in the present report indicate that paralysis was not related to skeletal involvement and microscopic lesions throughout the brain provide evidence that there were possible cord lesions.

Nosanchuk & Greenberg (1973) summarized 6 documented and 2 unpublished human protothecal infections. Five of these involved the olecranon bursa and they speculated that protothecal olecranon bursitis would become increasingly recognized as a distinct clinicopathological entity in man.

Eight descriptions or references to protothecosis in dogs have preceded this one. (Saunders, 1968; Migaki *et al.*, 1969; Povey *et al.*, 1969; Van Kruiningen *et al.*, 1969; Van Kruiningen, 1970; Carlton & Austin, 1973; Sudman *et al.*, 1973; Buyukmihci *et al.*, 1975). Five of these reports included ocular involvement (Saunders, 1968; Migaki *et al.*, 1969; Van Kruiningen *et al.*, 1969; Carlton & Austin, 1973; Buyukmihci *et al.*, 1975). Van Kruiningen *et al.* (1969) commented on ocular involvement in dogs, suggesting that future cases of protothecal ophthalmitis could be anticipated and this has happened. It appears that *Prototheca* has a definite affinity for the olecranon bursa in man and the eye in dogs, possibly because these sites provide a favourable environment. Another interesting possibility connected with the tremendous proliferation of the organism in the eye of the dog is that, even though the *Prototheca* is colourless and has lost its ability for photosynthesis, it may still be photophilic and it therefore reproduces or performs other physiological functions better in the presence of light. However, in support of such an hypothesis one would expect to find growth more pronounced in the anterior portions of the eye and systemic infections in other animals should also result in eye involvement. Unfortunately, in other spontaneous animal cases with a chance of generalization, eyes were not examined. Klintworth *et al.* (1968) were unable to infect rabbits by instilling organisms in the anterior chamber of their eyes.

The histopathological diagnosis of algae infection is not difficult once it is accepted that these organisms have the ability to invade and reproduce in mammalian tissue. They are large, easily visible and have morphologic characteristics which are readily recognized as being different from the pathogenic yeasts and fungi. Sudman (1974), however, stated that typical endosporulation does not always occur and *Prototheca* then could be confused with pathogenic fungi such as *Blastomyces, Cryptococcus, Paracoccidioides, Coccidioides* and *Rhinosporidium*, and the organism *Pneumocystis carinii*. In HE sections of this case the more basophilic unicellular examples were more apt to be confused with protozoans such as amoebae or certain stages of coccidia.

To date all but one algal infection have been attributed to the genus *Prototheca* by cultural and/or microscopic appearance. Cordy (1973) described a case of algal infection in a slaughtered lamb which had bright-green necrotic lesions in the liver and hepatic lymph nodes. On the basis of gross colouration, he concluded that the organisms belonged to the genus *Chlorella* and termed the condition chlorellosis. Other examples which may have belonged to a genus other than *Prototheca* were 2 bovine cases (Migaki *et al.*, 1969) with lymph nodes grossly described as having a green colour. In addition organisms from these could not be stained by the FA conjugates for *Prototheca* spp. (Sudman & Kaplan, 1973).

Finally, the lesions found in a frozen beaver pelt were pea-green in colour (Sileo & Palmer, 1973) and identification was based solely on morphology. Because most animal cases will only be diagnosed on the basis of histopathological examination, the development of an FA test for formalinized material (Sudman & Kaplan, 1973) is a useful tool for genus and species identification and was necessary for the species identification in this case.

In species other than dogs the inflammatory reaction has been shown to be predominantly necrotic with a granulomatous response. Except in 1 case, the cellular response in dogs has been minimal, consisting, as it does, of a few small and large mononuclear cells. The present case conformed to that general pattern; however, there were foci in the eyes where plasma cells were plentiful and the kidneys had moderate inflammatory, chiefly plasma cell infiltrates. The large numbers of giant cells seen between the choroid and remaining layers of the retina contained typical cholesterol clefts and fat necrosis and were obviously the products of retinal degeneration as described by Hogan & Zimmerman (1962). Only an occasional organism was found in this reaction.

Treatment for protothecosis following diagnosis has been limited to man and experimentally-produced bovine mastitis. In man, the organism has been

resistant to an array of antibiotic and antifungal drugs and chemicals administered both locally and systemically (Nosanchuk & Greenberg, 1973). Mars *et al.* (1971) reported some success with potassium iodide therapy but surgical removal has been the only effective curative method in other human cases.

Experimentally-produced protothecal bovine mastitis was treated by udder infusion with nystatin, streptomycin-penicillin, neomycin, hydrocortisone and by intravenous injection of sulphonamides, oxytetracycline and a combination of penicillin, dihydrostreptomycin, dexamethasone, chloropheniramine and oxytocin, but the number of organisms shed was not reduced. When protothecosis is spread through a dairy herd, it is probably best controlled by improved sanitation and the elimination of infected animals (Frank *et al.*, 1969).

For economical reasons effective treatment in animals is not important except where there is the herd problem of bovine mastitis. However, in man, with the threat of generalization occurring and the probability of eye involvement, it is essential that investigation into treatment other than surgery should be undertaken. It seems justified, therefore, to arrive at such treatment using experimentally-infected animals.

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