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ORIGINAL ARTICLE

Biochemical changes in cerebrospinal fluid of *Chlorocebus aethiops* naturally infected with zoonotic *Meningonema peruzzii*

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Abstract

Background Thirty-four wild *Chlorocebus aethiops* monkeys were trapped for research purposes.
Methods During routine quarantine check-up, cerebrospinal fluid (CSF) and blood were microscopically examined for parasites. Estimations of CSF protein levels were made by the biuret method and the white cell counts by the hemocytometer.
Results Seven monkeys demonstrated microfilariae in blood and CSF. This was accompanied by a two- and ninefold increase in CSF total protein and white cell counts, respectively. Necropsy of one of the blood and CSF microfilariae-positive animals revealed the presence of adult worms in the brain meninges. The parasites were identified as the zoonotic filaroid nematode *Meningonema peruzii*.
Conclusions Wild *C. aethiops* monkeys developed CSF changes resulting, most probably, from infection with *M. peruzii*. Moreover, the monkeys could be acting as an important reservoir. The study highlights the need for epidemiological and pathogenological studies of this parasite, which is of public health significance. Moreover, *C. aethiops* proved to be a useful primate model for the study of this zoonotic infection.

Introduction

Cerebrospinal fluid (CSF) analysis has been used to assess the central nervous system (CNS) disease involvement in parasitic and bacterial infections [2, 14]. In human trypanosomosis, increases in the CSF total white cell counts and protein levels have been used for diagnosis of CNS infection, as indicators for therapeutic decision making and for post-treatment follow-up [9].

Vervet monkeys used for *Trypanosoma brucei rhodesiense* infection studies at the Trypanosomosis Research Center (TRC) are acquired from the wild [12]. Upon arrival from the wild, the monkeys are isolated in quarantine for a period of 90 days, during which they are accustomed to laboratory conditions and handling. The animals are observed for any physiological manifestations of stress from their capture and restraint as suggested by Suleman et al. [7] and Hau and Shapiro [8]. They are also screened for various pathogens including zoonotic parasites. The most common infections encountered in wild-caught monkeys include those of endoparasites and ectoparasites [10]. In addition, zoonotic infections such as tuberculosis, simian immunodeficiency virus (SIV) [6], and *Campylobacter* diarrhea [12] have been encountered.

Another zoonotic parasite encountered in the CNS of wild-caught *Cercopithecus* monkeys is *Meningonema peruzzii* [16, 17]. The worm is parasitic in African monkeys with the normal habitat for the adult worm

being the subarachnoid spaces along the dorsum of the brainstem at the level of the medulla oblongata [17]. The microfilariae have been recovered in the CSF and blood of infected humans and monkeys [16]. Several cases of human infection with the parasite have been reported [1], indicating its zoonotic potential. However, no detailed CSF studies have been carried out in reported cases. *Meningonema peruzzii* has not been previously reported in Kenya, although wild-caught monkeys have been used for research for many years.

The present investigation was designed to determine the CSF changes and the causative agent for microfilariae observed in a batch of eight infected monkeys among a group of 34 wild-caught vervet monkeys. During the quarantine period, seven of the infected *C. aethiops* monkeys, those with microfilariae in the CSF and blood, showed elevated CSF total white cell counts and total protein. The eighth infected animal, with microfilariae in the blood and not in the CSF, had normal total protein and cell levels.

Materials and methods

Maintenance of animals

Thirty-four wild, non-human primates were caught at a new capture site in West Pokot region of Kenya. On arrival at the TRC quarantine facility, the animals were housed singly in stainless steel cages in the quarantine facility. The cages were placed in such a way that the animals had visual contact with each other. They were fed two rations daily (morning and afternoon) of commercial monkey pellets (Unga Feeds Ltd, Nakuru, Kenya), fresh fruits, and vegetables. Water was provided *ad libitum* in a bottle with a stainless steel ball-nozzle. The animals were allowed to settle down without any laboratory procedures for the first 2 weeks. A screen to prevent direct eye contact with humans was placed on the front of each cage.

The animals were examined for skin lesion and tested for tuberculosis, using the mammalian tuberculin test. Their serum was also tested for SIV antigens. At the same time, skin scrapings from the animals were microscopically examined for ectoparasites and their fecal materials examined for endoparasites by the Macmaster flotation test.

Once completely acclimatized, the entire monkey colony was anesthetized at 2-week intervals to enable the collection of blood to be used for screening for various diseases. At the same time CSF samples were aseptically collected by lumbar puncture using a sterile gauge 23 needle in order to establish normal protein and white cell levels prior to trypanosomosis studies. Similarly the CSF was microscopically examined to rule out the presence of trypanosomes and other parasites in the CNS.

Research approval to carryout the present study was obtained from the institutional Animal Care and Use Committee, and the animals used were cared for and used humanely through out the study.

Blood sample analysis

Direct microscopy was carried out on all blood samples to screen for hemoparasitic diseases. Giemsa stained slides were also examined to screen for intracellular hemoparasites. Buffy coat examination was performed to rule out the presence of extracellular hemoparasites.

CSF analysis and postmortem examination for adult worms

Cerebrospinal fluid was screened for parasites by direct microscopic examination. Centrifugation concentration of CSF in sealed Eppendorf tubes was performed to rule out presence of extracellular parasites. Total protein levels and white cell counts were determined as described previously [11].

One of the monkeys with microfilariae in blood and CSF was killed under anesthesia and a complete postmortem examination carried out in order to search for adult worms in the CNS tissues. Both microfilariae and adult worms were preserved in 10% buffered formalin for taxonomic studies.

Statistical analysis

Cerebrospinal fluid total cell counts and protein levels in the eight microfilariae-positive monkeys were compared to the levels in eight microfilariae-negative monkeys. The microfilariae negative monkeys served as controls and were matched to the infected animals for closeness in age, sex, and weight. Infected animals and controls were compared by a two-way ANOVA. Statistical difference was considered significant at a P-value of < 0.05.

Results

Clinical examination and determination of microfilariae in blood

Out of the 34 monkeys screened, eight had microfilariae in their blood. Seven of these monkeys had microfilariae in both blood and CSF while one had

microfilariae only in blood. All the seven monkeys with microfilariae both in blood and CSF also had various sizes of facial lesions. Four of the monkeys had ulcerated fibrous lesions on the cheeks, while the remaining three had similar lesions, but on the chin. Notably, the one monkey that had microfilariae in blood only had no visible lesions in any part of its body.

Cerebrospinal fluid cytology

Four CSF samples were collected from each of the eight microfilariae-positive monkeys at 2-week intervals and compared with eight microfilariae-negative monkeys within the same batch of monkeys that was sampled simultaneously.

Only the CSF microfilariae-positive monkeys showed an elevation in their CSF total white cell counts and total protein levels. The one monkey that had microfilariae in blood only showed CSF total white cell and protein levels similar to those of microfilariae-negative animals and was therefore not included in the comparison.

The CSF total white cell counts for microfilariae positive and negative monkeys are presented in Fig. 1.

All the monkeys with microfilariae in their CSF had elevated total white cell counts in all their four CSF samples collected biweekly. The total CSF white cell count mean ranged from 37 to 44 and 2 to 5 cells/dl for the CSF microfilariae-positive and microfilariae-negative monkeys, respectively. These represented a 9- to 16-fold increase in the CSF microfilariae-positive monkeys compared to the microfilariae-negative monkeys trapped from the same area ($P < 0.05$).

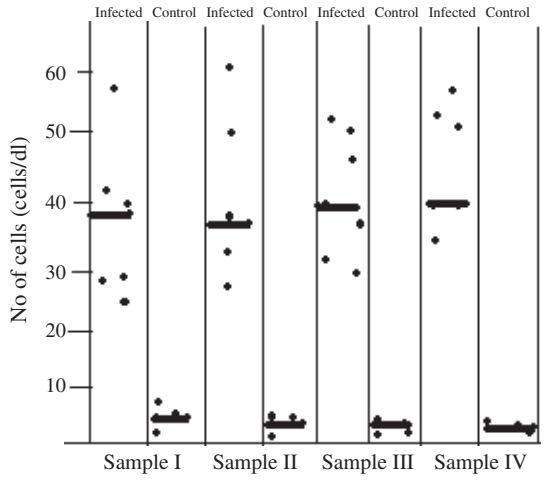

Fig. 1 Total white cell counts in cerebrospinal fluid of *Meningonema peruzzii* infected monkeys and uninfected controls.

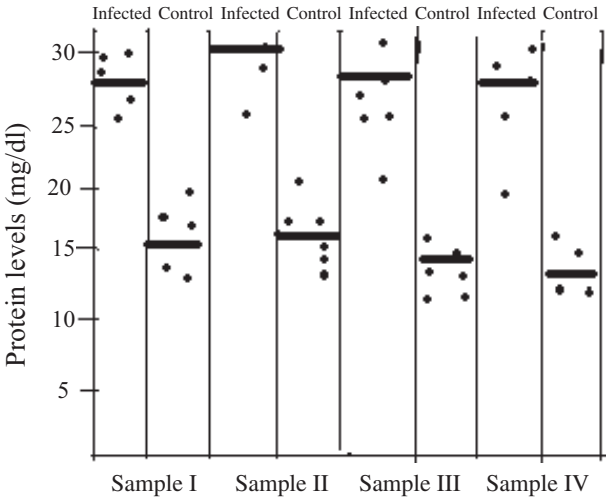

Fig. 2 Total protein levels in cerebrospinal fluid of *Meningonema peruzzii* infected monkeys and uninfected controls.

The CSF total protein levels for microfilariae-positive and microfilariae-negative monkeys are presented in Fig. 2.

All the monkeys with microfilariae in their CSF had elevated levels of total protein in all their four biweekly CSF samples. The CSF protein levels mean ranged from 26.1 to 28.7 and 13.7 to 16.14 mg/dl for the CSF microfilariae-positive and microfilariae-negative monkeys, respectively. This was a twofold increase in the mean total protein from the CSF of microfilariae-positive monkeys compared with the microfilariae-negative monkeys trapped from the same area ($P < 0.05$).

Necropsy of one of the monkeys with microfilariae in CSF revealed the presence of adult worms in the brain meninges, just under the brainstem. The microfilaria and adult worms were submitted to Professor Thomas Orihel's laboratory in the United States for taxonomic identification, where they were identified as *M. peruzzii*, a zoonotic filarial worm.

Discussion

The increased demand for captive-bred non-human primates for biomedical research has far outstripped their supply, resulting in the use of captive, wild, non-human primates in various countries including Kenya. The TRC in Kikuyu, Kenya, is one such institution that uses wild-caught vervet monkeys (*Chlorocebus aethiops*, syn. *Cercopithecus aethiops*) as an animal model of human African trypanosomosis [4, 5, 11, 13].

In *Trypanosoma brucei rhodesiense* infection of humans [9] and vervet monkey models [19, 18, 11], the

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CSF total protein and total white cell counts have been used as indicators of neurological changes during disease. The normal CSF total white cell counts and total protein levels for uninfected vervet monkeys have been established to be 0–5 cells/dl and 10–19 mg/dl, respectively [11]. However, in the present study, it was observed that the wild-caught monkeys from a new capture site in Kapenguria, Kenya, had elevated protein levels and white cell counts indicating the possible presence of infection of the CNS. Further investigations indicated that the CSF changes were due to the presence of microfilariae. It was noted that the elevated levels of total white cell counts and protein were displayed only by monkeys with microfilariea in both CSF and blood. These changes were also accompanied by the presence of ulcerated lesions on the face or the chin of affected monkeys. However, such lesions have not been documented in other reported cases of *M. peruzzii* infection of monkeys. The ulcerated wounds responded well to topical treatment with the antibiotic neomycin. This indicated that the lesions were possibly due to bacterial infection of traumatic injuries, resulting possibly from fights within the troops.

These findings are in agreement with other studies that showed that *M. peruzzii* could be found in blood or CSF or in both [1, 15, 17].

The present study provides the first report of the presence of filarial worm infection in wild-caught monkeys in Kenya and also provides a detailed report of CSF changes in the infected monkeys. In Equatorial Guinea, filarial infection with *M. peruzzii* in the CNS of a Talapoin monkey, *Cercopithecus talapoin* syn, *Miopithecus talapoin,* has previously been observed [17].

Some neurological disorders have been attributed to infection with *Mansonella perstan*s reported in Rhodesia (Zimbabwe) in humans [15]. Microfilariae recovered from the CSF of two patients, although similar to those of *M. perstans* in their gross appearance were found to have a striking resemblance to the microfilariae of *M. peruzzii*, a filarial worm found in the CNS of various African monkeys. Dukes et al. [3] reported two cases of cerebral filariasis, which were ascribed to *M. perstans*. The first case, a British soldier, displayed a severe neurological disorder best described as an acute neurological encephalomyelitis. The second case was a Zimbabwean who had a relatively mild illness characterized by headache, drowsiness, and fatigue. These symptoms were cleared spontaneously after 3 months. In both cases, microfilariae were recovered from the CSF and none from the blood.

Conclusion

Filariasis due to *M. peruzzii* appears to be prevalent in vervet monkeys in Kapenguria District, Kenya. The host range for this parasite remains undetermined. Due to the wide morphological variation produced by the different staining techniques, the microfilariae of *M. peruzzii* may have been misdiagnosed in the past as one of the endemic species in Africa, such as *Wuchereria bancrofti*, *Loa loa,* or *M. perstans* [15].

The presence of *M. peruzzii* in vervet monkeys in the current study is an indication that the monkeys within the West Pokot region of Kapenguria District could be acting as reservoir hosts of the zoonotic worm. Similarly, it is possible that this worm is of significant public health importance and efforts to conduct epidemiological studies in this area should be made. Furthermore, this study showed that the vervet monkey, *C. aethiops*, can be a useful animal model for the study and elucidation of the biology of this zoonotic parasite.

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