

Cerebrospinal angiostrongyliasis in five captive tamarins (*Sanguinus* spp)

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Four cotton-top tamarins (*Sanguinus oedipus oedipus*) and one emperor tamarin (*S imperator subgriseus*) housed in a zoo became depressed, anorexic, paraparetic and eventually paralysed. The animals died within 5 days to 18 months of the appearance of clinical signs. Histological examination showed nonsuppurative and eosinophilic meningoencephalitis, and metastrongyle nematode larvae were found within subarachnoid spaces of all animals and within the spinal cord of one. Intact larvae with features consistent with *Angiostrongylus cantonensis* were recovered from the brain of one animal. This parasite is the classical cause of eosinophilic meningoencephalitis in many parts of the world and the diagnosis can be strongly suspected on clinical grounds. In endemic areas like south-east Queensland, protection of captive animals against infection with *A cantonensis* is a difficult balance between providing a stimulating, natural setting and eliminating potentially infectious definitive, intermediate and paratenic hosts. This is the first report of cerebrospinal angiostrongyliasis in tamarins and nonhuman primates in Australia.

Aust Vet J 1998;76:167-170

L3 Third-stage larvae
CNS Central nervous system

L4 Fourth-stage larvae

Tamarins are small, omnivorous, arboreal, nonhuman primates native to a wide range of forest habitats in South America (Figure 1). They eat fruits, flowers, nectar and leaf buds and spend over 25% of their time foraging in trees for small animals such as insects, spiders, snails, frogs and lizards.¹

Angiostrongylus cantonensis lives in the pulmonary arteries of several rat species in many tropical and subtropical regions.^{2,4} Eggs released by adult female worms are trapped in pulmonary capillaries, where first stage larvae develop and hatch, then pass via alveoli and airways to the gut, to be eliminated in the faeces. When larvae are ingested by intermediate hosts, which include many species of snails and slugs, they develop to the infective L3 in various tissues. Rats acquire infection by eating intermediate hosts or paratenic hosts, such as terrestrial planarians or freshwater crustaceans that harbour L3. Once in the rat, the L3 enters the circulation and, on

arriving in the CNS, migrates through tissues (moulting to the L4 in the process) to reach the subarachnoid space, where the final moult occurs. Immature adult worms re-enter the venous circulation by penetrating veins exiting the skull or the spinal canal to reach pulmonary arteries.^{2,3}

In accidental hosts such as primates, larvae undergo a similar migration, but only rarely reach pulmonary arteries and do not mature to produce eggs.^{5,6} In

eastern Australia, neural angiostrongyliasis has been reported from humans,^{7,8} dogs⁹⁻¹¹ and macropods.¹² The clinical manifestations of eosinophilic meningoencephalitis caused by migrating larvae reflect the focal nature of the neurological trauma and the accompanying host inflammatory response to larval antigens.^{4,13}

We here report for the first time cases of cerebral angiostrongyliasis in nonhuman primates in Australia. This is also the first report of the infection in tamarins.

Clinical reports

All the tamarins were bred in captivity in Australia and housed in a lushly vegetated outdoor enclosure in a private zoo on the northern outskirts of Brisbane.

Cases 1 to 4

These were adult, cotton-top tamarins (*S oedipus oedipus*). Three males arrived at the zoo in July 1994. Two tamarins (cases 1 and 2), aged 3.2 and 4.2 years, were presented in December 1994 with anorexia, ptialism, lethargy, hindlimb ataxia and inability to climb. They spent most of the time lying on the floor of the enclosure and had lost a quarter of their body weight within a month, despite antibiotic therapy and special feeding. By January 1995 their condition had improved, and they were climbing again and catching and eating



Figure 1. A cotton-top tamarin (*Sanguinus oedipus oedipus*).

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grasshoppers. However, in April 1995 they developed diarrhoea and urinary incontinence and died within a day of each other.

The third male, aged 5.5 years (case 3), was presented in July 1995 with hind-limb ataxia. Although active, it was unable to climb and stayed on the floor of the enclosure. It had anorexia, rhinorrhoea and periorbital oedema and its coat was in poor condition. It was treated with flumethasone and was climbing again by September. Two weeks later, the tamarin was observed combing the tree branches and eating what appeared to be a snail. It became ataxic within 4 weeks of this. By January 1996 it developed hindlimb paralysis and tail chewing and died 5 days later.

Case 4, a 4.5-year-old female tamarin, arrived at the zoo in August 1995 and 2 months later developed hindlimb ataxia. Most of the time it layed stretched out on the enclosure floor, but maintained good appetite. Four days after treatment with flumethasone it developed tremors, became ataxic and disoriented and died a day later.

At this stage, all the cotton-top tamarins in the enclosure had died.

Case 5

This was a 6-year-old male emperor tamarin (*S imperator subgrisescens*), one of three acquired by the zoo in May 1993 and kept in an enclosure adjacent to the cotton-top tamarins. It developed diarrhoea, hind-limb ataxia and periorbital oedema in January 1996, but improved after treatment with flumethasone. It died unexpectedly in July 1997. The two remaining emperor tamarins are currently in excellent condition.

Pathological findings

Necropsy was carried out on all tamarins. Cases 1, 2 and 3 were emaciated while cases 4 and 5 were in good body condition. All tamarins had moderately congested cerebral meninges.

Case 1

Histological sections of the brain showed extensive, mild, multifocal meningitis over the cerebrum and cerebellum. Inflammatory cells in diminishing order of frequency included macrophages, plasma cells, lymphocytes and eosinophils. Nematodes were found

adjacent to cerebral blood vessels in the subarachnoid space. They were associated with minimal inflammation. The worm sections (Figure 2) appeared consistent with a metastrongyle nematode: coelomyarian-polymyarian musculature, prominent lateral cords, thin 3 to 4 μm cuticle, developing gonads and sparse, multinucleated intestinal cells.¹⁴

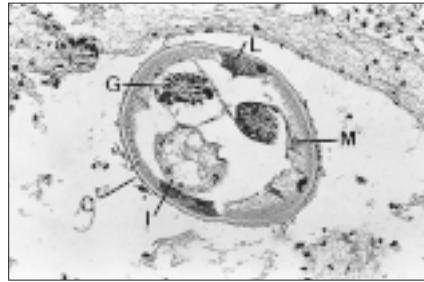


Figure 2. Section from brain of cotton-top tamarin (case 1), showing immature adult *Angiostrongylus cantonensis* (arrow) within inflamed meninges. G = gonad, L = lateral chord, M = somatic muscle, I = intestine, C = cuticle. Maximum diameter of nematode 220 μm . Haematoxylin and

Case 2

Multifocal meningitis was similar to that in case 1. Sections of a partially collapsed nematode, with intact refractile cuticle and coelomyarian-polymyarian musculature, were seen within an intense focus of inflammation in a similar pattern to case 1, adjacent to congested vessels in the cerebral subarachnoid space.

Case 3

As in previous cases, histological sections showed mild, diffuse inflammation within cerebral and cerebellar meninges. Immediately underlying meninges near the lateral cerebellar nucleus, there were six confluent 200 to 300 μm circular areas of malacia, each containing a few foamy macrophages and surrounded by mild inflammation. Deep within diencephalic white matter there were multiple sections of a worm with a collapsed refractile cuticle and with little associated inflammation. A similar section containing gonads was found within the caudal cerebellar peduncle.

Moderate meningitis was also found throughout the spinal cord, where

multiple foci of malacia often extended between the meningeal surface and grey matter. These foci contained mild haemorrhages, macrophages and eosinophils. There was also extensive haemorrhage within the central canal, multifocal Wallerian degeneration and mononuclear perivascular cuffing. A cluster of worms with collapsed cuticles, associated with intense focal meningitis, was found in the thoracic region.

Case 4

Inflammatory changes were as found in cases 1 and 2, and multiple sections of intact nematodes were found, similar to those described above, within the subarachnoid spaces of the cerebral occipital lobe and adjacent cerebellum.

Case 5

Histological sections showed mild, diffuse cerebellar meningitis and moderate choroiditis of the third ventricle. Within the mesencephalic subarachnoid space, adjacent to the hippocampus, multiple sections of nematodes with collapsed cuticles and degenerate internal structures were seen, surrounded by mild inflammation. Sections of immature metastrongyle nematodes surrounded by intense inflammation were also found in the superficial white matter of a cerebellar folium. In spinal cord sections, mild, multifocal, nonsuppurative meningitis was seen, but neither worms nor areas of malacia were found.

Identification of worms

About 80% of the brain of the tamarin in case 1 was gently pulverised in a loose-fitting Dounce glass homogeniser. The homogenate was centrifuged at 1000 g and the resulting loose pellet was examined under a stereo microscope. Two 7 mm long immature adult nematodes were found, indicating that at least 3 weeks had elapsed since ingestion.¹⁵ The head was simple, without a buccal capsule, and the mouth opened directly into the oesophagus (Figure 3). The tail tip of a female worm did not terminate in a mucron (pointed projection) (Figure 4), as seen in *A mackerrasae* (see Discussion). Spicules of a male adult worm were 1200 μm long, consistent with the anatomical features of *A cantonensis* (Figure 5).²



Figure 3. Anterior extremity of immature adult *Angiostrongylus cantonensis* removed from brain of cotton top tamarin (case 1). Oesophageal length 220 μm .

Environmental survey

Mammals of several different species housed near the tamarin enclosures have not been affected. These include the common marmoset (*Callithrix jacchus*), black-handed spider monkey (*Ateles geoffroyi*), rhesus macaque (*Macaca mulatta*), crab-eating macaque (*M. fascicularis*), fallow deer (*Cervus dama*), Arabian camel (*Camelus dromedarius*) and koala (*Phascolarctos cinereus*).

After necropsy of the tamarins in the first two cases, the immediate environment of the enclosures was inspected for molluscan and planarian habitats. All vegetation and plant pots containing molluscs were removed. All vegetable foods were carefully washed and the enclosure floor mulch was immediately replaced and renewed monthly. Although there was little physical evidence of rats, efforts were made to eliminate them from the area. In one adult *Rattus rattus*, trapped in vegetation near the enclosure and dissected, six nematodes were found in pulmonary arteries. This was strongly suggestive of *A. cantonensis* infection but the nematodes were not identified.



Figure 4. Immature adult female *Angiostrongylus cantonensis* removed from brain of cotton-top tamarin (case 1). Absence of mucron at tail tip (arrow) distinguishes this species from *Angiostrongylus mackerrasae*. Length from anus to tail tip 210 μm , maximal body diameter 230 μm .

Snails and slugs collected from the surrounding gardens were identified as *Helix aspersa* (an introduced European garden snail), *Bradybaena similis* (an introduced Asian snail) and *Limax maximus* (an introduced leopard slug).

Discussion

There is little doubt that the deaths of the tamarins described here resulted directly from eosinophilic meningoencephalitis secondary to intracranial nematode infection. While parasitic nematodes can not be definitively identified in tissue sections, our findings were entirely consistent with *A. cantonensis* infection and the morphological features of the dissected intact worms (Figures 4, 5 and 6) also indicated this species rather than *A. mackerrasae*.² The latter occurs in Australia,^{2,10,15,16} and can infect nonhuman primates, although its role in human infections has not been established.¹⁵

The five tamarins described here died at widely varying intervals after the onset of clinical signs. Fatal and chronic angiostrongyliasis have been described in experimentally infected nonhuman primates¹⁵ and in a naturally infected, captive white-handed gibbon (*Hylobates lar*) and howler monkey (*Alouatta caraya*) in south-eastern USA.¹⁷ It occurs occasionally in humans, dogs and some macropods.^{8-10,12,13,18}

The neurological signs exhibited by these tamarins were consistent with *A. cantonensis* infection,^{4,9,12,17} and suggest a combination of meningeal irritation with cerebral, cerebellar, cranial nerve and spinal nerve root lesions. Typically, clinical signs include depression and flaccid hind limb paresis or paralysis, which can extend to the forelimbs. The protracted nature of illness and its apparent relapsing course may have been the result of repeated exposure to infection (see below).

Urinary incontinence, as observed in the first two cases, has been reported in affected dogs⁹ and humans.¹⁹ Gastrointestinal signs observed in the tamarins, including poor appetite, vomiting and weight loss, agree with findings from humans^{4,19} and may be related to larvae penetrating the intestinal mucosa in early infection⁹ or to increased intracranial pressure.¹⁹ Refusal of food and weight loss has been seen in affected Taiwan monkeys

(*M. cyclopis*)¹⁵ and were a feature of some of the cases reported here.

Rhinorrhoea (case 3) and ptialism (cases 1 and 2) have also been reported in humans.¹⁸ Ptialism may be associated with difficulty in swallowing, which has been reported to occur 27 days after ingestion of infective larvae in humans.²⁰ Periorbital oedema (cases 3 and 5) is uncommon in human angiostrongyliasis.^{19,21}

Provisional diagnosis is suggested by clinical signs and eosinophilia in CSF in the appropriate epidemiological setting, for there are few other causes of eosinophilic meningoencephalitis.⁴ However, collection of CSF is very difficult in mammals as small as tamarins. Eosinophilia of the CSF in humans peaks at 25 to 30 days after exposure and again at 75 to 80 days.¹⁹ The peak in experimentally infected Taiwan monkeys occurs at 14 to 28 days.¹⁵ Immunoassay has been used to diagnose human cases, but there is cross reactivity with other nematodes⁸ and the tests are poorly characterised.⁴



Figure 5. Immature adult male *Angiostrongylus cantonensis* removed from brain of cotton-top tamarin (case 1). Spicule length 1200 μm (arrow).

The tamarins are highly predisposed to angiostrongyliasis because of their natural tendency to actively eat slugs and snails,¹ compared to the other, larger, nonhuman primates which eat fruit and leaves, with or without insects and small animals.^{22,23} The common marmoset is similar in size to the tamarin, but eats mainly fruit and flowers and, when fruit is in short supply, tree gum and small animal prey including spiders and insects.¹ Being well supplied with fruit, the zoo marmosets were less likely to eat molluscs than the tamarins, who actively seek out small animals despite adequate supplies of other foods.

Herbivorous fallow deer, Arabian camel and koala were not obviously infected, although inadvertent infection has been reported in herbivores and omnivores; for example cerebral angiostrongyliasis has been diagnosed in foals.²⁴

The necropsy and histological findings were similar to those reported from other accidental hosts.^{4,12,17} Incubation times in humans vary from 3 to 36 days²¹ and, in experimental infection of Taiwan monkeys, meningitis is most intense by 24 to 32 days.¹⁵ The long intervals from onset of first neurological signs to death in four of the tamarins (4.5 to 18 months) and the finding of intact and partially degenerating larvae in their brains suggest repeated infection. The extent of direct trauma and inflammation caused by migrating worms varies with the number of infective larvae consumed in each episode.¹⁵ Thousands of infective larvae can be harboured in a single snail.⁸

It is possible that these tamarins may have had many *A. cantonensis* in their brains, because larvae were readily detected in the small sample of brain examined histologically.

The onset of clinical signs in these cases indicate that primary infection occurred mainly in summer (December to January), which is similar the situation in dogs in this area.¹⁰ Rainfall data show daily precipitation of 6 to 94 mm in the period 5 to 22 days before onset of signs in affected tamarins, and of 12 to 26 mm over the 8 to 19 days before death in the chronically affected tamarins (cases 1, 2, 3 and 5).

Snails and slugs which became mobile immediately after heavy rainfall near the tamarin enclosure were identified as *H. aspersa*, *B. similaris* and *L. maximus*. *B. similaris* is an intermediate host for *A. cantonensis* in Malaysia¹⁵ and the other two species have been found in Sydney where several dogs have been diagnosed with neural angiostrongyliasis.¹¹ *A. cantonensis* undoubtedly occurred in *R. rattus* around the tamarin enclosure, as it does elsewhere in Brisbane.⁸

There is no definitive treatment for cerebral angiostrongyliasis.⁴ Anthelmintics are contraindicated in dogs⁹ and of doubtful efficacy in human infections.¹³ Corticosteroids are often used to reduce intracranial pressure and inflammation. They appear effective in dogs,⁹ but less effective in humans.^{4,13,19} Flumethasone did not prevent the death of these tamarins.

Theoretically, this infection could have been prevented by eradicating definitive, intermediate and paratenic hosts from the vicinity. However, this is an unrealistic goal given the need to maintain an interesting, forested habitat for captive tamarins. Measures to control rats, already in place at this zoo, are being improved in an attempt to achieve cost-effective long-term elimination of infection.

Acknowledgments

We are grateful to Mrs Jennifer Coombs for technical assistance, Mr Darryl Potter, of the Zoology Section of The Queensland Museum, for identifying molluscs, and the Climate and Consultancy section in the Queensland Regional Office of the Bureau of Meteorology, for rainfall data.

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(Accepted for publication 24 November, 1997)