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PATTERNS OF MICROFILARAEMIA IN *MESOCRICETUS AURATUS* CAUSED BY *DIPETALONEMA VITEAE*

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Mesocricetus auratus infected by *Dipetalonema viteae* and the patterns of microfilaraemia were observed for 193 days post infection. The hosts were necropsied and adult worms were recovered. Density of peripheral microfilariae was very low. Adult worms of both sexes were recovered from the amicrofilaraemic hosts. It was concluded that the reaction of the host against the adult stages probably has some sterilizing effects on the female worms.

INTRODUCTION

Detection of microfilariae in the circulating blood is the most commonly used criterion for the diagnosis of filarial infection. Filarial worms are found in several other vertebrate hosts besides man. Heartworm disease of dogs, caused by *Dirofilaria immitis*, is one of the serious concerns in the world. The discovery of the presence of another species of dog filariid in United States has provided the stimulus for taking a more critical look at the general subject of canine filariasis inthe country. Newton and Wright [1] reported that the newly found filariid belongs to the genus *Dipetalonema*. In a survey of over 250 dogs from Pennsylvania, Maryland and New Jersey, 18 dogs were found to be positive [2]. Only three out of these positives contained heartworm microfilariae, the rest were infected with *Dipetalonema*. Two Illinois studies indicated a 1.4 % infection rate for *Dipetalonema* sp. [3] and 7.2 % *Dipetalonema reconditum* [4]. Costa and Freitas [5] reported two species of *Dipetalonema* in dogs in Brazil. More incidence surveys are needed for accurate determination, however, the presence of *Dipetalonema* in dogs provides a positive objective for the detailed investigations of various aspects of this parasite.

The occurrence of adult filarial infection without a discernible microfilaraemia is called *Occult filariasis*. This state of infection is much more frequent and constitutes an even more important diagnostic problem. The range of clinical manifestations in broad, including an asymptomatic condition in which individuals manifest persistant microfilaraemia. This wide spectrum clinical disease, presumably reflecting an equally wide range of host responses to filarial infection, and the disappointing therapeutic results make filarial diseases particularly intriguing and important for study.

The adult of this particular filariid under investigation inhabits the subcutaneous area of the vertebrate host. The present studies were carried out in the golden hamster, *Mesocricetus auratus*. The adult deposits the microfilariae which make their way to the blood stream and are available to the vector. The tick *Ornithodoros tartakovskyi* was used as the arthroped vector. Microfilariae, called L1 are picked up by the tick and take approximately 30 days to molt into L2 and L3 stages. L3 being the infective stage, if transmitted to the vertebrate host, molts into L4 and then L5 or the adult.

MATERIALS AND METHODS

Ticks were obtained from U.S. Japan Programme Section, National Institute of Allergy and Infectious diseases, National Institute of Health of Bathesda, Maryland. These ticks were placed in a chamber adjusted at 80 % humidity and 27' C. The hamsters were obtained from the local commercial source and kept in small cages in the animal room. These were kept in groups of four or five in each cage and were provided with animal lab. food and water ad lib. The animal room was provided with humidity (30 %), temperature (22°) on a photoperiod regulator (12 hours light and 12 hours dark) system. The ticks were fed on the shaved abdomens of anaesthesized infected jirds. Infective larvae were recovered by teasing apart the tick tissue in 0.85 % saline. Approximately 25 larvae were injected subcutaneously into the uninfected hamsters by means of a 1 c.c. syringe and a 18 gauge needle. The hamsters were labelled by clipping their toes.

The density of microfilariae was determined by drawing blood from the inter orbital plexus by means of a 9 inch heparinized pasteur pipette. A sample of 20 μl was

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drawn out of this blood by a measured micropipette, fixed in a drop of modified Knott's solution on a slide and examined under a microscope at 40X. The blood was collected twice each week until the post patent period.

Recovery of the adult worms from the Host. The animals were killed and skinned with in five minutes of death. The carcass was completely soaked in saline while the under side of the skin was also soaked in the saline. This was left in the saline for approximately 3 hours in order to achieve the maximum recovery of the adults.

OBSERVATIONS AND RESULTS

Twenty four adult males and females were randomly chosen and infected with the inoculum. Some of the hamsters from the same colony were saved to serve as control. The hamsters seemed to be quite resistant to the infection. There were no early deaths and all infected animals remained in good health throughout the observations. Thirteen hamsters (approximately 43 %) showed no patency. Ten hamsters out of these thirteen animals were negative throughout the period of infection, while three showed microfilariae only once throughout the observation (Table 1). The recovery of adults on the days post infection is indicated in Table 1. Hamsters Nos. 7, 9 and 30 showed one, 13 and one microfilariae on 81, 60 and 78 days post infection, respectively. Two male and two female *D. viteae* were recovered from the hamster No. 9 on day 183 post infection while two females and one male was collected from the hamster No. 30 on day 193 post infection. The microfilariae did not appear after the day 97 post infection (range: 95–97 days post infection) in any of the infected hamsters. The highest peak levels in the infected hamsters were found to be ranging from only 5–22 mf/20 µl blood with a mean of only 12 mf/20 ul blood (Fig. 1). The overall pattern of the infection was very irregular in hamsters. Each individual showed its peak level at a different stage of the infection. Some of the hamsters were even found negative for short durations in the middle of the patent period. Standard errors of the means were also computed as illustrated in Fig. 2. This composite graph also indicates an irregular pattern. Very low peak levels in the

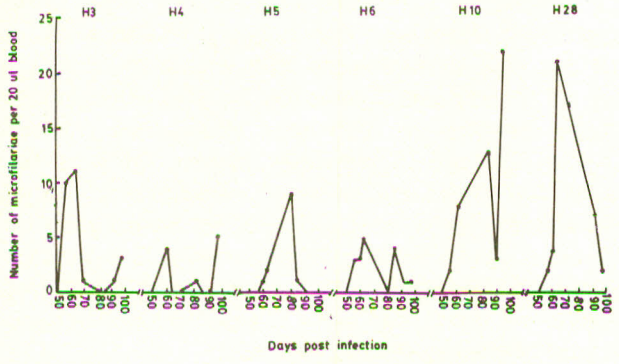

Fig. 1. Patterns of microfilaraemia in six representative hamsters infected with 25 L3 of *D. viteae* obtained from the same batch of ticks.

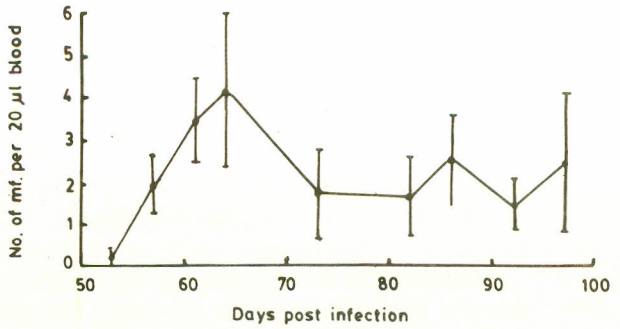

Fig. 2. Mean ± standard error of microfilarial levels in the infected hamsters.

Table 1. Hamsters showing no patency.

Hamster identification number	Presence of microfilariae	Adults recovered
1	Negative throughout the infection	None on day 179 post infection
2	Negative throughout the infection	No record
7	Negative (showed only one mf on day 81 post infection	No record
8	Negative throughout the infection	Only males were recovered on day 182 post infection
9	Negative (showed 13 mf on day 60 post infection)	2 males and 2 females recovered on day 183 post infection
11	Negative throughout the infection	None on day 186 post infection
12	Negative throughout the infection	None on day 88 post infection
13	Negative throughout the infection	Only males on day 188 post infection
14	Negative throughout the infection	Only males on day 188 post infection
17	Negative throughout the infection	Only one female recovered on day 187 post infection
18	Negative throughout the infection	None on day 185 post infection
27	Negative throughout the infection	None on day 190 post infection
30	Negative (showed only one mf. On day 76 post infection	2 females and 1 male recovered on day 193 post infection

composite graph are due to the irregularity in showing the highest levels in hamsters, The higher levels seem to deviate more from the mean than the lower levels.

DISCUSSION

The following conclusions were drawn from the above explained observations:

1. All the hamsters remained in good health which may have provided the parasite with optimum physiological environment but still the microfilaraemia was very low.

2. 43 % of these animal were found to be negative throughout the period, they were observed. Female worms were recovered from animals on day 183, 187 and 193 post infection from microfilaraemic hosts.

Considering these conclusions it is assumed here that the reaction of the host against the adult stages probably has a great deal of effect on the presence or absence of the peripheral microfilariae. This assumption is supported by the findings of Ponnuduri *et al.* [6] indicating that the sera from all of 33 cats infected with *Brugia pahangi* had adult worms anti-bodies, Ambroise-Thomas and Kein-Truong [7] also detected fluorescent anti-bodies against *Wuchereria bancrofti* adults in 95 % of the patients with filariasis.

If the host showed such a strong reaction to the adult worm, the number of females yielded during this experiment might not have been the correct picture. There is a possibility that there could have been more adult worms at the earlier stages of the infection. Some of the females might have died and disintegrated prior to day 179 post infection as a result of the strong response of the host (most of the hosts were sacrificed after day 179 post infection). Direct host resistance to the microfilariae does not seem to be the cause of low microfilaraemia in this situation. If this had been the case, there should have been microfilarial production during early potency, insufficient quantities to initiate the reaction in the host creating the resistance to the microfilariae at the later stages of the infection. The low microfilaraemia remaining constant throughout the period of infection in the hamsters supports the hypothesis that the adult must be initiating some kind of host response or responses resulting in a very low fecundity rate in female adults or perhaps even sterility.

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