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What's your diagnosis?

Thread-like Worms in the Abdominal Cavity of a Squirrel Monkey

In the 1980s, during a routine necropsy on a female squirrel monkey (*Saimiri sciureus*), we found large numbers of long, slender worms in the animal's abdominal cavity. The monkey belonged to a colony of squirrel monkeys used for malaria research. It had been group-housed in a large home cage with enrichment toys, as a member of a cohesive social group consisting predominantly of adult females with their young and some juveniles and adult males. The room temperature was kept at $74 \pm 3^\circ\text{F}$ and the humidity was $60\text{--}70\% \pm 10\%$, in an attempt to mimic warm, humid jungle conditions. Air changes were set at 12–15/h and the room was on a 12 h light-dark cycle. Animal technicians fed the monkeys a commercial New World nonhuman primate (NHP) diet fortified with vitamin D3, along with fresh fruits and vegetables. An automatic watering system provided water ad libitum.

This female monkey had been in the colony for eight years and its individual history record indicated the facility had received it as a wild-caught monkey. During the eight years in the colony, it had given birth several times. The record did not show where in South America the NHP importers had captured the monkey. Instead, it identified the animal only as *Saimiri sciureus*. All squirrel monkeys were once considered a single species (*S. sciureus*) with several geographically separated subspecies; however, karyotypic and

phenotypic information gathered in the early 1980s led Hershkovitz¹ to propose classifying squirrel monkeys as a single genus with four species and nine subspecies. Further studies have supported this taxonomic classification. Although precise identification of squirrel monkeys often requires both phenotypic and karyotypic examination, we can generally divide squirrel monkeys into two groups based on the shape of the non-pigmented hair patch above the eyes. We now classify those belonging to the *S. sciureus* group as 'gothic arch' squirrel monkeys because they possess a pointed arch of whitish hair above each eye. We refer to the *S. boliviensis* group of squirrel monkeys as 'roman arch' because of the more shallow semicircular patterns above the eyes. Unfortunately, this phenotypic characteristic was not used in the individual history records to identify different species of squirrel monkeys. In retrospect, the breeding colony may have consisted of interbreeding Peruvian, Bolivian, and Guyanese squirrel monkeys.

The Principal Investigator had attempted to serially passage blood-stage malaria parasites to the monkey five years before, to induce a parasitemia. However, the animal did not develop a parasitemia and the investigator decided not to use the animal experimentally again, and kept it for breeding. Animal care technicians had recently noticed that the monkey would often sit on the floor of the cage, instead of the shelves or branches like the other monkeys in its group.

When we examined the monkey, it was bright and alert and appeared hydrated. We

FIGURE 1. Exposed abdominal cavity of an 8-year-old laboratory-housed, wild-caught, female squirrel monkey showing long, thread-like white worms on the liver. The worms can also be seen on the intestine and on the stomach.

weighed the animal and compared its present weight (0.72 kg) with a previous weighing six months earlier (0.85 kg). We notified the investigator of our findings and asked if he wanted further diagnostic work-up. Due to the animal's age, the investigator decided to euthanize the monkey and use its fresh organs for cell culture studies and her serum for immunological studies. We euthanized the monkey with an intravenous dose of phenobarbital after heavy sedation with ketamine.

When we opened the abdomen, we noticed the long slender worms on the surface of the liver and within the cavity (Fig. 1). There were no abnormal gross findings at necropsy. Can you identify the worms based on their gross appearance? What types of worms are most likely to be found in the abdominal cavity of New World monkeys? Where do the worms come from? Are the worms associated with the animal's weight loss? Do the worms pose a hazard to other monkeys or humans?

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Diagnosis:

Filariasis

The worms are known as 'filariae' and are adults of filarial nematodes that live in tissues outside the digestive tract. They are among the most highly evolved of the parasitic nematodes and belong to the superfamily Filarioidea. Within this superfamily are the family Filariidae (e.g., *Wuchereria bancrofti*, the cause of human elephantiasis) and the family Onchocercidae (e.g., *Onchocerca volvulus*, the cause of human river blindness; *Dirofilaria immitis*, the cause of canine heartworm).

Infection by these parasites, known as filariasis, is a common finding in necropsies of imported, wild-caught, South American monkeys. However, monkeys born and raised in an animal research facility are free of these parasites due to the absence of an arthropod vector. Long and slender adult filariae of *Dipetalonema* sp. are most often found (usually in large numbers) in the abdominal cavity of squirrel monkeys, spider monkeys, and cebus monkeys (Fig. 2). Sometimes a mild chronic peritonitis with fibrous adhesions is associated with the filariae. Adults of other filarial species, such as *Tetrapetalonema marmosetae*, can be found in the subscapular connective tissue of tamarins and marmosets.

The length of the adult worms can vary depending on the NHP host. For example, McCoy² has reported female worms of the species *Dipetalonema gracile* to range from 16 to 21 cm in a cebus monkey and up to 50 cm in a black spider monkey. The male

worm is much smaller than the female and distinguishable by its corkscrew-shaped tail.

The adult female filaria is viviparous and produces larvae, known as microfilariae, that are seen in the circulating blood or sequestered in tissues. Large numbers of microfilariae are routinely seen in blood smears of infected New World monkeys. Standard techniques identify microfilariae by measuring their length, width, and morphology. In some species, the first-stage larva retains the egg membrane and the morphology is described as 'sheathed'. In other species, the egg membrane ruptures and the larva is 'unsheathed' or 'naked'. Histochemical demonstration of acid phosphatase is used as an ancillary technique to aid in distinguishing microfilariae³; the naphthol AS-TR method activates a red azo dye at the site of acid phosphatase activity that is unique for each species of microfilaria⁴.

Chalifoux⁵ identified 11 distinct types of microfilariae in seven species of New World monkeys from 282 blood smears stained for acid phosphatase. Four species of microfilariae were specifically identified, and tentative identification was made for four other species. Multiple infections occurred in up to 62% of the blood samples examined. She reported that adults responsible for many of the microfilariae identified in blood smears were not found on necropsy of affected monkeys, despite careful searching.

FIGURE 2. Close up of adult filariae on the liver surface of the squirrel monkey shown in Fig. 1. Although the worms were not identified at the time, they most likely belong to the genus *Dipetalonema*.

An intermediate arthropod vector is necessary in the transmission of filariae. A bloodsucking arthropod ingests the circulating microfilariae and the second and third larval stages evolve within the arthropod. The third-stage larva is the infective stage that the arthropod then transmits to the vertebrate host. Many species of microfilariae are periodic and found in circulating blood only at certain times, usually nocturnally, when the arthropod vector is most likely to feed. Mosquitoes and black flies transmit filariasis to humans; black flies transmit onchocerciasis.

Although filariasis can be a serious disease in humans and other species, in New World monkeys the parasites and their hosts seem to have evolved a relatively compatible relationship. Chalifoux⁵ considers that despite the large numbers of circulating microfilariae found in some monkeys, they are surprisingly benign. In New World monkeys, a mild chronic peritonitis or pleuritis may occur from the presence of adult filariae and an occasional granuloma with foreign body giant cells occurs in response to a dead adult parasite.

There are many filarial parasites of animal species. The best known is *Dirofilaria*

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immitis, the dog heartworm. The adults are found in the right ventricle and cause right heart failure and pulmonary embolism. Acid phosphatase staining also differentiates the circulating microfilariae of *D. immitis* from the harmless, subcutaneously found canine filarial parasite *Dipetalonema reconditum*⁴.

Wuchereria bancrofti, found worldwide in tropical areas, and *Brugia malayi*, limited to tropical Southeast Asia, cause human lymphatic filariasis. About 90% of cases are bancroftian and < 10% are brugian filariasis. Cats and monkeys (macaques) can serve as reservoirs of strains of *B. malayi*. The World Health Organization (WHO) estimates that lymphatic filariae infect more than 120 million people, and the disease has seriously incapacitated and disfigured 40 million⁶. One-third of the people infected live in India, one third are in Africa and the remainder in South Asia, the Pacific, and the Americas. The prevalence of infection is continuing to increase. A primary cause of this increase is the rapid and unplanned growth of cities, which creates numerous breeding sites for the mosquitoes that transmit the disease. In its extreme manifestations, lymphatic filariasis causes elephantiasis, an enlargement of the entire leg or arm, and the genitals (noticeably the scrotum)⁶.

Onchocerca volvulus causes onchocerciasis, the world's second leading infectious cause of blindness. The disease manifests as a reaction to the presence of microfilariae in the skin (itching, acute and chronic papular onchodermatitis, fibrosis, atrophy, and depigmentation), and the eye (acute and chronic lesions in the anterior and posterior segment)⁷. Ninety-nine percent of cases are found in Africa and the other 1% in the Americas. At present, the WHO estimates that onchocerciasis affects more than 17.7 million people of whom approximately 500,000 have visual impairments, and 270,000 are blind⁷. Onchocerciasis is

often called 'river blindness' because of its most extreme manifestation and because the blackfly vector abounds in fertile riverside areas that frequently remain uninhabited for fear of infection. Other filarial species may cause dermatitis, eosinophilia, and fever in humans but seldom cause serious disease problems. Zoonotic filariae, such as the dog heartworm *Dirofilaria immitis*, rarely produce lesions in humans. Filariae from New World monkeys are not zoonotic.

The control of filariasis has relied on chemotherapy, vector control, and reduction of human-vector contact. Death of the adult worms produces a severe inflammatory response by the host that can be fatal. Treatment of onchocerciasis consists of an annual single dose of ivermectin, the same drug used to treat heartworm in dogs⁷. Merck and Co, Inc. (Whitehouse Station, NJ) has donated this drug for mass treatment "for as long as necessary" since 1987. The treatment strategy of lymphatic filariasis now consists of an annual, single-dose,

two-drug regimen of ivermectin+albendazole in countries that are coendemic for onchocerciasis, and of diethylcarbamazine + albendazole in all other countries⁶.

References

1. Hershkovitz, P. Taxonomy of squirrel monkeys genus *Saimiri* (Cebidae, Platyrrhini): a preliminary report with description of a hitherto unnamed form. *Am. J. Primatol.* **7**(2), 155–210 (1984).
2. McCoy, O.R. Filarial parasites of the monkeys of Panama. *Am. J. Trop. Med.* **16**, 383–403 (1936).
3. Chalifoux, L.V., Hunt, R.D., Garcia, F.G., Sehgal, P.K. & Comiskey, J.R. Filariasis in New World monkeys: histochemical differentiation of circulating microfilariae. *Lab. Anim. Sci.* **23**(2), 211–220 (1973).
4. Chalifoux, L. & Hunt, R. D. Histochemical differentiation of *Dirofilaria immitis* and *Dipetalonema reconditum*. *J. Am. Vet. Med. Assoc.* **158**(5), 601–605 (1971).
5. Chalifoux, L.V. in *Nonhuman Primates* (eds. Jones, T.C., Mohr, U. & Hunt, R.D.) 206–214 (Springer-Verlag, Berlin, 1993).
6. Mabey, D., Peeling, R.W., Ustianowsk, A. & Perkins, M.D. Diagnostics for the developing world. *Nat. Rev. Microbiol.* **2**(3), 231–240 (2004).
7. Lazdins-Helds, J.K., Remme, J.H.F. & Boakye, B. Focus: Onchocerciasis. *Nat. Rev. Microbiol.* **1**(3), 178–179 (2003).