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MYOSITIS AND DEATH IN BOBWHITES, *COLINUS VIRGINIANUS* (L.), DUE TO HEMORRHAGIC CYSTS OF A HAEMOSPOROZOAN OF UNDETERMINED TAXONOMIC STATUS

C. H. Gardiner,[1] H. J. Jenkins,[1] and Kerry S. Mahoney[2]

ABSTRACT: An epizootic of myositis and death in pen-reared bobwhites occurred at a hunting club in California. The myositis was caused by myriads of elongate protozoan cysts. The cysts were in various stages of development and when mature, contained spherical zoites 1 μm in diameter. Sinuous compartments were present in all cysts. The walls of the compartments were composed of a material similar to the cyst walls. Mature cysts were filled with blood. The histologic and ultrastructural morphology of the parasite revealed the parasite to be a haemosporozoan. The parasite has numerous similarities to both *Akiba caulleryi* (Mathis and Leger, 1909) and organisms that cause aberrant leucocytozoonosis in other species of birds. Further studies are needed to determine if the parasite is a part of the normal parasite fauna of quail or if it represents a parasite in an aberrant host.

INTRODUCTION

The protozoan blood parasites of birds include members of *Plasmodium, Haemoproteus, Parahaemoproteus, Leucocytozoon* and *Akiba* (Bennett et al., 1965; Fallis and Desser, 1977). All are sporozoans in the suborder Haemosporina (Baker, 1977). Herein we report an outbreak of myositis which caused morbidity and ultimately mortality in bobwhites from California.

During the fall of 1979 the manager of a hunting club in Pine Valley, California (San Diego County) noticed morbidity in his pen-reared bobwhites. The birds developed weight loss, acute diarrhea, convulsions, torticollis, and lateral recumbency. In a 2-wk period approximately 700 died. Linear hemorrhages were present in all skeletal muscles at necropsy.

MATERIALS AND METHODS

Pooled tissue from eight bobwhites were available for study. All tissues were fixed in buffered formalin. Representative tissues were processed for histologic sections (i.e., dehydrated in ethanol series, embedded in paraffin, sectioned at 5 μm). For electron microscopy formalin-fixed muscle was post-fixed in Dalton's osmium-dichromate solution (Dalton, 1955), embedded in Epon, and cut at 8 nm. Sections were stained with lead citrate and uranyl acetate.

RESULTS

Histologic sections of paraffin embedded skeletal muscles revealed numerous protozoan cysts in every tissue section. Most cysts were filled with blood (Figs. 1, 2). The cysts were elongate and appeared to be contained within muscle fibers. The length of cysts could not be determined. The maximum diameter of the cysts was 270 μm. The walls of the cysts were thin (1 μm) and were eosinophilic when stained with hematoxylin and eosin. Sinuous compartments were present in all cysts. The walls of the compartments were composed of material similar to the cyst walls. Cysts in all stages of development were present. When immature or just beginning to develop (Fig. 4) the sinuous compartments were filled with granular basophilic cytoplasm. Clear clefts were often present in the cytoplasm. A homogeneous, darker basophilic material was often present adjacent to the interior of the cyst wall. As the cysts matured, the cytoplasm rounded up into spherules (approximately

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4 μm in diameter) (Fig. 5). In these cysts the dark homogeneous material was often present as large globules at the periphery of each compartment. Mature cysts were filled with 1 μm spherical zoites and often contained host red blood cells (Fig. 6).

Inflammatory cells from the host were found associated only with those cysts that reached maturity and ruptured. Cysts first appeared to fill with blood and then zoites were released; the cysts were subsequently invaded by neutrophils and macrophages. The inflammatory infiltrate often extended to the periphery of the cysts. Numerous degenerating and regenerating muscle fibers were present in all sections of muscle examined (Figs. 1–3).

The spleen, liver and lungs contained numerous intracellular schizonts. Schizonts had a maximum diameter of 30 μm and were spherical. Some schizonts contained cytomere-like bodies (Fig. 7). Others, possibly mature ones, contained uninucleate, elongated zoites (Fig. 8). The nucleus of the host cell was markedly hypertrophied. The cell type could not be determined but probably was either a macrophage or endothelial cell. A few schizonts were lobulated.

Blood smears were not available for study but examination of erthrocytes in histological section revealed many cells infected with a pigmented protozoan. Cells most commonly contained one organism but some cells contained up to four small parasites. When mature, the parasite was elongate, contained pigment and surrounded the nucleus in a horseshoe pattern (Fig. 9), a morphology consistent with *Haemoproteus lophortyx* O'Roke, 1930.

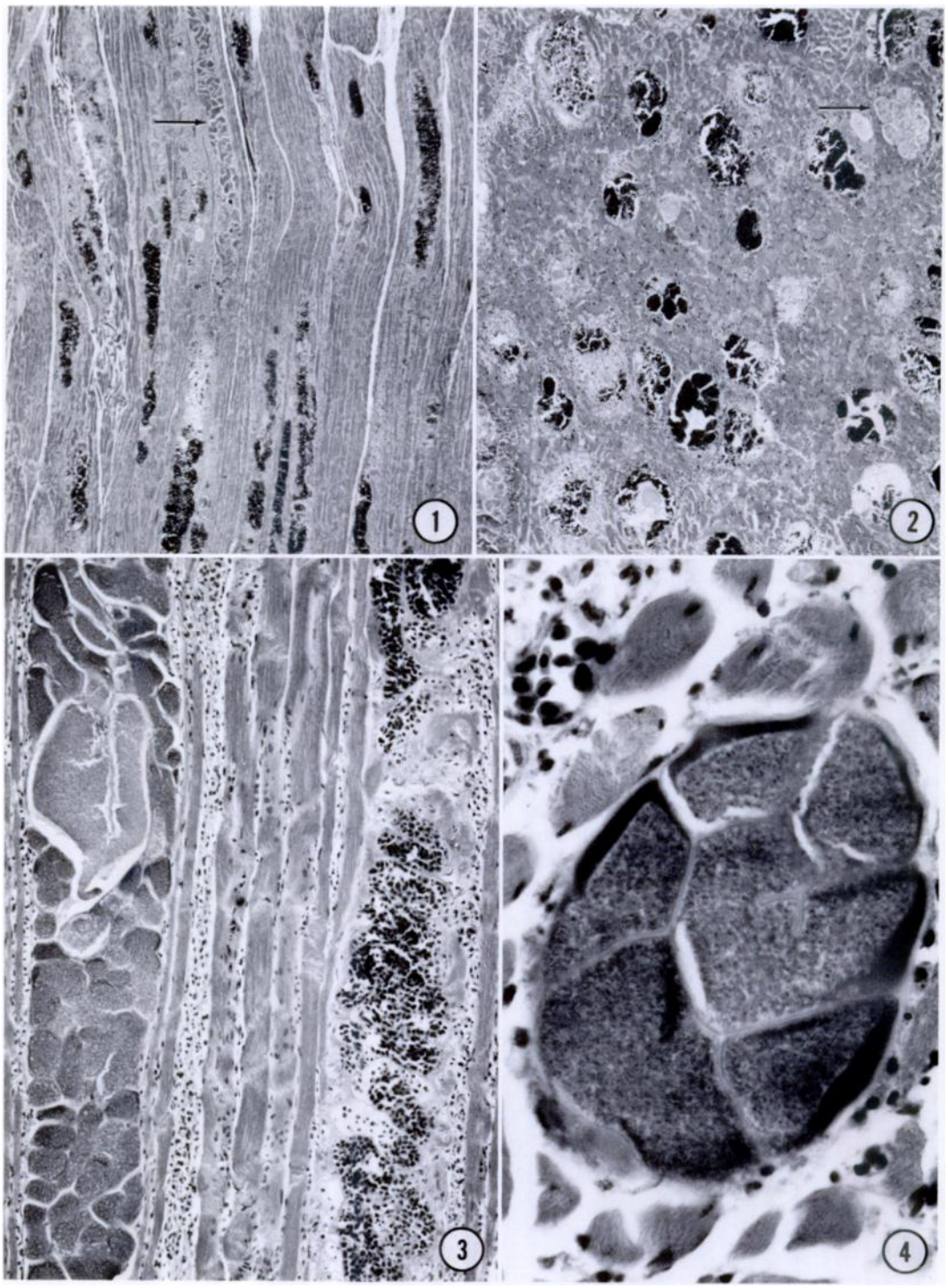
Electronmicroscopy revealed the wall of the cyst to be electron dense and have irregularly spaced villous processes (Figs. 10, 11). Immature cysts contained a mass of electron-dense cytoplasm. The homogeneous basophilic material noted in histologic section appeared less electron dense than the cytoplasm (Figs. 10, 12). As the cysts matured, the cytoplasm rounded up into spherical masses (cytomeres) (Fig. 13). Small zoites budded from the interior of the cytomeres. Each zoite had several nuclear masses or a mass that was lobulated (Fig. 14).

Ultrastructurally, the intramuscular organism resembled megaloschizonts of *Leucocytozoon* (Desser, 1970). However, the central hypertrophied host cell nucleus often seen in megaloschizonts of *Leucocytozoon* was never seen in paraffin or Epon sections.

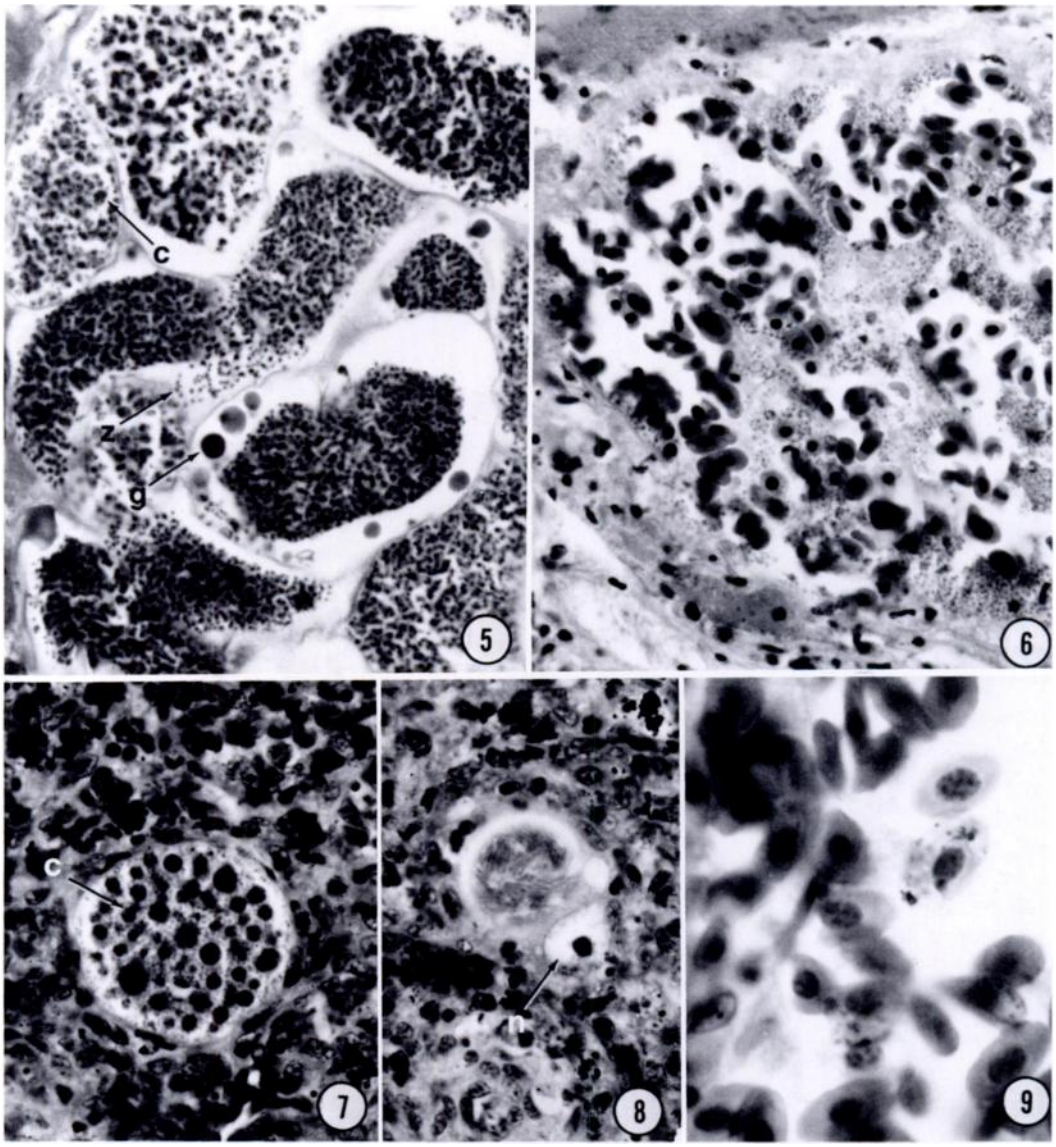
DISCUSSION

There are approximately 90 named species of *Leucocytozoon* infecting over 1,000 species of birds (Hsu et al., 1973; Bennett et al., 1982). *Akiba*, a closely related genus erected in 1965, has only one species, i.e., *A. caulleryi* (Mathis and Leger, 1909). Although once classified as *Leucocytozoon caulleryi* (Mathis and Leger, 1909) this species differs from other members of the genus in the following ways (Bennett et al., 1965): 1) The gametocyte of *Akiba* pushes the host cell nucleus to the side but does not noticeably distort it in its maturation. In addition, this nucleus eventually disappears from the cell when the parasite is fully grown. Many mature parasites are free of host cells. 2) The prepatent period for *Akiba* is approximately 2 wk whereas it is 1 wk for *Leucocytozoon*. 3) *Leucocytozoon* is vectored by black flies of the genus *Simulium*, *Akiba* by biting midges of the genus *Culicoides*. 4) The "central body" (which is the hypertrophied nucleus of the host cell) in megaloschizonts of *Akiba* is lateral, rather than central, as seen in *Leucocytozoon* (Fig. 15).

Akiba caulleryi (Figs. 16, 17) has been incriminated as the causative agent of Bangkok hemorrhagic disease of chickens (Campbell, 1954; Akiba, 1970). The most striking gross lesions in this disease are petechial hemorrhages of the skin, muscles and viscera. Blood films reveal numerous extracellular oval organisms (1.5–2.0 μm



FIGURES 1–4. Cysts of a haemosporozoan in skeletal muscle of a bobwhite. 1. Longitudinal section of muscle with numerous linear hemorrhages. An intact cyst (arrow) is present. H&E, ×25 (AFIP MIS #83-6863). 2. Cross-section of muscle. Note lobulation of hemorrhages due to sinuous tubes in empty cyst. An immature, intact cyst is present (arrow). H&E, ×60 (AFIP MIS #83-7077). 3. Higher magnification of muscle with intact, maturing cyst (left) and empty mature cyst filled with blood (right). Note minimal



FIGURES 5–9. Haemosporozoan in a bobwhite. 5. A maturing cyst in skeletal muscle. The granular basophilic material has rounded up into cytomeres (c). Mature zoites (z) are numerous. The dark basophilic material contained within the tubes is now globular (g). H&E, ×630 (AFIP MIS #83-7065). 6. A mature cyst which has filled with blood. The mature zoites are 1 μm in diameter. Individual tubes can still be discerned. H&E, ×630 (AFIP MIS #83-7070). 7. Maturing schizont in spleen. Note the cytomere formation (c) within the schizont. H&E, ×630 (AFIP MIS #83-9499). 8. Mature schizont in spleen. Note that the schizont is contained within an enlarged cell. The nucleus of the host cell (n) is hypertrophied. The zoites within the schizont are elongate, uninucleate, and arranged in whorls. H&E, ×630 (AFIP MIS #83-9500). 9. Pigmented gametocyte surrounding nucleus of erythrocyte within hepatic vessel. H&E, ×1,500 (AFIP MIS #84-6850).

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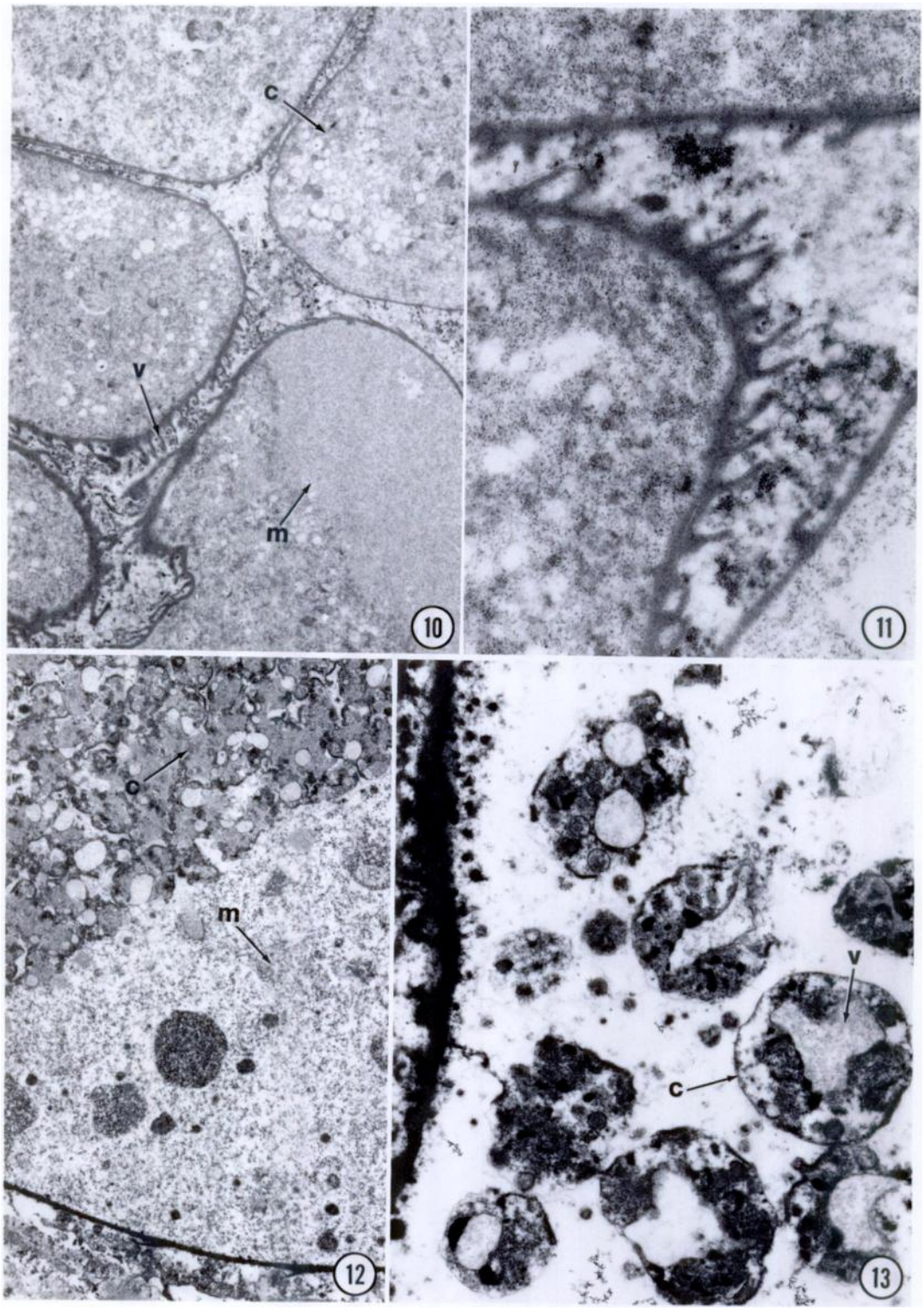
inflammatory infiltrate. H&E, ×160 (AFIP MIS #83-6864). 4. Cross-section of maturing cyst. Note invaginations of cyst wall to form tubes. A dark homogeneous basophilic material is at the sides of several tubes. H&E, ×630 (AFIP MIS #83-7068).



in diameter). Studies of the life cycle (Akiba, 1970; Akiba et al., 1971; Morii and Kitaska, 1971; Novilla et al., 1971) in naturally and experimentally infected chickens have shown that schizonts occur within endothelial cells in many organs. The schizonts grossly enlarge the host cell and cause hypertrophy of the host cell nucleus. These schizonts are released from the host cells in approximately 9 days and spread to all organs of the chickens by the blood stream where they lodge and develop into individual or clusters of schizonts. After lodging, the schizonts develop a thick capsule. These schizonts rupture 2 wk after infection and the zoites are liberated. The zoites then enter erythrocytes or erythroblasts where they form gametocytes.

In addition to *Akiba* there are three other genera of protozoans that have been reported to form cysts in muscles of birds. 1) Desser et al. (1968) reported an elongate cyst of *Leucocytozoon simondi* Mathis and Leger, 1910 in the heart of a wild black duck (*Anas rubripes* Brewster) experimentally infected and then allowed to "relapse." 2) *Sarcocystis* has been reported in over 60 species (Springer, 1978; Keymer, 1982). 3) Two outbreaks of *Arthrocystis galli* Levine, Beamer, and Simon, 1970 have been reported from chickens in India and Malaysia (Levine et al., 1971; Optiz et al., 1982). Levine et al. (1971) found protozoan cysts measuring up to 1,400 μm long and 126 μm wide in skeletal and cardiac muscle. The cysts had thin walls and were septate in a bamboo-like fashion. Mature merozoites were spherical and 1 μm in diameter. The authors believed the organism most closely resembled *Sarcocystis* sp. Optiz et al. (1982) reported myopathy in skeletal and cardiac muscle in over 150 chickens. The authors felt that the parasite was identical to *A. galli* but caused more severe lesions than those reported initially by Levine et al. (1971).

Numerous cases of aberrant leucocytozoonosis have been reported in various genera of wild and caged birds. Graham-Smith (1907) was first to report this entity. He found protozoan cysts (which he likened to *Rhinosporidium*) in three budgerigars (*Melopsittacus undulatus* (Shaw)). Cysts were most numerous in the muscle of the gizzard but were also present in cardiac and skeletal muscle. Since this report, over 12 species of parakeets and one of a woodpigeon (*Columba palumbus* L.) have been reported infected with this entity from England (Fowler and Forbes, 1972; Smith, 1972; Walker and Garnham, 1972; Garnham 1973a, b). In addition, cases have been reported from Germany (Frank, 1965, 1967; Frank and Kaiser, 1967; Schuppel and Kronberger, 1976), central Europe (Minarik, 1972), Netherlands (Borst and Zwart, 1972), and New Zealand (Hartley et al., 1981). These infections can be summarized as follows: Most cysts are in the gizzard (Figs. 18, 19), skeletal muscle, and/or cardiac muscle. Cysts are usually in groups and often align to form a bamboo-like appearance (Figs. 20, 21). Walls of the cyst are thin but do thicken and form invaginations. Mature zoites are approximately spherical and 1

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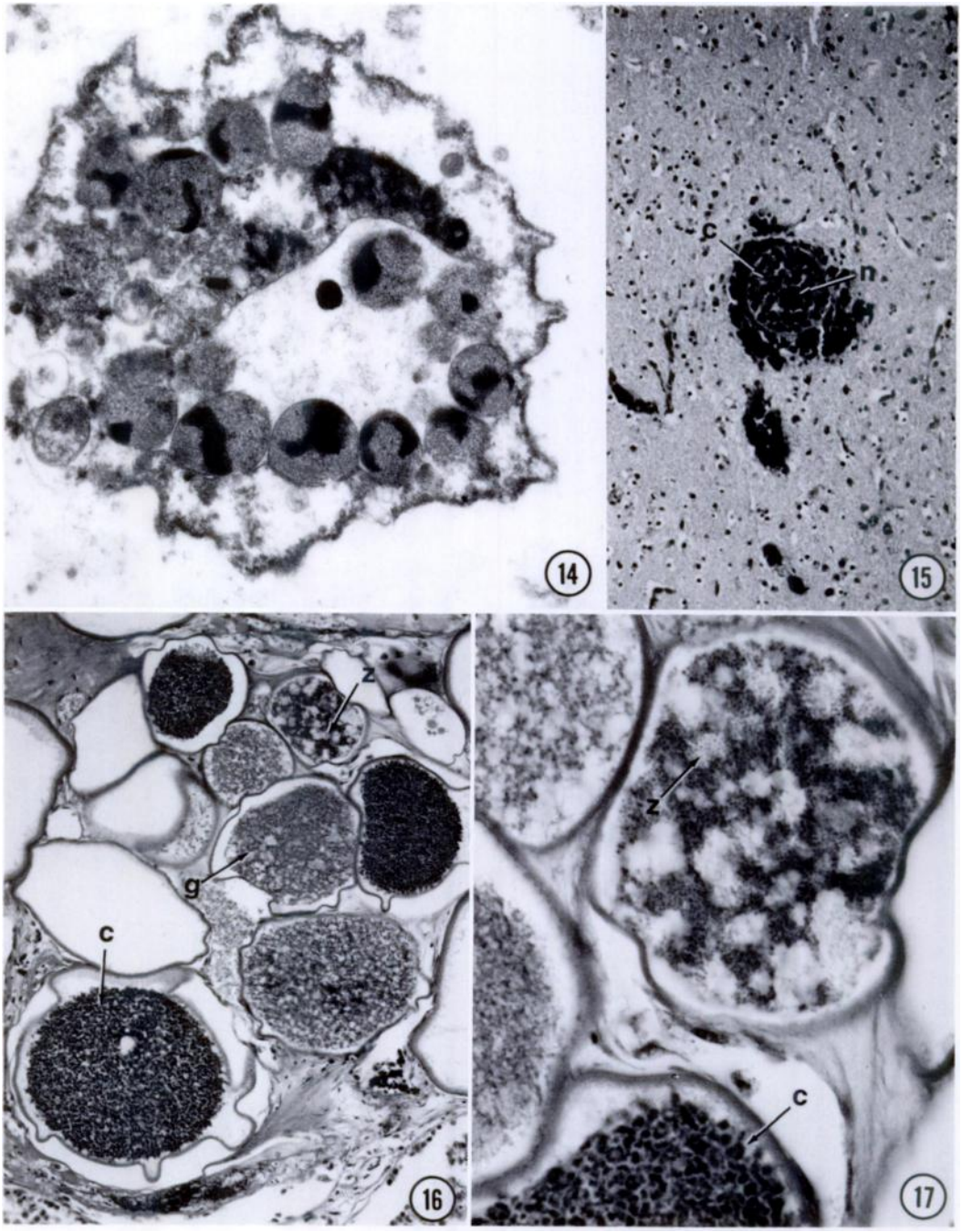
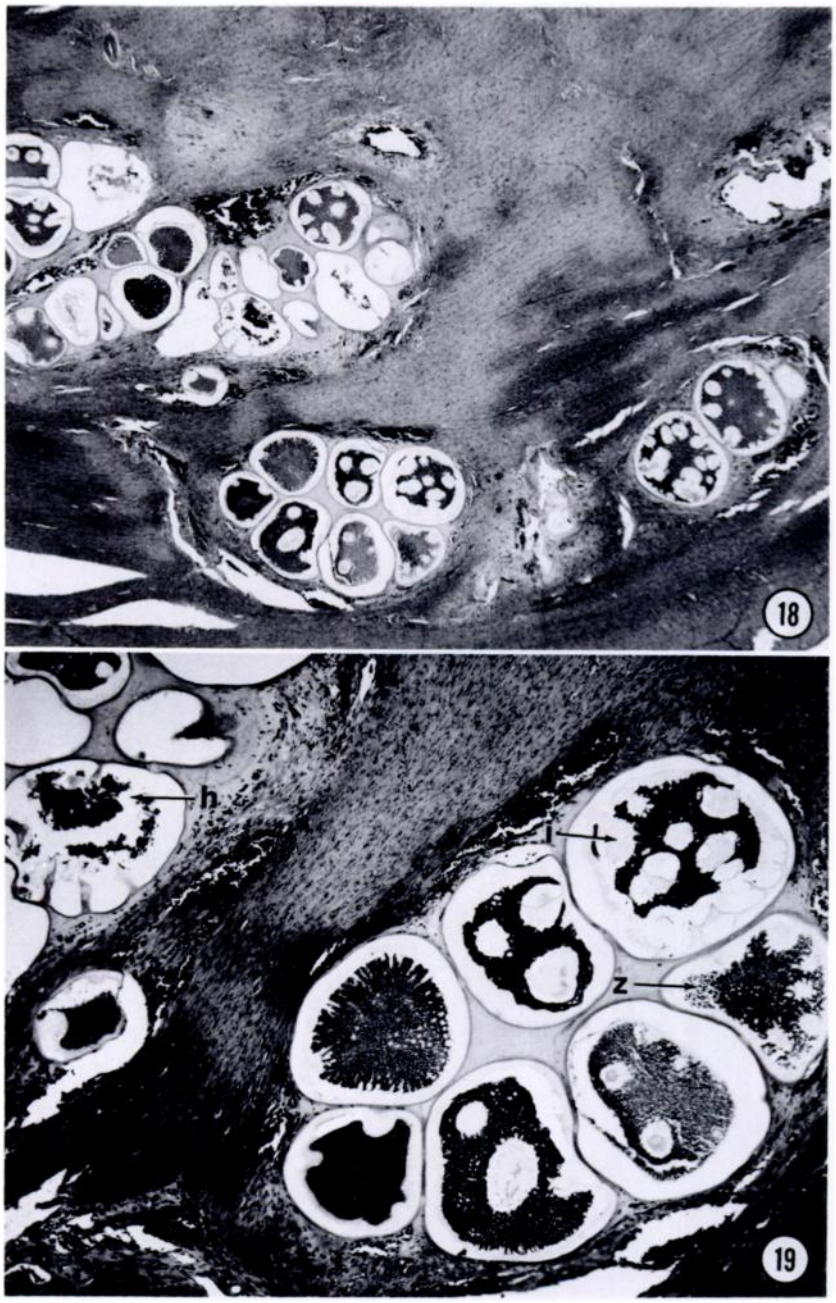
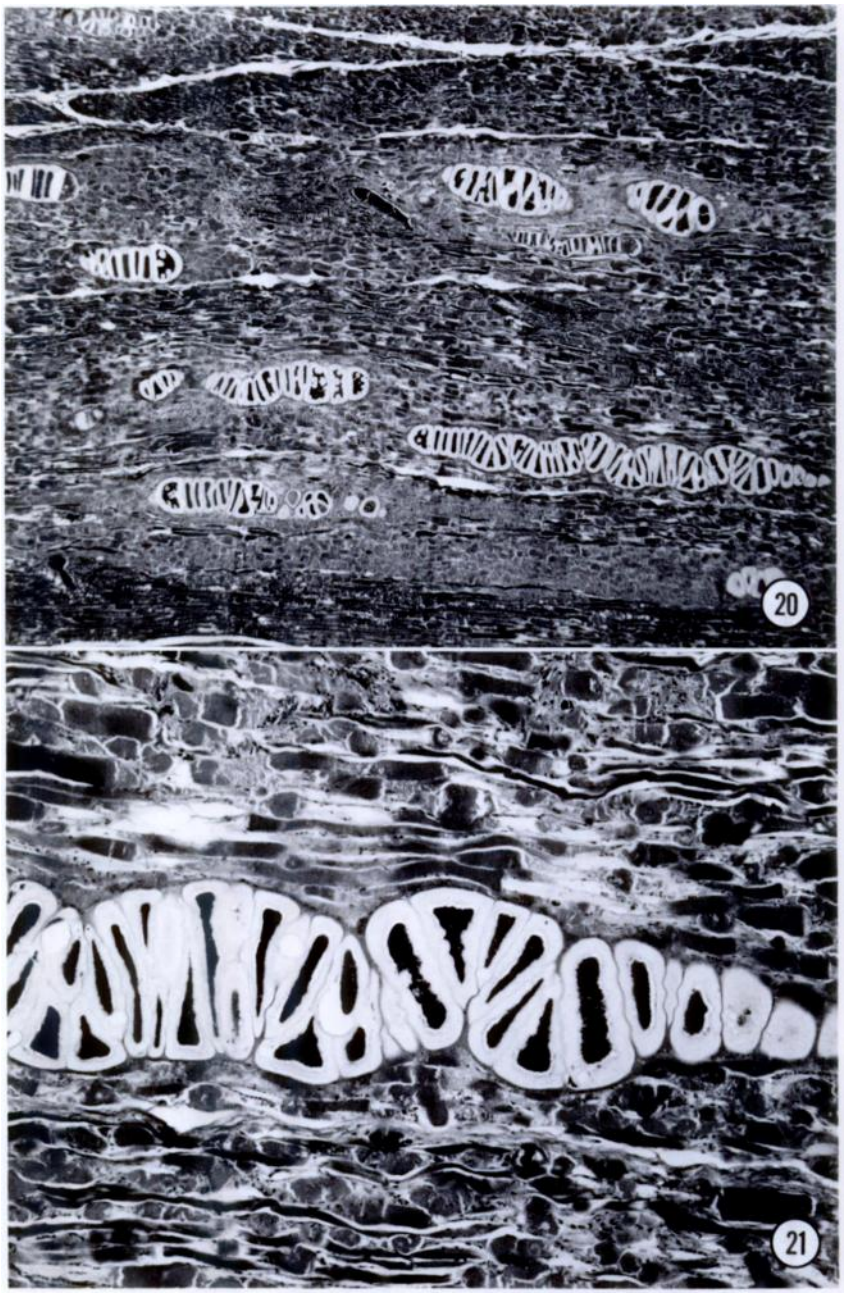
FIGURES 10–13. Transmission electron micrographs of cysts of a haemosporozoan from muscle of a bobwhite. 10. An immature cyst with undefined granular cytoplasm (c). The darker basophilic material (m) seen with H&E is less electron dense. Villous processes (v) extend from the exterior of the tubes. TEM, ×3,860 (AFIP MIS #83-7969). 11. Higher magnification of villous processes of tubes. TEM, ×18,200 (AFIP MIS #83-7965). 12. Maturing cyst with cytoplasm (c) rounding up into cytomeres. The dark basophilic material (m) is external in tube. TEM, ×3,860 (AFIP MIS #83-7967). 13. Maturing cytomeres (c) have large vacuoles (v) in the interior. TEM, ×9,355 (AFIP MIS #83-9501).



FIGURES 14–17. 14. Transmission electron micrograph of cytomere of a haemosporozoan of bobwhites. Note zoites budding into interior vacuole. TEM, ×18,200 (AFIP MIS #83-7966). 15. Megaloschizont of *Leucocytozoon simondi* in brain of a mallard (*Anas platyrhynchos* L.). Note numerous cytomeres (c) and central hypertrophied nucleus of host cell (n). H&E, ×160 (AFIP MIS #83-9498). 16. Cysts of *Akiba caulleryi* in testicle of chicken. Numerous spherical cysts at various stages of development. Immature cysts contain granular cytoplasm (g), maturing cysts contain cytomeres (c), and mature cysts contain 1-μm zoites (z). H&E, ×160 (specimen compliments of Dr. H. M. Optiz) (AFIP MIS #83-6865). 17. Higher magnification of cysts of *Akiba caulleryi*. Note mature zoites (z) and cytomeres (c). H&E, ×630 (specimen compliments of Dr. H. M. Optiz) (AFIP MIS #83-6866).



FIGURES 18, 19. Aberrant leucocytozoonosis in a Port Lincoln parrott (*Barnardius zonarius* Shaw). 18. Gizzard with "aberrant leucocytozoonosis." Note numerous oval cysts in muscle. H&E, ×25 (specimen compliments of Dr. P. C. C. Garnham) (AFIP MIS #83-9496). 19. Higher magnification of cysts in gizzard. Note thick walls of cyst often have thick invaginations (i). Cysts are at various stages of development. Mature zoites (z) are 1 μm in diameter. Hemorrhage (h) is present within empty, mature cysts. H&E, ×60 (specimen compliments of Dr. P. C. C. Garnham) (AFIP MIS #83-9497).



FIGURES 20, 21. Aberrant leucocytozoonosis in a pied currawong (*Strepera graculina* White). 20. Elongate cysts of aberrant leucocytozoonosis in skeletal muscle. Note bamboo-like arrangement of cysts. H&E, ×25 (specimen compliments of Dr. W. J. Hartley) (AFIP MIS #83-9494). 21. Higher magnification of muscle. Note parasite has thick walls and the zoites are spherical and 1 μm in diameter. H&E, ×100 (specimen compliments of Dr. W. J. Hartley) (AFIP MIS #83-9495).

μm in diameter. Myopathy is mild to severe with cysts surrounded or filled with blood.

The parasite in this report has many morphologic similarities to several genera, i.e., *Leucocytozoon*, *Akiba* and *Arthrocystis*. It resembles all three in that the mature zoite is spherical and 1 μm in diameter. It has ultrastructural morphology very similar to *Leucocytozoon*. It is found in muscle as is *Akiba* and *Arthrocystis*. The presence of smaller schizonts in visceral organs and the fact that these schizonts cause hypertrophy of the host cell nucleus closely aligns it to *Akiba caulleryi*. Clinically and histopathologically this organism closely resembles Bangkok hemorrhagic disease of chickens, a disease caused by *A. caulleryi*. Morii and Kitaoka (1971) tried unsuccessfully to infect nine species of gallinaceous birds (such as quail and pheasants) with *A. caulleryi*. Although similar to *A. caulleryi* the parasite in this case differs in that this parasite has sinuous compartments with a cyst limited by a thick cyst wall. The causative organism(s) of "aberrant leucocytozoonosis" has (have) many similarities to the parasite in this case. Both organisms are found in muscle, have thick cyst walls, and contain zoites that are 1 μm when mature. The cysts in aberrant leucocytozoonosis, as in *A. caulleryi*, lack compartments.

The pigmented gametocytes in this case resemble those of *Haemoproteus*. The cysts in other organs (Figs. 7, 8), however, are not morphologically compatible with schizonts of *Haemoproteus*. The cyst in Figure 7 resembles *Leucocytozoon* (Desser et al., 1968) but lacks a hypertrophied host cell nucleus. The elongated zoites within the cyst in Figure 8 do not resemble *Leucocytozoon* sp. or *Haemoproteus* sp. It is possible that the pigmented gametocytes are a result of the cyst replication seen in muscle. If this is true the organism within the muscle would be a new member of the Haemosporina. Further studies are needed to determine if this parasite is a part of the normal parasite fauna of quail or if it represents a parasite in an aberrant host.

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