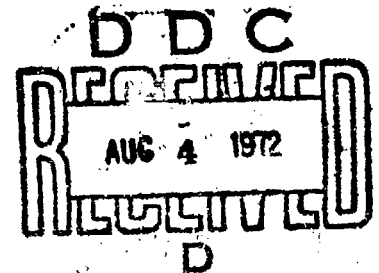


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Special Report 72-1

**NOSEMATOSIS IN A SQUIRREL MONKEY (Saimiri sciureus):
FIRST REPORTED CASE**

Richard J. Brown, Donald K. Hinkle, Walter P. Trevelyan, James L. Kupper,
and Adam E. McKee



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13. ABSTRACT A 2-month old squirrel monkey (<u>Saimiri sciureus</u>) succumbed following a month of frequent petit mal seizures. At autopsy the only gross abnormality was a separation of the parietal suture line of the cranium. Hematoxylin and eosin stains revealed multiple focal glial nodules throughout the brain. Granulomatous hepatitis and nephritis were also present. These lesions were not typical of any reported disease in the squirrel monkey. Special stains for mycosis, tuberculosis, and toxoplasmosis were unrewarding. The bacterial stains, however, revealed gram positive, slightly curved bacilli typical of the protozoal parasite <i>Nosema cuniculi</i> . Electron microscopy also revealed the <i>Nosema</i> organism. <i>Nosema</i> causes a clinically silent granulomatous encephalitis and meningitis in rabbits, rats and mice, and occasionally in other animals including man. The authors believe this to be the first case of nosematosis in the squirrel monkey.			

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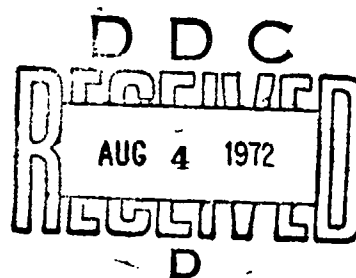
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Assistant for Scientific Programs

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Naval Aerospace Medical Research Laboratory
Naval Aerospace Medical Institute
Naval Aerospace Medical Center
Pensacola, Florida 32512

SUMMARY PAGE

THE PROBLEM

A 2-month old squirrel monkey (Saimiri sciureus) succumbed following a month of frequent petit mal seizures. At autopsy the only gross finding was a separation of the parietal suture line of the cranium. Hematoxylin and eosin stains revealed multiple-focal glial nodules throughout the brain. Granulomatous hepatitis and nephritis were also present. These lesions were not typical of any disease reported in the squirrel monkey.

FINDINGS

Special stains for mycosis, tuberculosis, and toxoplasmosis were unrewarding. The bacterial stains, however, revealed gram positive, slightly curved bacilli typical of the protozoal parasite *Nosema cuniculi*. Electron microscopy also revealed the *Nosema* organism. *Nosema* causes a clinically silent granulomatous encephalitis and meningitis in rabbits, rats, and mice, and occasionally in other animals including man. The authors believe this to be the first case of nosematosis reported in the squirrel monkey.

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Dr. Hinkle is Director of Animal Resources, Perrine Primate Research Laboratory, U. S. Public Health Service, Environmental Protection Agency, Perrine, Florida. Dr. McKee is the Comparative Pathologist, Naval Medical Research Laboratory, Bethesda, Maryland.

The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science-National Research Council.

INTRODUCTION

Nosema (*Encephalitozoon*) *cuniculi* is a gram positive, rod shaped, slightly curved protozoan with polar vacuoles. The microorganism usually elicits a striking granulomatous encephalitis and meningitis and focal interstitial nephritis in rabbits. Rats and mice are also affected with nosematosis but the granulomatous encephalitis is usually not seen (11).

The disease is nearly always clinically silent (2) and becomes apparent only upon the histological examination of the tissue. An occasional case has been reported in the dog, guinea pig, hamster (F. M. Garner, personal communication) shrew (6), and in man (5). The purpose of this documentation is to report the first case of nosematosis in the squirrel monkey.

PROCEDURE

An infant male squirrel monkey (*Saimiri sciureus*), orphaned at birth, was raised by hand in an incubator. At 1 month of age it began having spontaneous petit mal seizures several times a day for 3 to 4 days. There was no evidence of an infectious process or a history of trauma. In an effort to preclude a deficiency of vitamin D₃ this substance was provided orally. Five days following the first vitamin D₃ administration the seizures stopped. During the next 3 weeks the seizures appeared intermittently, and although the animal continued to eat, it failed to make expected weight gains. Approximately 1 month from the time of the first seizure the animal died. An autopsy was performed, with the only gross finding being a separation of the parietal suture line. The tissues were fixed in 10 per cent cold buffered formalin.

For light microscopy the tissues were embedded in paraffin. Seven-micron sections were stained with hematoxylin and eosin, MacCallum-Goodpasture gram stain, periodic acid Schiff stain, Ziehl-Neelsen acid fast stain, and the Gridley fungus stain.

For electron microscopy the formalin-fixed tissues were post-fixed in 1 per cent osmium tetroxide and Millionigs phosphate buffer, pH 7.4. Fixation was followed by dehydration in a graded series of ethanols and propylene oxide. Durcupan araldite was used as the embedding epoxy. Embedded tissue was cut at a thickness of 60 to 90 mμ on an LKB III ultramicrotome, stained with Reynolds lead citrate and 4 per cent uranyl acetate, and examined with a Philips 200 electron microscope at 40 kV.

RESULTS

The hematoxylin and eosin stain revealed multiple, focal, glial nodules within the brain (Figure 1), as well as a patchy lymphocytic meningitis and perivascular cuffing. Multiple, focal granulomatous hepatitis (Figure 2) and multiple, focal interstitial nephritis were also present. *Nosema* organisms were not identifiable on the hematoxylin and eosin stain; however, the MacCallum-Goodpasture technique demonstrated the *Nosema* organisms quite well (Figure 3) (9). *Nosema* has a strong gram-positive

affinity and contains easily seen polar vacuoles and chromatin granules. It is rod shaped, slightly curved, and with rounded ends. Its dimensions range from 1μ to 2μ in length and 0.5μ in width.

The organisms were consistently seen in the center of many of the glial nodules scattered throughout the brain (Figure 3) and in many cystic spaces within the brain (Figure 4). The organisms in the cystic spaces or vacuoles had not elicited an inflammatory response while other organisms in the same general area had caused the glial nodules to form. These may represent different stages of a developing lesion. The histological features of these nodules were essentially similar wherever they were encountered. Cysts or pseudocysts containing the organisms were found in the small granulomas of the liver (Figure 5) and kidneys. The mycotic, acid fast, and periodic acid Schiff stains were negative. A negative periodic acid Schiff stain is important in differentiating this organism from another protozoan that frequently affects the central nervous system, *Toxoplasma gondii*. *Toxoplasma* is periodic acid Schiff positive and can also usually be identified with the hematoxylin and eosin stain.

Details of the *Nosema* ultrastructure have been amply discussed by Petri and Schiodt (7), Hunger (1), and Kudo and Daniels (3). Only a few of the major characteristics of nosematosis will be pointed out here.

The outermost membrane of the parasite is a single undulant, uneven membrane (Figure 6, 7, and 8). Immediately beneath is a wide electron-lucid space. Below this is a delicate, double electron dense membrane enveloping a coarse granular substance with the appearance of ribosomes. This granular substance fills the entire parasite except for two well-defined internal structures. Figure 7 (parasite two), and Figure 6 display the circumferential laminations of the polarplast. In its center is a cross section of the polar filament. Figure 7 (parasite one) and 8 demonstrate the cross sections of the polar filament as it courses obliquely through the coarse granular substance. The outer membrane of the polar filament also has a double electron dense configuration with a more electron-lucid halo just inside it (Figure 8). The core of the filament is electron dense. The polar filament is reported to be unwound and extruded for germ material transfer during one phase of the life cycle (4).

DISCUSSION

Nosematosis has a long history of vitiating experimental results since the clinical diagnosis is difficult or impossible. In the early days of syphilis research, arsenicals were administered to rabbits in varying dosages for different periods of time to determine the effects of this compound on the experimental animals. The granulomatous encephalitis of nosematosis was present in the animals used in that toxicological research, and initially these central nervous system lesions were considered to be due to the effects of the arsenic compound (F. M. Garner, personal communication). The disease was again manifested in the 1920's when rabbits were used as experimental animals for research involving sleeping sickness. In the early months of World War II, the effect of high-altitude flying was being evaluated by the Army Air Corps; rabbits again were used as experimental models to determine what effects, if any, there would be on

living beings existing for extended periods of time at high altitudes. Once again the central nervous system lesions of nosematosis were present in those experimental animals, and for a time the granulomatous encephalitis was believed attributable to exposures to altitudes above 20,000 feet (T. C. Jones, personal communication).

Nosema has been known for many years to cause disease in so-called lower animals such as the bee (Isle of Wight disease) and the silkworm (pebrine disease). Information on the clinical signs of these diseases is lacking, but presumably a decrease in the production of honey or silk would be an early symptom. In primates (our case here) and the human patient of Matsubayashi et al. (5), clinical signs were apparent. The human patient was a Japanese boy with fever and cerebral symptoms. The organism was isolated by inoculating cerebrospinal fluid and urine into laboratory mice. The organism was recovered from the inoculated mice and maintained by serial passage in mice. Thirty control mice remained negative. Encephalitozoon-like organisms were identified in the cerebrospinal fluid and the urine of the patient. The patient's serum was negative for the Sabin-Feldman dye test (for toxoplasmosis), and he made an uneventful recovery 3 weeks later.

The life cycle or the manner in which nosematosis is perpetuated in nature is not clearly defined, but the evidence is strongly in favor of both contact and transplacental contagion. The occurrence of central nervous signs 1 month after birth in the present case suggests a transplacental infection. Since urine of infected animals usually contains organisms, and these can be identified in kidney sections stained by the MacCallum-Goodpasture method, the urine probably is the main route of excretion from the infected animals. Transmission of the disease between rabbits by ectoparasites has been suggested by one investigator (10).

In 1964 a 24- μ polar filament that is extruded by *Nosema cuniculi* was described (4). The end of this polar filament had a sarcoplast that was responsible for injecting sarcoplasm from the parent cell into an uninfected host cell. Binary fission is also reported to be a part of the life cycle. A host septicemia probably develops following binary fission. *Nosema* has not been cultivated on artificial media (8).

CONCLUSION

The authors believe this to be the first case reported of nosematosis in the squirrel monkey (*Saimiri sciureus*). The disease is usually a clinically occult disease in rodents and rabbits and thus vitiates experimental results that are based upon light and electron microscopic examination of the central nervous system, liver, and kidneys. *Nosema* is considered to be a protozoan and to have gram-positive characteristics.

A brief review of the one human patient has been included, as well as some of the facts known about the life cycle of *Nosema*. It is not improbable that nosematosis is an emerging zoonotic disease likely to be found more frequently. Hopefully, future work will develop a serological technique capable of diagnosing past or present infections and the antibiotic sensitivity of nosematosis can be determined.

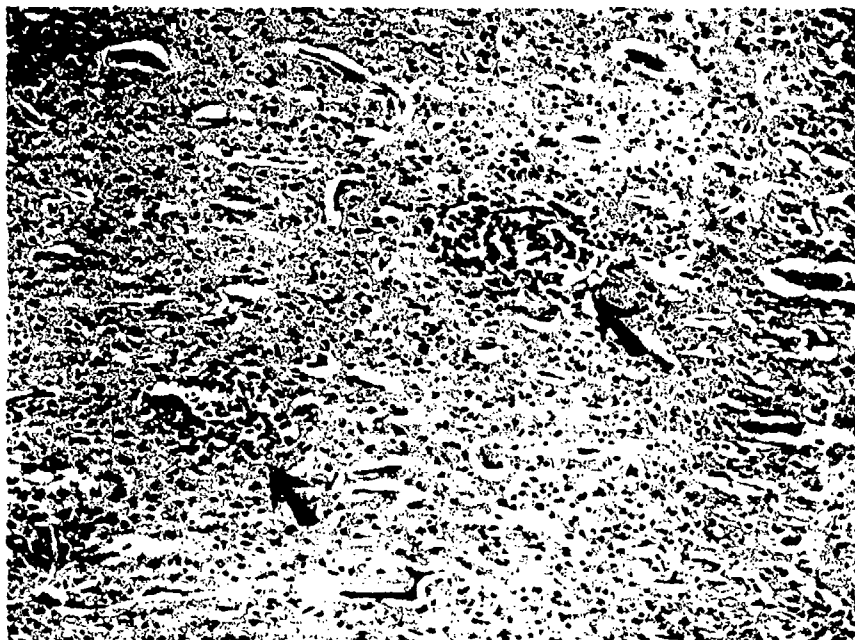


Figure 1

Multiple glial nodules typical of *Nosema* in the squirrel monkey cerebrum (arrows).
H & E. X 100

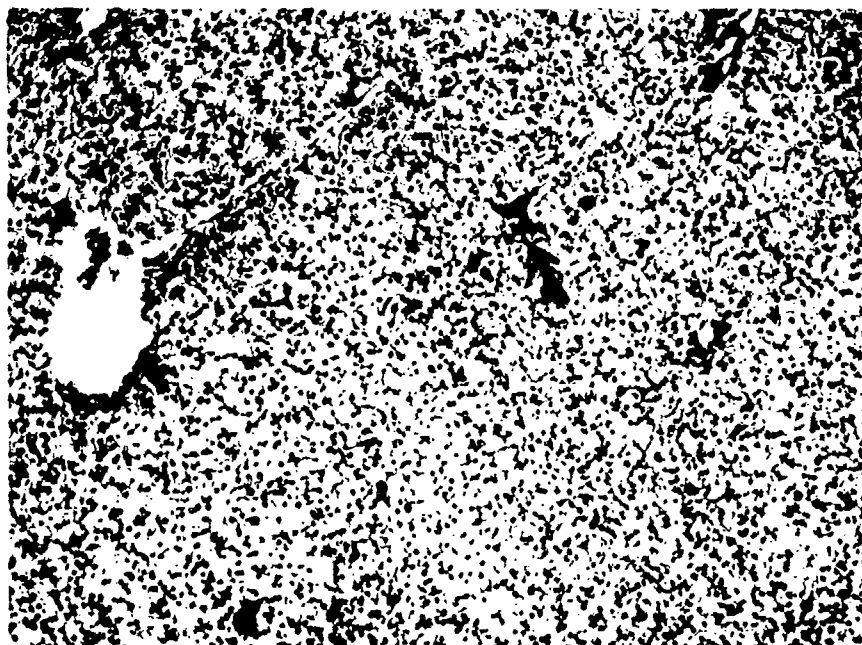


Figure 2

Multiple accumulations of lymphocytes and giant cells (arrow) containing the *Nosema* organisms, liver, squirrel monkey. MacCallum-Goodpasture. X 40.



Figure 3

Nosema organisms (arrow) in the center of a cerebral glial nodule, squirrel monkey. MacCallum-Goodpasture stain. X 1000.



Figure 4

Nosema parasites from a vacuole (V) in the central nervous system of the squirrel monkey. They are seen in longitudinal as well as in cross sections. (X 9100).

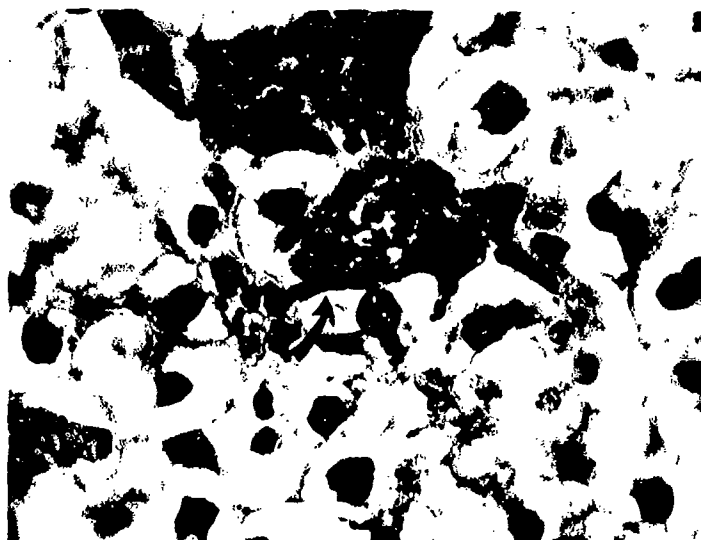


Figure 5

Liver, squirrel monkey, cyst adjacent to giant cell containing several *Nosema* organisms (arrow).
MacCallum-Goodpasture stain. X 1000.

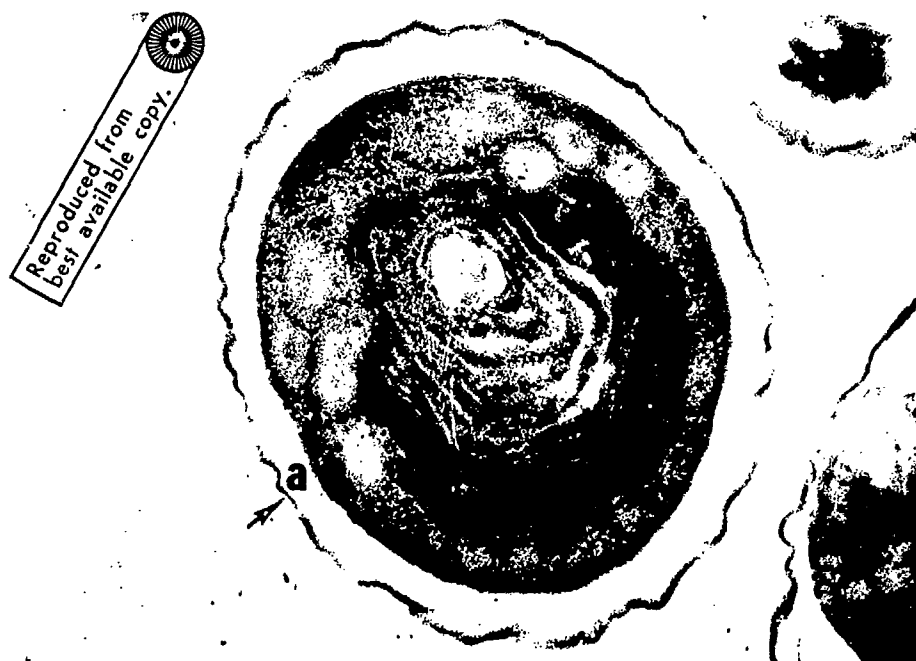


Figure 6

Higher power (X 46,600) of a single *Nosema* parasite. Below the undulant outer membrane (arrow) is an electron-lucid zone (a). Immediately below this is a delicate double electron dense membrane that appears to contain the coarse granular substance, suggesting ribosomes, which fills the parasite. The laminated polaroplast with the central polar filament is clearly visible here.

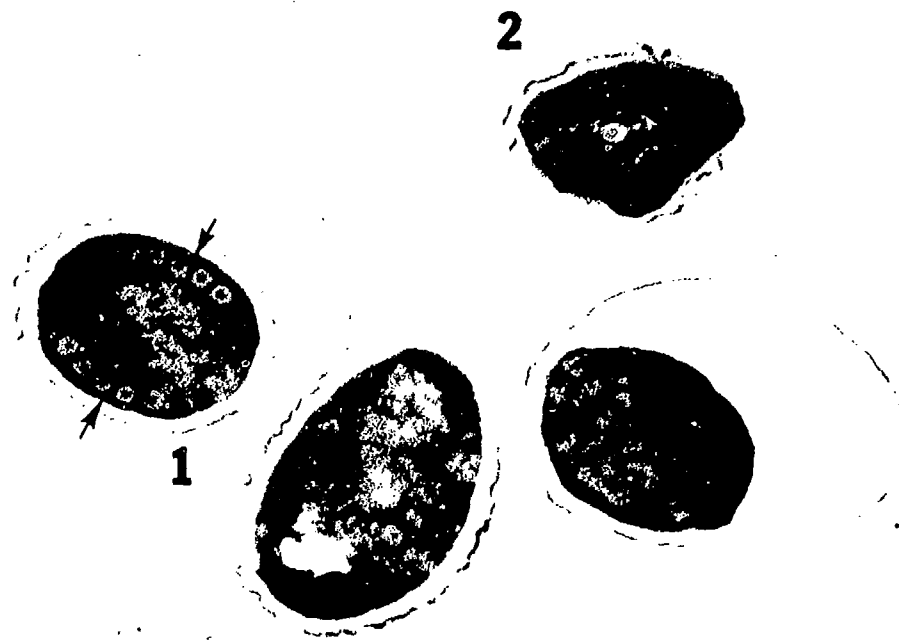


Figure 7

Nosema organisms at X 27,000. The cross section of parasite 1 demonstrates five cross sections of the polar filament (see also Figure 8) near each lateral border (arrow). A coarse granular substance with the appearance of ribosomes fills the diffuse part of the parasite. Parasite two demonstrates the circumferential laminations of the polarplast with presumably the anterior part of the polar filament in the center. (See also Figure 6.)

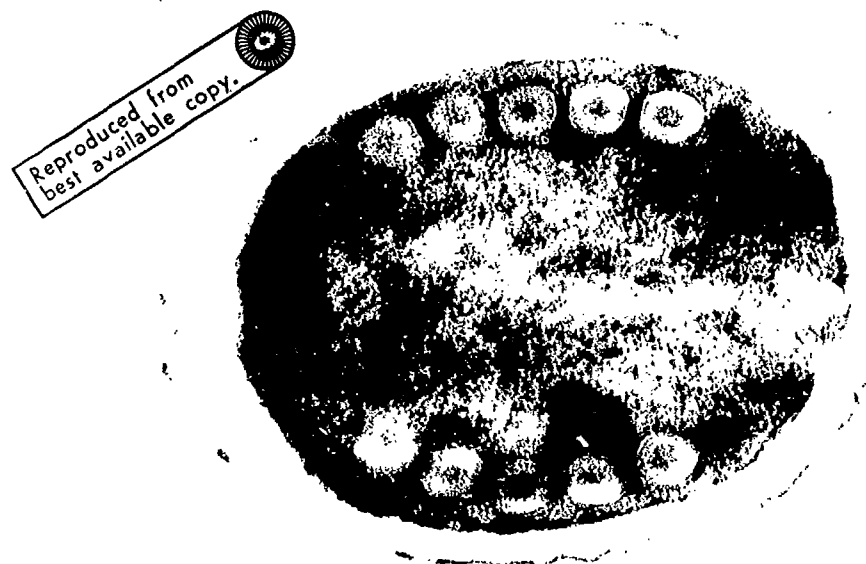


Figure 8

High power (X 46,600) view of the polar filament details. Its outer membrane is a double electron dense layer which surrounds an electron-lucid halo. The core of the filament is electron dense.

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