PNEUMOCYSTOSIS IN A DOMESTIC GOAT*

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ABSTRACT

McCONNELL, E. E., BASSON, P. A. & PIENAAR, J. G. Pneumocystosis in a domestic goat. *Onderstepoort J. vet. Res.*, 38 (2), 117-126 (1971).

A fatal case of an infection by the parasite, *Pneumocystis carinii* Delanoë & Delanoë, 1914, is described in a young domestic goat. The disease was manifested as a severe diffuse interstitial pneumonitis accompanied by filling of the alveolar air spaces by large numbers of organisms. Light and electron microscopic studies revealed the parasite to be identical to previously described cases in man and other animals. This is apparently the first case recognized in an animal in Africa.

INTRODUCTION

Pneumocystosis is a disease of man and animals characterized by interstitial pneumonia and filling of the terminal air spaces by the parasite, *Pneumocystis carinii* Delanoë & Delanoë, 1914.

P. carinii was first observed by Carlos Chagas (1909) in the lungs of guinea pigs during his studies on American trypanosomiasis, caused by *Trypanosoma cruzi* Chagas, 1909. *P. carinii* was subsequently observed by Chagas (1911) in a man who had died of Chagas' disease. Another Brazilian scientist, Carini (1910, according to Carini & Maciel, 1914) discovered the parasite in rats, also during studies on *T. cruzi*. Shortly thereafter two French scientists, Delanoë & Delanoë (1914), described similar organisms in the lungs of rats.

Wenyon (1926) in describing *P. carinii* as a sporozoan parasite also mentioned that the organisms had been observed in numerous mammals including dogs, guinea pigs, mice, rats, rabbits, goats and sheep. The accounts of the disease in ruminants, however, are difficult to document as they apparently were published as references to personal communications only. Since then reports of the disease in domestic animals have been limited to pigs (Bondy, 1958, according to Kučera, Slesingr & Kadlec, 1968; Kučera *et al.*, 1968). The latter report is especially significant in that the authors specifically searched for *P. carinii* in pigs which had respiratory distress. Of 24 affected animals three had pneumocystosis. These pigs had light infections but all had positive complement fixation reactions to the organisms. Barta (1969) has proved this reaction to be a relatively accurate method of establishing recent infections in man. He found complement fixing antibodies in 75 to 85 per cent of clinical cases and in 90 per cent of patients who died from the disease, but in only 3 per cent of the control group.

The second report of the disease in man was described by Van der Meer & Brug (1942), who diagnosed it in three infants in the Netherlands. In the course of their studies they also found the parasites in lung smears from guinea pigs, white rats, wild rats, white mice and wild mice.

Even though it may appear that pneumocystosis was not a problem during the period following its first recognition until 1942, there were isolated reports in Europe of a disease entity referred to as "interstitial plasma cell pneumonia". Gajdusek (1957) stated that most of these cases were caused by *P. carinii*. The organisms are, in fact, clearly noticeable in some excellent photomicrographs in a report by Ammich (1938).

The disease is apparently most important when it occurs in epidemic form as it did in central Europe, especially Czechoslovakia and Yugoslavia (Vaněk & Jirovek, 1952). Gajdusek (1957) stated that in 1954, during a tour of a hospital in Ljabljana, Yugoslavia, he found a special isolation ward set aside for infants suffering from pneumocystosis. He also reported that over 700 cases had been observed in Switzerland between 1941 and 1948. In these epidemics, in contrast to other areas of the world where it is sporadic (Le Clair, 1967), the disease is found usually in infants, especially in premature babies and infants 6 to 16 weeks of age who suffer from debilitating disease. Mortality is in the range of 20 to 25 per cent regardless of the therapy (Sheldon, 1959). The incubation period is 20 to 30 days (Faust & Russell, 1964) and the duration of the disease usually 4 to 6 weeks (Gajdusek, 1957). Kučera (1967) observed a direct relationship between the disease and environment and poor hygiene. He maintained that the disease is probably a zoonosis since the epidemics coincided with cyclic increases of the field vole [*Microtus arvalis* (Pallas, 1779)] populations. Removal of sick infants from such areas significantly decreased the incidence of the disease. The relationship between the infection in man and animals was supported by Sheldon (1959), who produced pneumocystosis in rabbits by intranasal inoculations of lung material from a fatal human case. The rabbits had been pre-treated with cortisone and antibiotics. In a recent report Frenkel, Good & Schultz (1966) pointed out that while *P. carinii* organisms from different species are microscopically identical, immunological differences suggest that they may be host specific.

It should be emphasized that the disease in man is not restricted to infants. In adults it is invariably secondary to some predisposing disease or the administration of some chemotherapeutic agent that interferes with the immune response. Examples include leukaemia (Hamlin, 1968), primary agammaglobulinaemia (Burke, Krovetz & Good, 1961), and chemotherapy associated with organ transplants or malignant disease such as long-term corticosteroid therapy, irradiation and administration of cytotoxic drugs (Rifkind, Faris & Hill, 1966). Frenkel *et al.* (1966) suggested that corticoid

preparations have a much greater capability for predisposing rats to pneumocystosis than other immunosuppressants or cytotoxic agents.

Pneumocystosis has been recognized in Africa on two occasions to date. The first report was by Pepler (1958), who diagnosed it in a 3-month-old child in South Africa, and the second by Kibel & Page (1967), who encountered the condition in a 3-month-old infant in Rhodesia. To our knowledge, however, it has not been reported previously in African animals. Geographically, beside southern Africa, Le Clair (1967) stated that the disease has now been observed in continental Europe, Great Britain, the United States of America, Canada, South America and Australia.

Pertinent reviews on pneumocystosis in man are by Vañek & Jirovek (1952), Gajdusek (1957), Sheldon (1962), Le Clair (1967) and Robbins (1968) and in animals by Timofeev, Petrouskii, Kazakov. & Zabloskii (1966) and Kučera (1967).

The purpose of this presentation is to document a case in a goat. This is believed to be the first observation of the disease in a ruminant on the African continent.

MATERIALS AND METHODS

On December 10, 1969 a 4-month-old female Boer goat was forwarded to the Veterinary Research Institute, Onderstepoort, for post mortem examination. It originated from a farm in the Vaalwater area of the Transvaal. The animal was *in extremis* at the time of shipment to the laboratory, and died en route shortly before arrival; no further history was available. A necropsy was performed and specimens were fixed in 10 per cent buffered glucose formalin. These tissues were processed in a routine manner, viz., embedded in paraffin wax and sectioned with a sliding microtome at 3 to 6 μ thickness. Sections were stained with haematoxylin and eosin (HE), Giemsa, Gram's stain (MacCallum & Goodpasture), Gomori's methenamine silver (GMS), Feulgen-Schiff-naphthol yellow (FSNY) (Himes & Moriber, 1956) and Hotchkiss' periodic acid-Schiff reaction (PAS) were employed as special staining techniques. Specimens of lung, liver and spleen were cultured on routine bacteriological media.

Portions of lung were examined by electron microscopy (EM). Blocks for EM were cut from the formalin-fixed lung, postfixed in 2 per cent osmium tetroxide in Millonig's buffer (Millonig, 1961), dehydrated and embedded in Araldite* (Luft, 1961). Sections 1 to 2 μ thick were cut, stained with toluidine blue pyronin (Ito & Winchester, 1963) and examined under a light microscope for the selection of appropriate areas for ultra-thin sectioning. The thin sections for EM were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963).

Macroscopic findings

The goat was in fairly good condition. The autopsy examination took place less than 1 hour after death.

There was a marked hydrothorax and hydropericardium and the lungs retained their contour when the thorax was opened. They were reddish-pink and the interlobular septa were distended by clear straw-coloured fluid. The lungs were fairly firm and foamy material was present in the trachea and bronchi. The appearance suggested diffuse bilateral interstitial pneumonia with pulmonary oedema.

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The liver was large and soft and had a mottled reddish-brown surface with diffusely scattered small slightly raised foci. The prescapular, mediastinal, mesenteric and periportal lymph nodes were prominently enlarged and oedematous, especially the mediastinal node. The spleen was enlarged. The mucosa of the abomasum contained several petechiae and ecchymoses. The intestines were congested and contained some blood. The kidneys were light brown and slightly enlarged. A slight increase of clear synovial fluid was apparent in several joints.

Microscopic findings

Microscopic examination of HE stained sections of the lung revealed a diffuse interstitial pneumonitis [Plate 1 (1 and 2)], with histiocytes and lymphocytes predominating. A small but increased number of neutrophiles was also present. No plasma cells were observed. Many of the alveoli and terminal bronchioles were filled or lined by a foamy eosinophilic coagulum [Plate 1 (2)] containing faintly staining clusters of organisms [Plate 1 (3 and 4)]. By light microscopy the predominent form of the organism was either circular (5 to 7 μ diameter) or oval (8 to 10 $\times$ 4 to 6 μ) within which there was a well-defined vacuole containing a small (1,0 to 1,5 μ diameter) basophilic nucleus. Approximately 20 to 30 per cent of the organisms were cysts which were composed of groups of 2, 4, 6 or 8 tightly packed intracystic bodies surrounded by a spherical (8 to 10 μ diameter) capsule [Plate 1 (3 and 4)]. Some cysts appeared empty. Occasional organisms were present within the alveolar macrophages but none were found in the interstitium. A few organisms were present in the mucocellular debris of some of the larger bronchioles. The GMS technique stained the cyst walls intensely [Plate 1 (5 and 6)]. This intense staining of the wall of the organism, however, obscured internal structures. An occasional cyst appeared to have a darker staining area (pore) on its surface. This technique also demonstrated a few crescent-shaped cysts [Plate 1 (6)]. Giemsa was more useful than HE in differentiating the intracystic bodies. With PAS the entire foamy mass was positive but this reaction obscured internal details. Gram's stain and FSNY did not stain the organisms.

Lesions in other organs included a marked diffuse lymphocytic infiltrate in the myocardium, especially near the endocardium. There was also a mild hydropic degeneration of the myocardium, accompanied by mild fibroplasia. A moderate number of Anitschkow cells were seen. The liver contained areas of marked centrilobular lytic necrosis, midzonal fatty degeneration and congestion. A narrow band of lymphocytes and neutrophiles was found at the edge of the necrotic areas. The spleen was mildly congested. The Malpighian corpuscles were numerous and moderately active.

The mononuclear infiltrate of the lamina propria in both the small and large intestine was reduced and consisted almost entirely of lymphocytes. Only an occasional plasma cell was found. The Peyer's patches were few in number and were smaller than normal. The lymph nodes were markedly hyperplastic and oedematous and had large lymph follicles. The surrounding cortex was heavily populated mainly with large and very small lymphocytes whereas the medullary cords contained the large ones only and no plasma cells. A small number of neutrophiles were observed in the medullary sinuses.

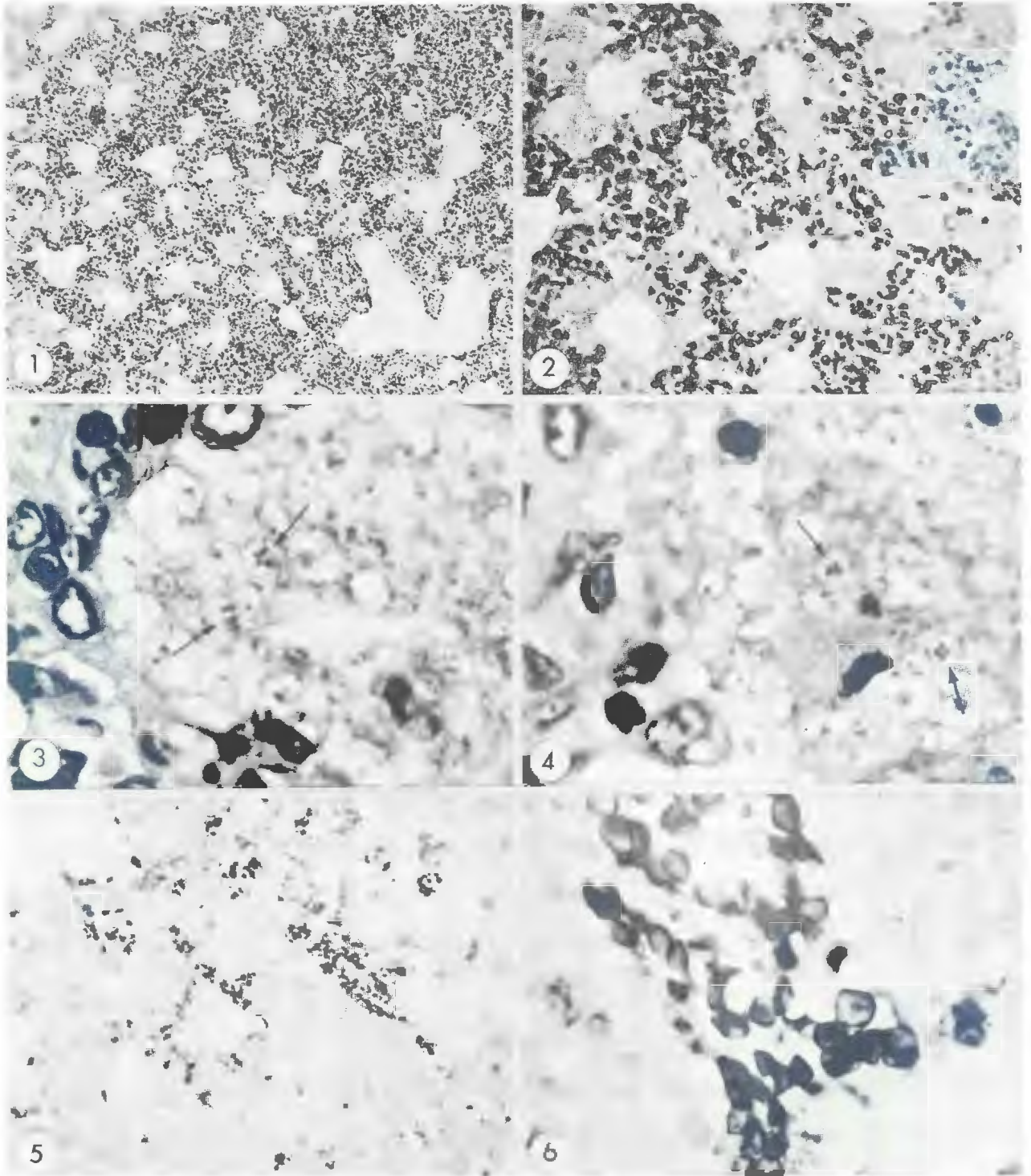The interstitium of the renal cortex contained a few foci of lymphocytes and the glomerular capillaries con-

PLATE 1   1. Diffuse interstitial pneumonitis. HE × 75.   2. Mononuclear infiltration of the pulmonary interstitium and foamy material (parasites) filling and lining the alveolar spaces. HE × 375.   3 and 4. Higher magnification of a portion of 2 showing numerous parasites which comprise this material. Note the cyst forms with multiple intracystic bodies (arrows). HE × 750.   5. Silver stain showing a significant proportion of the parasites as being argentophilic. GMS × 375.   6. Higher magnification of a portion of 5 showing several cysts, including several crescentshaped forms. GMS × 750.
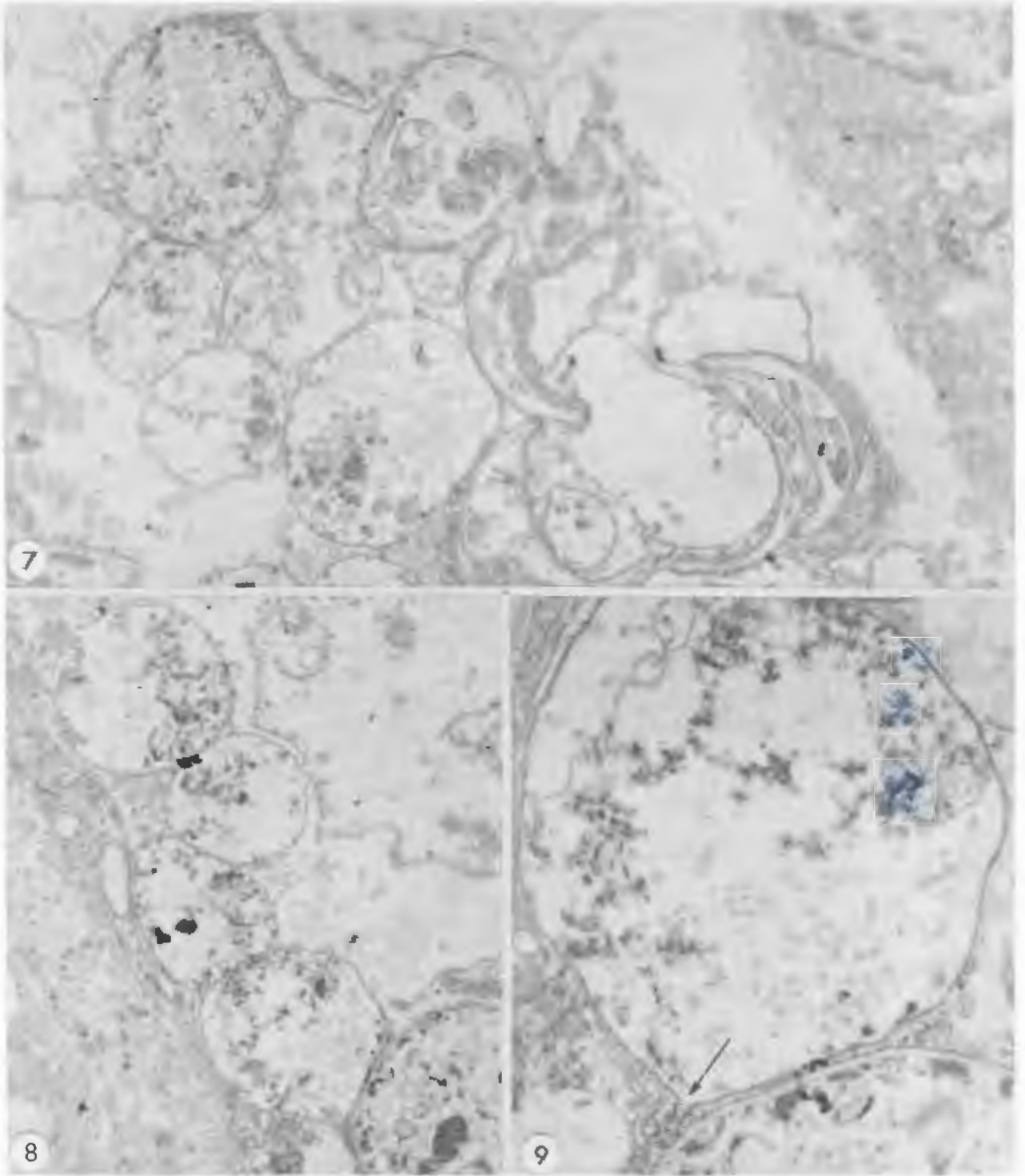
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PLATE 2   7. A group of large trophozoites, one cyst (middle top) containing intracystic bodies and empty collapsed crescent-shaped cysts in an alveolus. × 9 000.   8. Large trophozoites lying closely against the epithelial lining cells. × 7 500.   9. A large trophozoite showing a filopodium (arrow). × 24 500
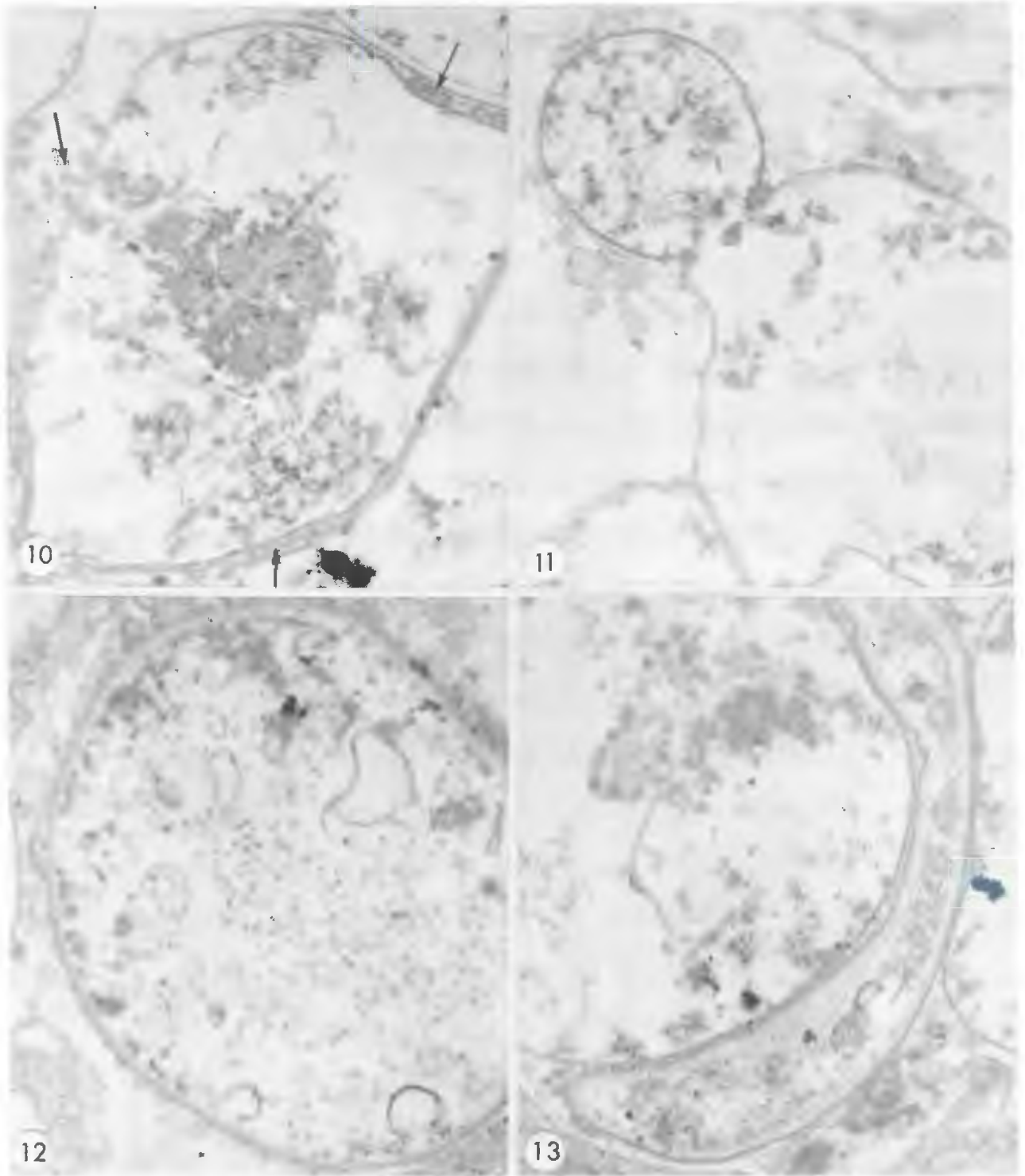
PLATE 3   10. Large trophozoite containing a mass of nucleoplasm that is not surrounded by a nuclear membrane. Numerous filopodia are present (arrows) at periphery of organism. × 24 500.   11. Cytoplasm of two adjoining trophozoites communicating through an opening in the pellicle. × 20 800.   12. Large oval-shaped trophozoite with thickened wall. Note large numbers of glycogen-like particles and vacuoles in cytoplasm. × 24 500.   13. Collapsed crescent-shaped thick-walled cyst containing cytoplasmic debris. × 23 300.
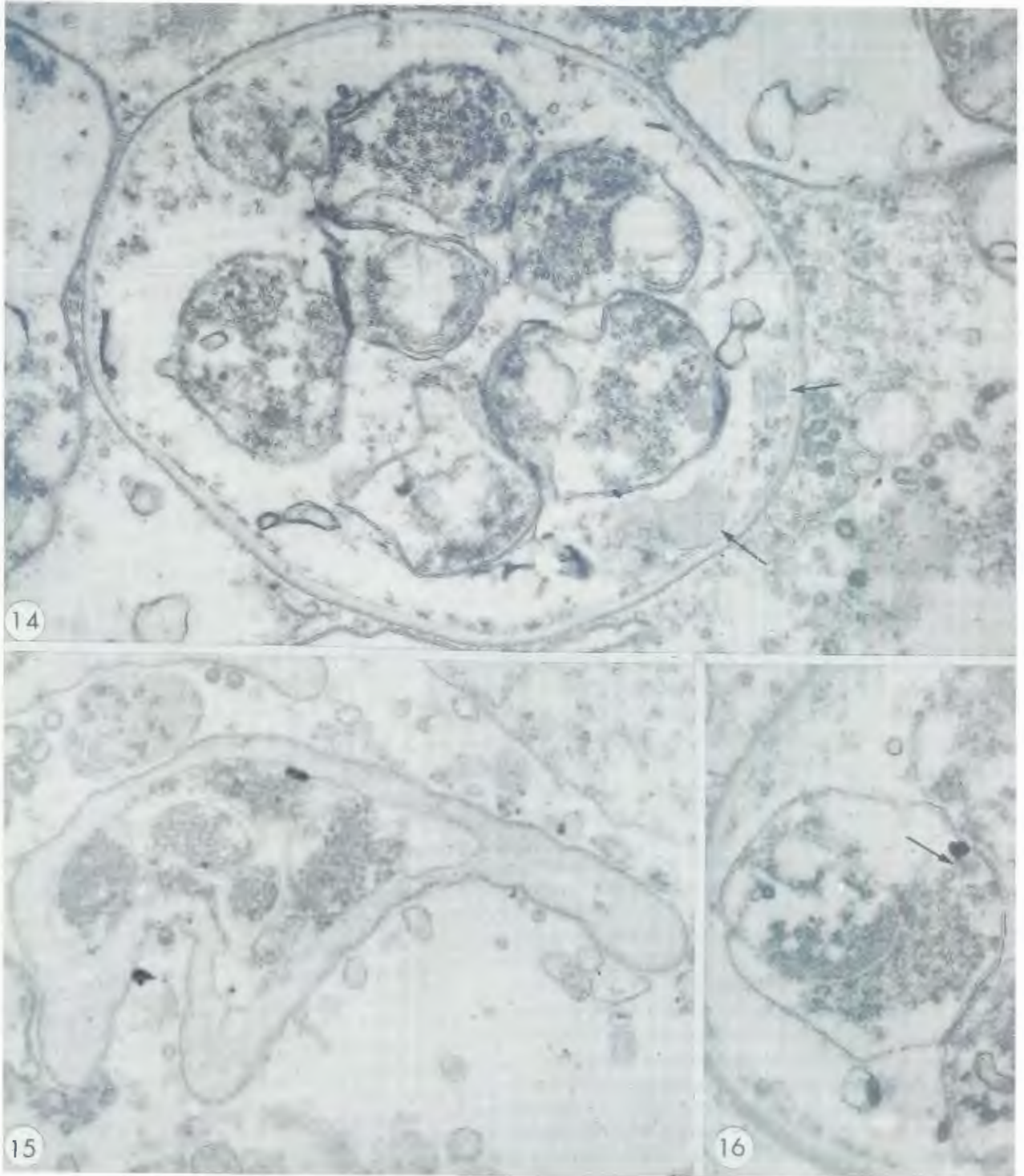
PLATE 4   14. Cyst with intracystic bodies. Lipid-like bodies (arrows) are also present. × 29 400. 15. Irregularly shaped collapsed cyst with a very thick wall. × 24 500. 16. Intracystic body. Nucleus surrounded by a nuclear membrane. Note narrow electronlucent area around nucleus, mass of granular particles and osmiophilic body (arrow). × 27 000.

tained many small lymphocytes. There were a few haemorrhages in the submucosa of the abomasum, but the gastrointestinal tract was otherwise normal.

There was mild micro-cavitation and rarefaction of the white substance of the cerebral cortex and chromatolysis of some neurones. Many of the small blood vessels revealed leukostasis. The sections of the brain and kidneys were also specifically examined for the presence of *Cowdria ruminantium* (Cowdry, 1926) but only one colony was observed in an endothelial cell.

P. carinii was not found in any tissue other than the lung.

The bacteriological examination proved unsuccessful as all the cultures were overgrown by a *Proteus* sp.

ELECTRON MICROSCOPY

On finding cysts of *P. carinii* in paraffin sections it was decided to prepare specimens of lung for electron microscopy. The delay in fixation, however, rendered many of the cytoplasmic organelles, e.g. mitochondria and rough endoplasmic reticulum, barely recognizable.

The following different morphological forms of the organism were identified:

1. *Trophozoites*

Large trophozoites, [Plate 2 (7, 8 and 9)] measuring 1,7 to 5 μ in diameter were the most frequent form encountered and often lined the alveolar septa [Plate 2(8)]. The pellicle consisted of two electron-dense membranes with an electron-pale area interposed between them and was often folded to form pseudopodia or numerous filopodia [Plate 2(9) and Plate 3(10)]. Glycogen-like particles and lipid-like bodies were encountered in the cytoplasm. Masses of nucleoplasm frequently were encircled by an incomplete nuclear membrane. Forms were occasionally seen in which the cytoplasm of two adjoining trophozoites communicated through a small opening in the pellicle [Plate 3(11)].

Some of the larger trophozoites were more rounded or oval with no pseudopodia. Their pellicles were also thickened and consisted of an inner and outer electron-dense layer with a thick electron-pale layer between them [Plate 3(12)]. Large numbers of glycogen-like particles and vacuoles were present in the cytoplasm of these larger trophozoites.

Smaller trophozoites measuring approximately 1 to 1,5 μ in diameter were infrequently found. They contained granules similar to ribosomes, round bodies, vacuoles and nucleoplasm and their pellicles consisted of an inner and outer electron-dense unit-type membrane. In appearance they were similar to the intracystic bodies present in the cysts (*vide infra*).

2. *Cysts*

The pellicles of the cyst were similar in structure to those of the larger trophozoites but with a much thicker electron-pale layer.

In addition to the intracytoplasmic structures already described for the trophozoites, most of these thick-walled organisms or cysts contained varying numbers of large intracystic bodies measuring 0,7 to 1,6 μ in diameter [Plate 4(14)]. These bodies had a nucleus, enveloped by a nuclear membrane. A narrow electron-lucent area surrounded the nucleus. Masses of granular particles, similar to ribosomes, small round bodies and osmiophilic bodies were present in their cytoplasm [Plate 4(16)]. The intracystic bodies were limited by a pellicle consisting of two unit-type membranes with a clear space in between. In some the outer membrane

was continuous with membranes in the cytoplasm of the cyst that were external to the intracystic bodies.

No intracystic bodies were found in the crescent-shaped and other odd-shaped thick-walled cysts. These forms only contained cytoplasmic debris and apparently represented cysts which had ruptured expelling the intracystic bodies [Plate 3(13) and Plate 4(15)].

The poor fixation precluded any critical observations on the fine structure of most of the organelles of the various forms of the organism under review. However, the general appearance of the different morphological stages of the organism observed in this case seems to be identical with those of *P. carinii* described in rats by Barton & Campbell (1969).

DISCUSSION

The histopathological findings in this case of caprine pneumocystosis conform to the lesions of the disease in man and other animals. Both the morphological and tinctorial features of the organisms as seen with light microscopy were identical to those of *P. carinii*. Most reports described an interstitial pneumonitis (of variable intensity) characterized by plasma cells, although Swiss workers (Loustalot, 1951; Tobler, 1953) felt that these cells are actually monocytes (histiocytes), as we also did in the case under discussion. The "foamy" or "honey-comb" material in the terminal air spaces is diagnostic for pneumocystosis. Although the parasite had a low affinity for HE stains, careful examination revealed their morphology adequately to make a diagnosis. Esterly & Warner (1965) reported that *P. carinii* is most easily identified by the use of special stains, especially GMS, which delineated the cyst form quite clearly. This finding was confirmed in our case. A more detailed analysis of the staining characteristics has been published by Le-Tan-Vinh, Cochard, Vu-Trieu-Dong & Solonar (1963).

The marked similarities between the findings in the present case and those reported previously do not necessarily imply that the organism is the same species in all the various hosts. Frenkel *et al.* (1966) have shown that pneumocystosis has different immunological features in different animal species. Whether this indicates that various distinct species occur is still somewhat speculative because, as Robbins (1968) pointed out, the immunological differences could have been due to lung tissue contamination of the antigen. The definitive work on this aspect must await the development of a pure antigen, which at this time is not possible since the organism has not been successfully cultured on artificial media.

The life-cycle of *P. carinii* has been illustrated by Vaňek & Jírovek (1952) and Wenyon (1926). Based on his experiments in rabbits, Sheldon (1959) believes that the organism is inhaled directly into the lung. Frenkel *et al.* (1966) also suggested that man and animals may harbour *P. carinii* in their lungs in a latent form and when an individual becomes susceptible (in one of the various ways described previously) overt disease develops. Once in the lung the organism first develops into an oval amoeboid form (2 to 4 μ length) which contains a nucleus and lies in a fragile mucoid envelope (5 to 12 μ diameter) (Vaňek & Jírovek, 1952). The nucleus then multiplies by binary fission with the mucoid capsule also dividing at this time. Another method of multiplication is by sporogony. Here, a firm membrane develops around the mature trophozoite forming a cyst. The nucleus undergoes division forming 2, 4 and then 8 bodies within an individual spherical

cyst which measures 7 to 10 μ in diameter. These cysts are argentophilic (with the GMS stain). The nucleus of the mature cyst undergoes segmentation into the individual spores (intracystic bodies) which, after rupture of the cyst, probably give rise to the amoeboid forms.

Further insight into the finite structure of the organism and the pathogenesis of the disease was provided by the electron microscopic studies of Barton & Campbell (1967, 1969) and Vavra, Kučera & Levine (1968). According to these authors the pleomorphic trophozoites are probably motile since their pellicle shows numerous evaginations into filopodia or pseudopodia. This pellicle then thickens (with loss of the filopodia and pseudopodia) and the cyst stage is thus initiated. They felt that the remainder of the life-cycle is essentially the same as described with light microscopy. The above life-cycle is valid only if *P. carinii* is indeed a protozoon. Recent work by Vavra & Kučera (1970) suggests rather convincingly that the organism is actually a fungus, possibly of the class Ascomycetes, subclass Hemiascomycetidae. Again, *in vitro* cultivation of the organisms will be necessary before proper classification is possible. Our electron microscopic findings, even though hindered by poor fixation, agree in all respects to those of the above authors.

The manner by which *P. carinii* causes disease appears to be mainly mechanical, in that the organisms interdigitate and thus form a membrane in close apposition to the alveolar lining cells. This would then interfere with gas exchange which would in turn stimulate thickening of the alveolar tissues. The parasites have not been reported to invade the lung tissue itself although in rare instances they have been seen within alveolar macrophages. Barton & Campbell (1969) suggested that the lack of cytosomes, Golgi apparatus, lysosomes and the large numbers of glycogen-like bodies in the trophozoites indicate that the organisms metabolize principally low-molecular weight substances from alveolar fluid or adjacent alveolar lining cells and that they do not rely on phagocytosis. They also postulated that the glycogen bodies probably provide a source of energy for the formation of the intracystic bodies while the lipid-like globules provide material for the formation of membranes. Furthermore, the possible release of proteinaceous or other substances during rupture of the cysts could also explain some of the host response in the alveolar septa.

The goat under discussion was a young animal apparently suffering from concurrent disease, as suggested by the marked lymphoid hyperplasia and mild lymphadenitis of all the nodes examined, but this could not be proved. However, the striking lack of plasma cells in the lung and lymphoid tissues, correlated with the young age of this animal, would suggest a possible primary hypogammaglobulinaemia (Robbins, 1968). It is therefore most unfortunate that serum globulin studies were not carried out on this case. The detection after diligent search of only one colony of *C. ruminantium* is not regarded as significant as such a finding in an endemic heartwater area is by no means diagnostic for the disease.

Summary

A case of pneumocystosis in a young domestic goat is described. It was manifested as a diffuse interstitial pneumonitis with numerous typical organisms filling the air spaces. This is apparently the first case of pneumocystosis in an animal to be recognized on the continent of Africa.

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