

## TOXOPLASMOSIS IN CAPTIVE SAIGA ANTELOPE

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**Abstract:** Disseminated toxoplasmosis was diagnosed in saiga antelope (*Saiga tatarica*) from a Canadian zoo. Multiple foci of necrosis were found in most tissues of the animals. Toxoplasma cysts were found in association with these lesions. Toxoplasma trophozoites were recovered from mice injected with infected tissues. Subsequent to his examination of the infected saigas, enlarged lymph nodes in the author were diagnosed as toxoplasmic lymphadenitis.

### INTRODUCTION

*Saiga tatarica* is a member of the family *Bovidae*, and is native to Eurasia.<sup>8</sup> It roams in herds from the down reaches of the Volga River to Kazakhstan and Zungaria; and some are present in Mongolia. Because of near extinction due to hunting, the animal has been protected since 1920. The population of over 1 million today, is controlled by annual planned cropping.<sup>9</sup> The animal does not breed well in captivity and consequently enjoys quite a high monetary value in zoos of the world.

This paper describes the diagnosis of toxoplasmosis in three saiga antelopes submitted to the Manitoba Veterinary Laboratory, Winnipeg, Canada from a local zoo. Confirmation of the pathological diagnosis of toxoplasmosis by mouse inoculation is also described.

### MATERIALS AND METHODS

Three antelopes; two females 14 months of age and 6 years of age, and a small male 2 years of age were submitted for post-mortem examination. The two female animals had died following a short illness (less than 24 hours), and the male was euthanized after a protracted illness

of 6 days duration. The three animals had been anorectic and depressed. Terminally the male was weak and incoordinated. Various tissues were harvested, fixed in 10% formalin, paraffin embedded and stained with hematoxylin-eosin for histological examination. Ziehl-Neelsen's modified acid fast stain was applied to sections of mineral-containing lymph nodes of the male. Brown and Brenn's tissue Gram stain was applied to several sections which contained suspected toxoplasma cysts. Feces collected from the rectums post-mortem were examined for the presence of internal parasites by a flotation technique. Lungs, spleens, mesenteric lymph nodes, and small intestines were cultured aerobically at a temperature of 37 C for 24 hours on standard blood agar and on MacConkey's agar. Tissues (liver, spleen, kidney) from the two females were harvested and stored frozen at 10 F for 14 days and tissues (liver and lymph node) collected from the male were refrigerated at 40 F for 3 days. These tissues were then ground and 0.5 ml of the suspension from each sample was injected intraperitoneally into each of two mice. Three of the mice (one representing each sample) were euthanized 3 days post-injection and the other three were euthanized 10 days

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post-injection. A drop of peritoneal fluid collected from each mouse at necropsy was smeared onto a glass slide. These smears were air dried, stained overnight in Giemsa and examined for the presence of toxoplasma trophozoites.

## RESULTS

### Gross Pathology

The three saiga were deficient in body fat. The small amount of perirenal fat in the male had undergone serous atrophy. The thoracic cavities each contained 1 or 2 litres of serosanguinous fluid, and there was a slight excess of fluid in each of the peritoneal cavities and pericardial sacs. The lungs of all three animals were congested and very edematous. The airways contained copious, whitish froth. The heart of the male was dilated and flabby and there were numerous whitish 2 to 4 mm irregularly-shaped foci, subepicardially along the coronary grooves. The livers and spleens were swollen and congested, and there was an irregular whitish area of necrosis approximately 1 cm in size on the surface of the liver of the male. Visceral lymph nodes in the three animals were variably enlarged and the mesenteric nodes had large areas of necrosis (Figure 1). Adjacent lymphatic vessels were distended by a whitish cheese-like exudate. Gross intestinal lesions were confined to the younger female. The intestine of this animal was turgid and contained watery fluid. There were petechial and ecchymotic hemorrhages visible from mucosal and serosal surfaces.

### Histopathology

Microscopic foci of necrosis and inflammation were observed in all tissues examined including the brains, lungs, myocardium, skeletal muscle, subepicardial fat, lymph nodes, spleens, livers, bone marrow, intestines and adrenal glands. These foci were infiltrated by a variety of inflammatory cells (Figure 2), including lymphocytes, plasmacytes, mononuclear macrophages, occasional foreign body giant cells (Figure 3), and numerous neutrophils.

The kidneys also contained interstitial foci of lymphocytes and plasma cells. Very small (100 to 200  $\mu$ ) cerebral lesions (Figure 4) were found only in the laminae of the cerebral cortices. Inflammatory cellular reaction to the intracerebral lesions were minimal and consisted of focal areas of gliosis. The brain of the male was not examined.

The pulmonary lesions were extensive in two of the three animals and were consistent with those of granulomatous and interstitial pneumonia with extensive alveolar edema.

The hepatic and lymph node lesions were similar in all three animals. The early lesions in these tissues were typical of those seen in other organs but as the lesions aged they became very large (grossly visible in the male's liver and lymph nodes), caseous, partly mineralized, and were surrounded by a narrow band of granulomatous tissue. The portal triads in all three animals were infiltrated by lymphocytes and plasmacytes and in the more chronically affected male, there was considerable periportal fibrosis and bile duct proliferation.

Cystic structures, varying from 20 to 60  $\mu$  and containing numerous small (2 to 4  $\mu$ ) gram negative nucleated bodies were common (Figures 5 and 6). Occasionally a small number of these merozoite-like bodies was found within the cytoplasm of mononuclear macrophages. These phagocystosed bodies were usually within or closely associated with inflammatory foci in the involved tissue.



FIGURE 1. An incised mesenteric lymph node from the male saiga showing a large area of caseous necrosis (arrows).

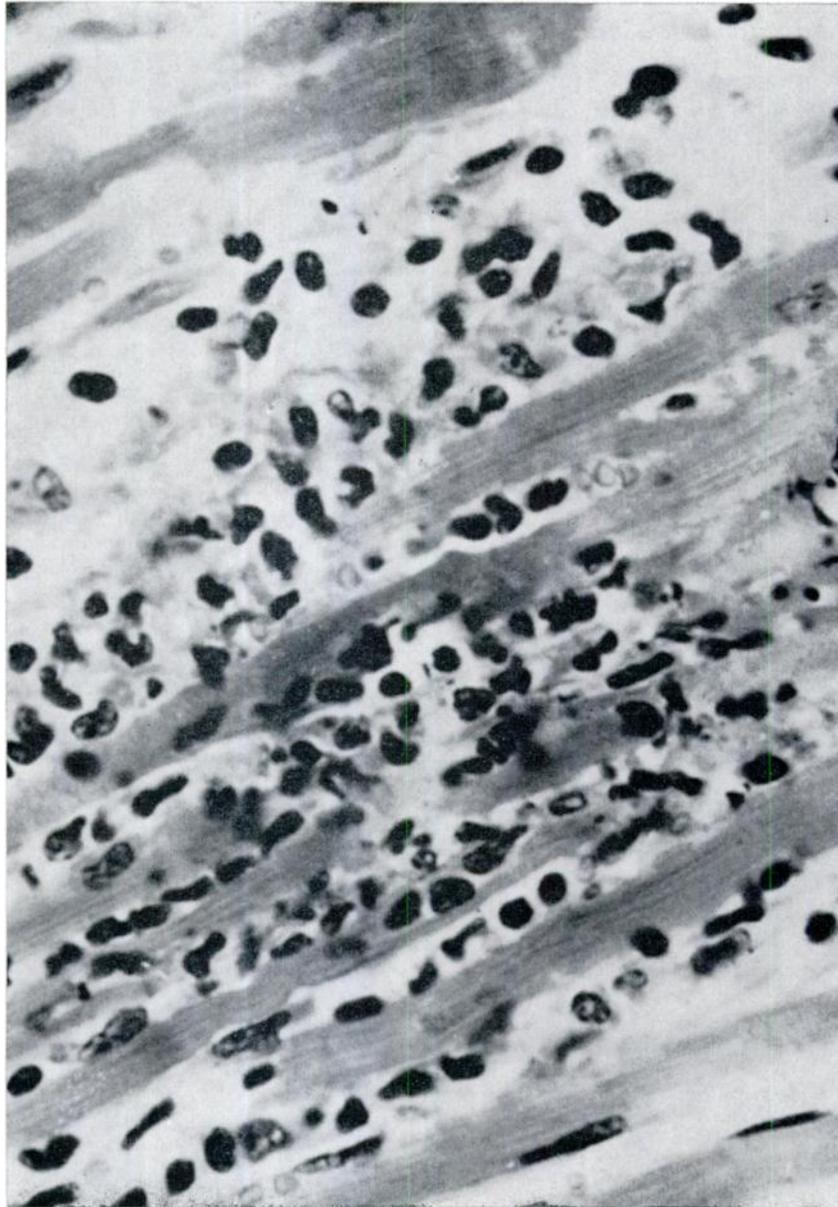


FIGURE 2. Fragmented, necrotic myofibres in the myocardium of the male saiga. Large numbers of inflammatory cells have infiltrated the area. H & E 400x.

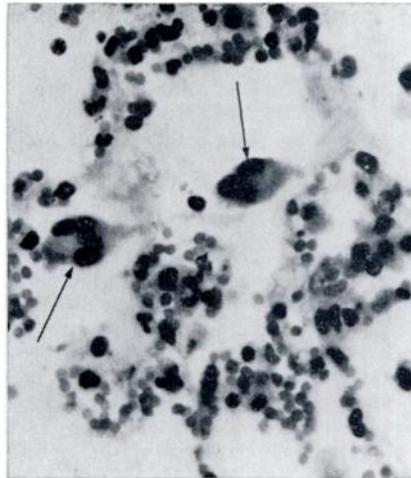


FIGURE 3. Two multinucleated giant cells in pulmonary alveoli. H & E. 400x.

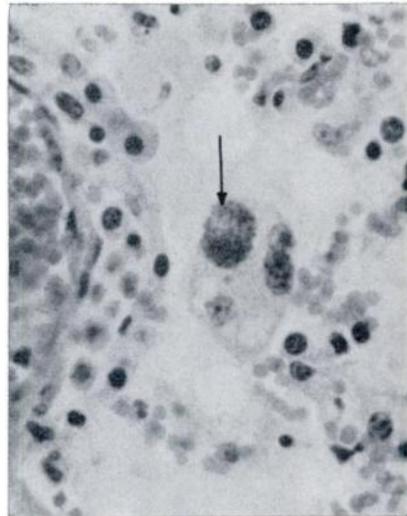


FIGURE 5. An intra-alveolar macrophage containing a toxoplasma cyst (arrow). H & E. 400x.

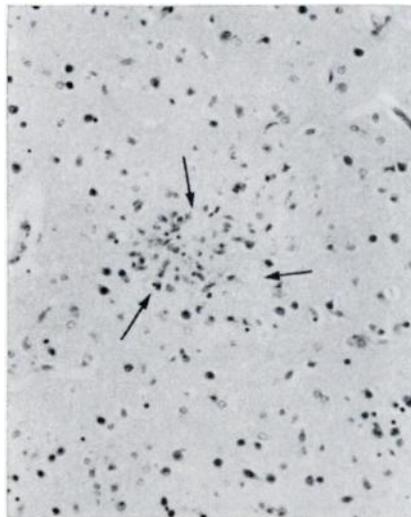


FIGURE 4. A typical lesion found in the brains of the two female saigas. The lesion consists of a focal area of necrosis infiltrated by glial cells. H & E. 100x.

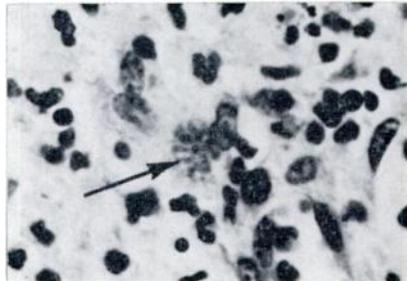


FIGURE 6. An area of lamina propria below an intestinal erosion in the 14 month female saiga. Showing an inflammatory reaction and a toxoplasma cyst (arrow). H & E. 630x.

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#### Bacteriology

A few colonies of hemolytic and non hemolytic *Escherichia coli* were cultured from the small intestine of the younger female. No significant bacterial growth occurred from other tissues of any of the animals.

### Fecal Examination

Few to moderate numbers of gastrointestinal helminth ova were found in the feces of the three animals. Parasite eggs were most numerous in the younger female and were in low numbers in the male. No attempt was made to identify the species of parasites which were present. Oocysts of coccidia were not found.

### Mouse Inoculation

Smears of peritoneal exudate from the mice inoculated with the refrigerated tissue suspension of the male saiga were air dried and stained overnight with Giemsa stain. These smears contained poorly-stained elongate crescent-like forms with an estimated length of 5 or 6  $\mu$  and breadth of 2 to 3  $\mu$ . An eccentric nucleus stained red and the cytoplasm was pale blue in color.

Organisms were not recovered from the peritoneal exudate of the four mice injected with fluid from the frozen tissues of the two female saigas.

Histological examination revealed no detectable lesions in any of the mice, including those from which peritoneal fluid yielded organisms.

### CONCLUSIONS

Based on the histopathological findings and the results of the mouse inoculation tests, a diagnosis of disseminated toxoplasmosis was made. Acid fast staining of tissues did not reveal acid fast rods, which diminishes the possibility of a mycobacterium being the causative agent. Organisms found in the various tissues were gram negative. This finding eliminates gram positive *Nosema* (*Encephalitozoon* sp.) as the causative organism.

### DISCUSSION

*Toxoplasma* has been known as a parasite of warm blooded animals including birds and man for more than 60 years.<sup>7</sup> A general pattern of infection occurs in most species. Following entrance of the infective organism into the

host's body a variable incubation period is followed by multiplication of the organism at the site of entry and then by parasitemia.<sup>8</sup> The parasitemic phase is followed by localization in various tissues and organs.<sup>8</sup> Then, depending on the ability of the strain of the organism to produce lesions in mice or other experimental animals,<sup>7</sup> the number of organisms involved, the species of the animal infected, its age and resistance, any of a number of syndromes will occur.<sup>9</sup> The expression of toxoplasmosis can vary from asymptomatic seroconversion, asymptomatic lymph node enlargement (local or generalized), intrauterine infection with abortion and/or various congenital defects, to a fulminating disease with fever, cachexia, variable nervous symptoms, and sometimes death.<sup>6,8</sup> All of these various expressions of infection occur in humans as well as in other animals.<sup>4,5</sup> However, only a small percentage of cases in humans result in severe disease,<sup>5</sup> the great majority result only in seroconversion and a lesser percentage in lymphadenopathy.<sup>5</sup>

Until recently, *Toxoplasma gondii* has been considered to be a protozoan having only two known forms, a trophozoite stage found in acute infections and a cystic stage found in chronic infections or coincidentally in tissues of animals that have died of other causes.<sup>2,6,8</sup>

Recent work<sup>2,1,5,7</sup> indicates that experimentally infected cats excrete in their feces an oocyst stage which resembles the oocyst of *Isospora bigemina*. Based on this finding, these workers<sup>1,2</sup> have postulated a life cycle for the organism with the cat as a primary host and other animals, including man, as intermediate hosts. They have classified *Toxoplasma* in the suborder *Eimeriorina* or *Eimeriina* as a member of the family *Toxoplasmiidae* with specific characteristics of the genus *Toxoplasma*.

The source of infection in the three saiga antelopes described in this report was not determined. However, 2 years previous to this case, the author had diagnosed toxoplasmosis in one of a number of wild *Felidae* kept in the same zoo, indicating the presence of the disease in the saigas' immediate environ-

ment. Evidence from previous work,<sup>1,2,5</sup> suggests that the saiga acquired their infection by ingesting either toxoplasma cysts or toxoplasma oocysts which had accidentally contaminated their food supply. The saiga enclosure at the zoo contained a very meagre growth of grass. To consume this grass it would have been difficult for the animals to avoid accidental consumption of their own feces, or fecal material from other enclosures confining members of the cat family and which could have been transferred by the feet of attendants or by wild birds.

The first of the saigas (the younger female) had intestinal ulcerations which contained the organism (Figure 6). It is possible that she encountered the infection first and then infected her pen mates by excreting infective organisms in her feces. This latter means of transmission between carnivorous animals has been suggested.<sup>6</sup> Perhaps it is also a method of transmission between herbivorous animals such as saiga antelope.

It is the author's impression that disseminated toxoplasmosis is a rare occurrence in members of the family *Bovidae*. Although statistics are not readily available, abortion and neonatal death in cattle, sheep and goats is reported more frequently than is death in adult animals of the *Bovidae*.

The infectivity of toxoplasma cysts in tissues can be eliminated by prolonged chilling or freezing of the infected tissues.<sup>3</sup> This fact is demonstrated here, in that suspensions from the male saiga produced toxoplasma trophozoites in injected mice, whereas tissues which had been frozen from the females produced no infection in mice.

An interesting sequel to this report is that, approximately 45 days after post-mortem examination of the three saiga antelope, the author developed painless enlargement of his left submandibular and left supraclavicular lymphnodes. No particular illness could be associated with the lymphadenopathy at the time it was first noticed, nor could any illness be recalled in the previous several months.

Finding nothing abnormal in routine examinations of peripheral blood or in

radiographs of affected lymph nodes and thorax, the author's physician recommended biopsy and histological examination of the affected supraclavicular nodes.

Lesions in these lymph nodes led to a presumptive diagnosis of toxoplasmic lymphadenitis which was later confirmed by serology. A Sabin and Feldman dye test performed 3 months after the enlarged lymph nodes were observed detected toxoplasma antibody in serum dilutions greater than 1:1024.

The lymph node lesion which is seen in cases of human toxoplasmic lymphadenitis is described<sup>7</sup> as being characteristic, but not pathognomonic. Similar lesions are seen in sarcoidosis and early Hodgkin's disease. In toxoplasmic lymphadenitis there are numerous prominent clusters of large epithelioid macrophages (Figure 7) which frequently occupy the center of germinal centers. There is a marked hyperplasia of lymphocytes which camouflage the normal architecture of the node, and lymphocytes and plasmacytes frequently invade the capsule of the affected node.



FIGURE 7. Photomicrograph of cortical area of the author's lymph node showing increased numbers of lymphocytes which invade the node capsule, (1) and clusters of plump epithelioid macrophages. (2) H & E. 100x.

The author was probably infected while performing postmortem examinations on one of the three animals. Although the usual precautions were taken to avoid contamination, it is recalled that while examining the last antelope, accidental splashing caused the author's face, including his left eye, to be sprayed with small droplets of thoracic fluid. One report in the literature<sup>5</sup> points out that workers in two laboratories worked for years with

the cystic and trophozoite forms of toxoplasma and maintained negative sera, but all have become serologically positive since they have been working with oocysts. This observation suggests the possibility that infection may have been acquired by contact with cat feces containing oocysts from any of a number of cats examined in the laboratory, rather than by contact with the infected tissues of the saigas.

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