Histological and Ultrastructural Appearance of Severe *Sarcocystis Fayeri* Infection in a Malnourished Horse

Richard J. Cawthorn, Maurice Clark, Robert Hudson and Dianne Friesen

J VET Diagn Invest 1990 2: 342
DOI: 10.1177/104063879000200418

J Vet Diagn Invest 2:342-345

Histological and ultrastructural appearance of severe *Sarcocystis fayeri* infection in a malnourished horse

Richard J. Cawthorn, Maurice Clark, Robert Hudson, Dianne Friesen

Sarcocysts are highly prevalent in the musculature of horses *(Equus caballus)* in various regions; however, the systematics of *Sarcocystis* species in equids is confusing.[3] There are 4 reported species of *Sarcocystis* in equids: *S. asinus, S. bertrami, S. equicanis,* and *S. fayeri*. All utilize canids as definitive hosts; however, transplacental infection can occur.[2,4] The interrelationships and validity of the above species are unclear. Pathogenicity of infection is variable, ranging from mild to severe chronic illness in naturally and experimentally infected horses.[5–7] We describe the histologic and ultrastructural appearance of intense *Sarcocystis fayeri* infection in a malnourished horse.

A 3-4-year-old mare was euthanized for humane reasons, having been in deteriorating condition for 2-3 months. At necropsy, the animal was severely emaciated; depot fat was absent from all organs. Bone marrow appeared wet and without fat. The liver and spleen were small and shrunken. Musculature of the diaphragm and especially the inner thigh muscles was pale with marked white streaking. Portions of cardiac and skeletal muscle were fixed in 10% neutral buffered formalin and forwarded to the Atlantic Veterinary College. Some samples were processed for histologic examination: tissues were embedded in paraffin, sectioned to 5-μm thickness, and stained with hematoxylin and eosin (HE) or Masson's trichrome or reacted with periodic acid-Schiff reagents. Other samples were processed for ultrastructural study: tissues were transferred to a buffered solution of 1% glutaraldehyde and 4% formaldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in epon-araldite. Thin sections were stained with lead citrate and uranyl acetate and examined using a Hitachi transmission electron microscope.

Histologically, the normal fat component of adult marrow was absent, replaced with amorphous pink-staining proteinaceous fluid with small shrunken adipocytes.

In skeletal muscle and diaphragm, there was marked variation in diameter of muscle fibers, with most containing large

From the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island CIA 4P3, Canada (Cawthorn, Clark, Friesen), and the Newfoundland Department of Rural, Agricultural and Northern Development, Clarenville, Newfoundland AOE 1JO, Canada (Hudson).

Received for publication April 19, 1990.

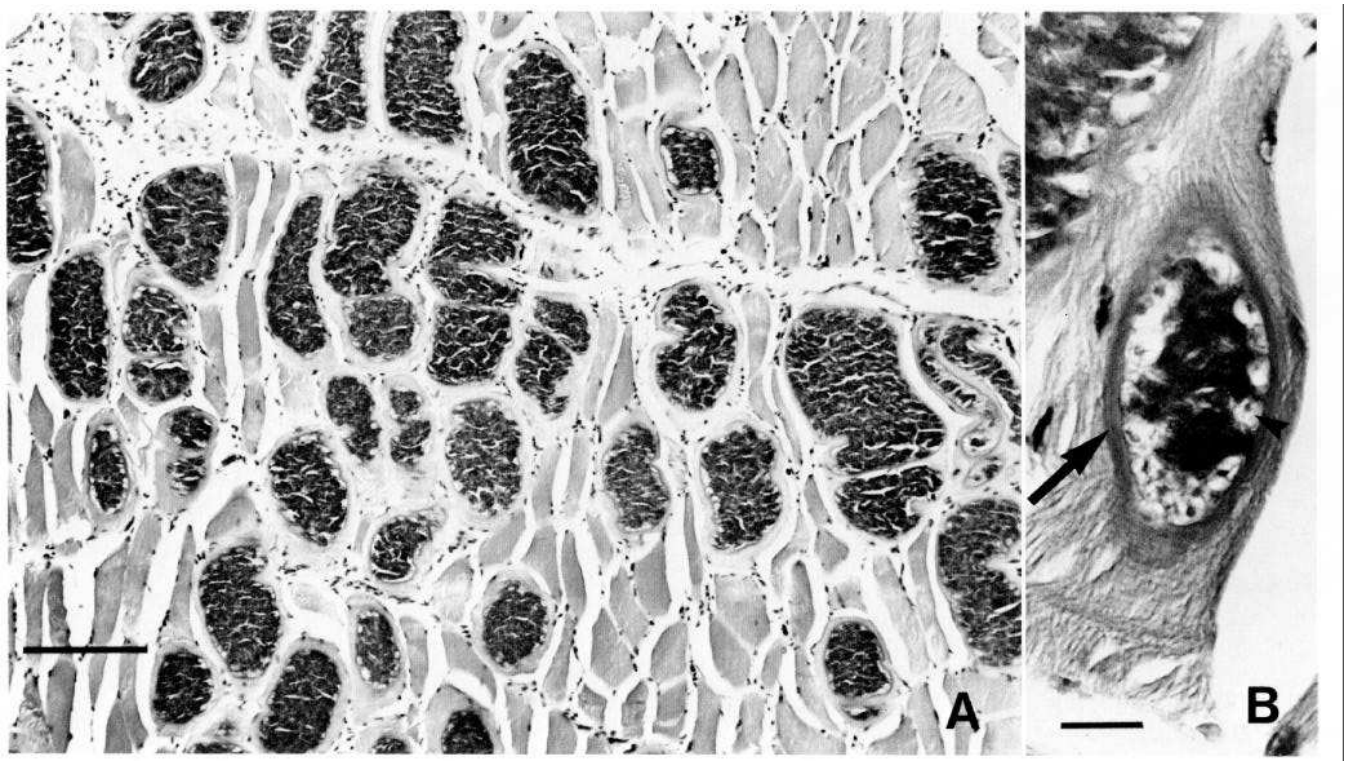

Figure 1. Photomicrographs of sections of skeletal musculature of malnourished horse. HE stain. **A.** Numerous sarcocysts of Sarcocystis fayeri are present, and there is acute muscle degeneration. Bar scale = 100 μm. **B.** Higher magnification of a single sarcocyst. Note prominent wall with fingerlike protrusions (arrow) and pale-staining metrocytes (arrowhead). Bar = 10 μm.

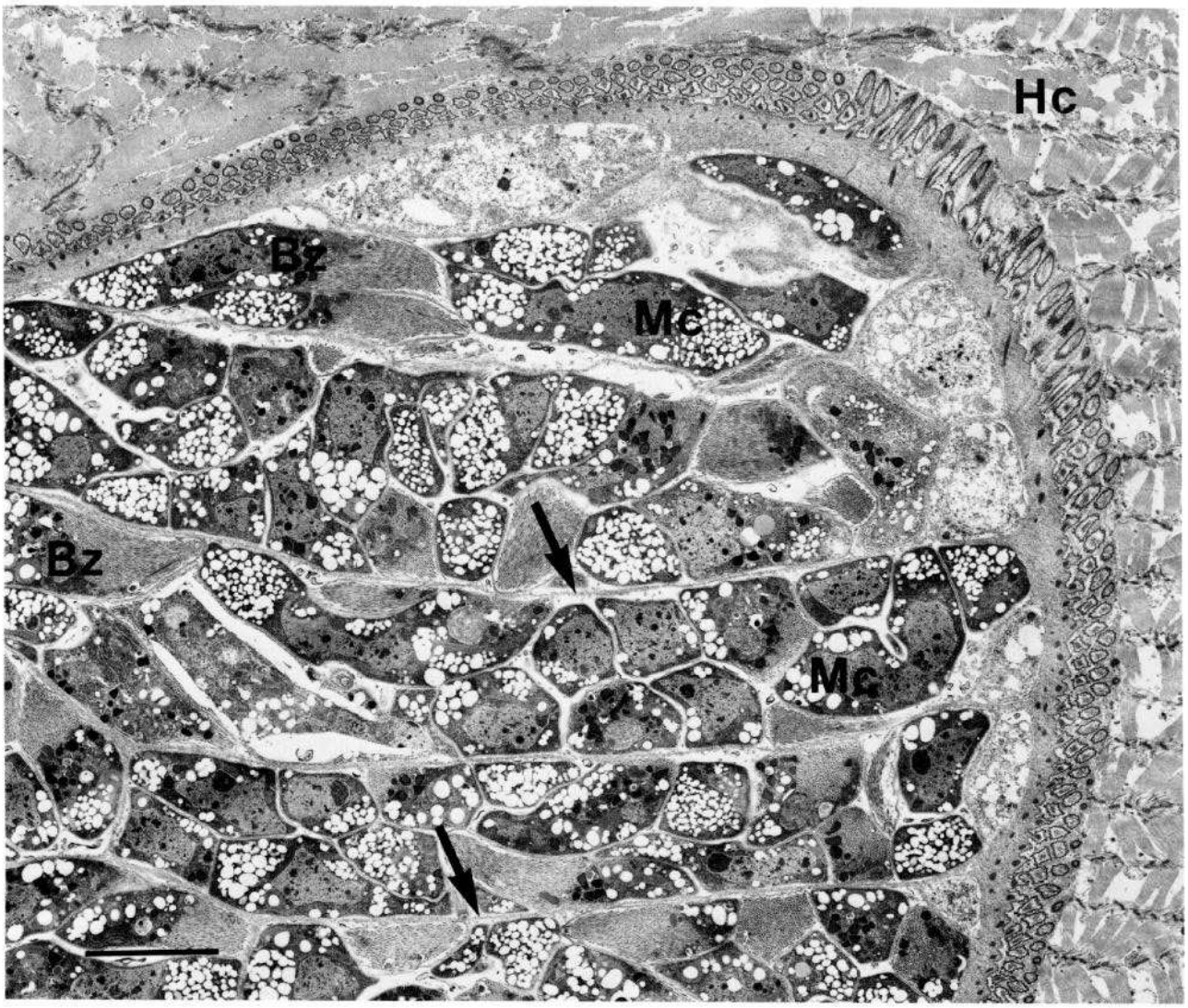

Figure 2. Transmission electron micrograph of a sarcocyst of *Sarcocystis fayeri* from the horse. Uranyl acetate and lead citrate stain. Note variable appearance of sarcocyst wall, depending on angle at which protrusions were cut. Septa (arrows), bradyzoites (Bz), metrocytes (Mc), and host myocyte (Hc). Bar = 5 μm.

sarcocysts (Fig. 1A). Sarcocysts were 50-500 μm long by 50-150 pm wide. There was no associated inflammatory response. There was acute muscle degeneration, ranging from loss of striations and a smooth homogenous appearance to fracture and fragmentation of muscle fibers.

Sarcocysts had a prominent wall with fingerlike protrusions, pale-staining globular metrocytes peripherally in the cyst, and numerous elongate bradyzoites filling the remainder of the sarcocyst (Fig. 1B). Ultrastructurally, sarcocysts had numerous protrusions forming the wall, the appearance of which varied with section, a few metrocytes peripherally, and numerous bradyzoites (Fig. 2). Septa divided sarcocysts into compartments. Bradyzoites were 10.6 ± 1.3 (8.8-12.3) μm long and 2.3 ± 0.3 (1.9-2.7) μm wide at the nucleus (n = 8) (Fig. 3A). Protrusions were 1.6 ± 0.16 (1.3-1.8) μm long and 0.3 ± 0.1 (0.2-0.4) μm wide (n = 10) at the midpoint (Fig. 3B).

The horse described herein had lesions consistent with prolonged cachexia associated with inadequate nutrition or chronic illness (i.e., sarcocystosis). Sarcocystosis is a variable mild disease of equids with signs and lesions including fever, apathy, anorexia, myositis, muscle weakness, autoimmune disease, or hair loss.[3] The association of sarcocysts and clinical myositis is unclear.[1]

Chronic sarcocystosis was previously described in a 3-year-old quarterhorse filly naturally infected with sarcocysts of *S. fayeri*.[8] The filly was ill for 2 weeks with weight loss, difficulty in chewing and swallowing, depression, ataxia, emaciation, and generalized muscular weakness. Histologically and ultrastructurally, there were numerous sarcocysts of *S. fayeri* in skeletal muscles. However, the mild lesions of myositis did not correlate with the profound muscular weakness. Although sarcotoxins and lectins are associated with bradyzoites from sarcocysts, the role of these substances in the pathogenesis of chronic sarcocystosis is not well defined. The interaction of tumor-necrosis factor and growth-regulating

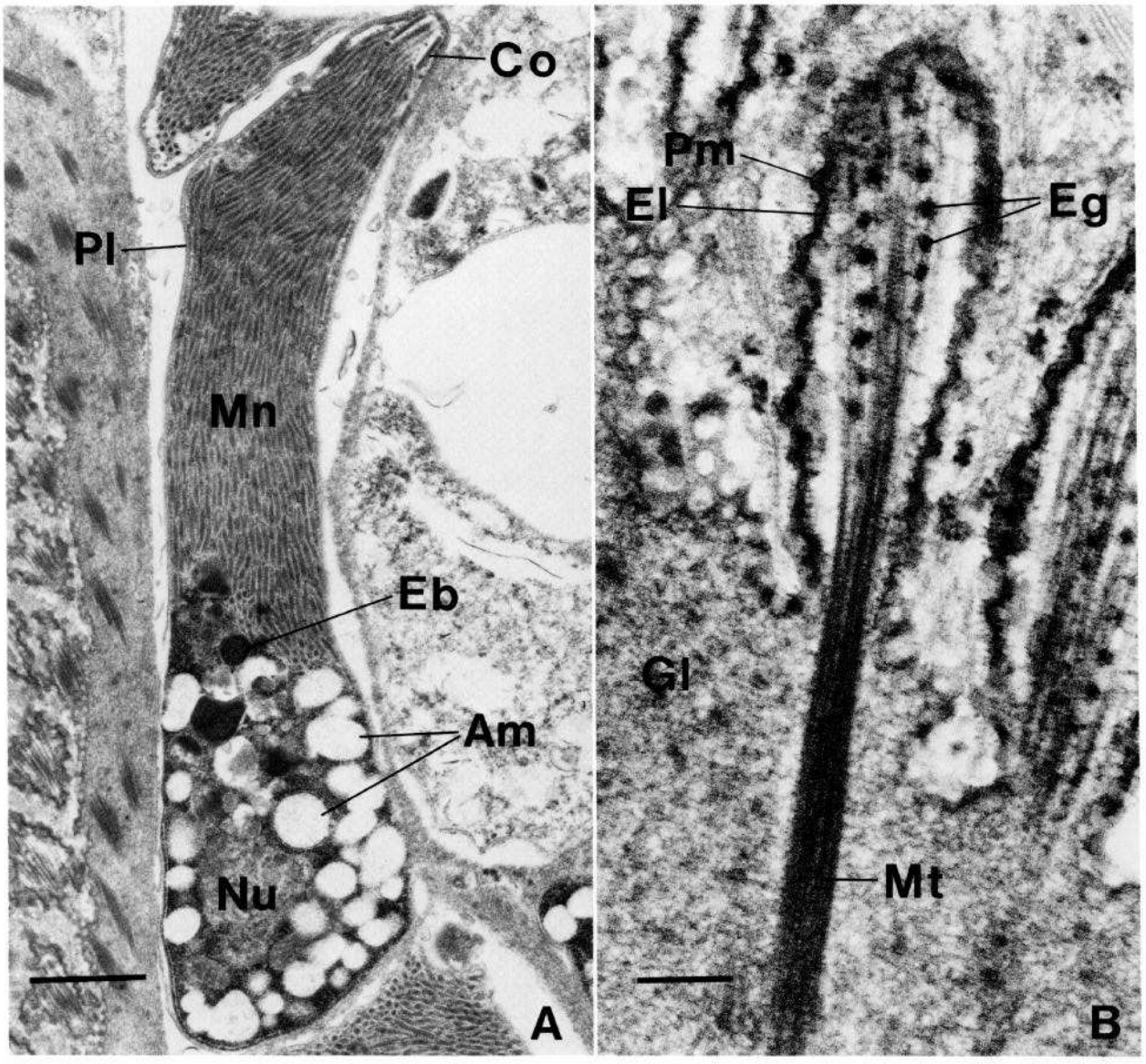

Figure 3. Transmission electron micrographs of a horse with sarcocystosis. Uranyl acetate and lead citrate stain. A. Bradyzoite of *Sarcocystis fayeri*. Note conoid (Co), plasmalemma (Pl), micronemes (Mn), electron-dense bodies (Eb), amylopectin (Am), and nucleus (Nu). Bar scale = 1 μm. B. Detail of protrusion of sarcocyst. Note parasitophorous vacuolar membrane (Pm), electron-dense layer (El), electron-dense granules (Eg), microtubules (Mt), and granular layer (Gl). Bar = 0.25 μm.

hormones in chronic sarcocystosis is unclear,[3] and the pathogenesis of chronic sarcocystosis and the influence of malnutrition on the effects of such parasitism are not known.[1]

We consider the parasite in the above case to be *S. fayeri*. We recognize, however, that the systematics of *Sarcocystis* species in equids is very confusing. This dilemma is complicated and perpetuated by inadequate descriptions, misinterpretations of original descriptions, the long growth period of sarcocysts, measurement techniques, and age (stage of maturity) of sarcocyst examined.[3] There is great need for complete transmission experiments to validate species descriptions of *Sarcocystis* in equids.

Acknowledgements. Drs. Holland and Wilcox, University of Guelph, critiqued our histopathologic interpretation. The study was supported in part by an operating grant from the Natural Sciences and Engineering Research Council of Canada to R. J. Cawthorn.

References

1. Cawthorn RJ, Speer CA: 1990, Sarcocystis: infection and disease of humans, livestock, wildlife and other hosts. In: Coccidiosis in man and domestic animals, ed. Long PL, chap. 6. pp. 91-120 CRC Press, Boca Raton, FL.

2. Cunningham CC: 1973, Sarcocysts in the heart muscle of a foal. Vet Rec 92:684.
3. Dubey JP, Speer CA, Fayer R: 1989, Sarcocystosis of animals and man, p. 215. CRC Press, Boca Raton, FL.
4. Edwards GT: 1984, Prevalence of equine *Sarcocystis* in British horses and a comparison of two detection methods. Vet Rec 115: 265-267.
5. Fayer R, Dubey JP: 1982, Development of *Sarcocystis fayeri* in the horse. J Parasitol 68:856-860.
6. Fayer R, Hounsel C, Giles RC: 1983, Chronic illness in a *Sarcocystis*-infected pony. Vet Rec 113:216-217.
7. Schneider T, Zimmermann U, Matuschka FR, et al.: 1985, Clinical signs, serum-enzyme activity and the dynamics of antibody titres in horses with experimental sarcosporidial infections. Zentralbl Veterinaermed Reihe B 32:29-39.
8. Tinling SP, Cardinet GH III, Blythe LL, et al.: 1980. A light and electron microscopic study of sarcocysts in a horse. J Parasitol 66:458-465.

J Vet Diagn Invest 2:345-347 (1990)

Fatal necrotizing encephalitis in a raccoon associated with a *Sarcocystis*-like protozoon

J. P. Dubey, Amir N. Hamir, Cathleen A. Hanlon, Michael J. Topper, Charles E. Rupprecht

Toxoplasma gondii and *Neospora caninum* are the only known coccidia to cause fatal encephalomyelitis in carnivores.[1,2] We report necrotizing encephalitis in a raccoon *(Procyon lotor)* caused by a protozoa distinct from *T. gondii* and *N. caninum*.

A juvenile female raccoon was one of 45 raccoons transported from Ohio for experiments concerning the development of an oral wildlife rabies recombinant vaccine for wildlife.[3,5] Upon arrival in Philadelphia, Pennsylvania, all animals appeared normal. Two days later, 1 raccoon exhibited neurologic signs. It was unsteady on its feet, had difficulty in maintaining its balance, and its head was constantly turned to the left. Over the next 4 days, the severity of illness became progressively worse. On day 7, it was anorectic, had a mucopurulent nasal and serous ocular discharge, and was moribund. The animal was euthanized and necropsied.

The carcass was dehydrated, the mucous membranes were pale, the lungs were consolidated, and the gastrointestinal tract was empty. A few tapeworms were seen in the intestines.

For diagnosis of rabies, samples of proximal cervical spinal cord and salivary gland were collected for virus isolation, and serum was used for the rabies fluorescent antibody test.[3] The whole brain and representative tissue samples of other organs were fixed in 10% neutral buffered formalin for histologic evaluation.

The fixed brain was cut into 5-mm-wide coronal sections. None of these sections revealed any gross lesions. However, when the sections were examined under ultraviolet light, several had randomly distributed 1-4-mm diameter foci of fluorescence in both the cerebral hemispheres. Some of these sections were stained with hematoxylin and eosin (HE) for histologic examination, and others were used for transmission electron microscopy.

Histologically, within the cerebrum there were multifocal and coalescing areas of necrotizing pyogranulomatous encephalitis (Figs. 1-3). These areas were characterized by infiltrates of neutrophils and foamy macrophages with fewer numbers of multinucleated giant cells, eosinophils, lymphocytes, and plasma cells. The neuropil, especially adjacent to the malacic foci, had marked perivascular cuffs consisting mostly of eosinophils. The cortical inflammatory reaction extended into the overlying meninges where the infiltrate was comprised predominantly of eosinophils and mononuclear cells. Multifocal eosinophilic meningitis was also seen in the cerebellum and brain stem where the necrosis was not present.

Within the malacic foci in the cerebrum, numerous developing stages of a protozoa were seen in the cytoplasm of macrophages, neurons, and multinucleated giant cells (Figs. 2, 3). The parasite divided by schizogony, a process of multiple nuclear division preceding cytoplasmic division. Uninucleate schizonts, with a darkly staining lobed nucleus, resembled pyknotic host cells (Fig. 3). Some multinucleated schizonts contained more than 20 nuclei. In some mature schizonts, merozoites were arranged in a rosette around a residual body (Fig. 3). Individual merozoites (4-5 × 1 μm) were also seen in mononuclear cells located within meningeal blood vessels. Organisms were located in the host cell cytoplasm without a parasitophorous vacuole. Individual merozoites had no rhoptries (Fig. 4). No organisms were found in other organs. The organism stained with anti-*Sarcocystis cruzi*[a] but not with anti-N. *caninum* and anti-T. *gondii* sera prepared in rabbits using an avidin-biotin complex immunohistochemical test.[4] Reagents and sera used for *T. gondii* and *N. caninum* have been described.[4,5] The *T. gondii* antibody titer in the raccoon's serum was 10 in the agglutination test using formalinized antigen.[2]

The epithelial cells of many organs (salivary, pancreatic and bile ducts and stomach) had variable numbers of eosin-

From the Zoonotic Diseases Laboratory, Livestock and Poultry Sciences Institute, BARC-East, ARS, USDA, Beltsville, MD 20705 (Dubey), New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348 (Hamir), Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104 (Hanlon, Rupprecht), and Pathology Division, US Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, MD 21702 (Topper).

Received for publication March 15, 1990.