# Indian langur (*Presbytis entellus*) as experimental host for *Brugia malayi* infection

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The susceptibility of Indian langurs (*Presbytis entellus*) to a subperiodic strain of *Brugia malayi* infection was observed using 22 male animals. Each animal receiving 250 or 500 infective larvae ($L_3$) in divided doses, spread over one to five weeks period developed microfilaraemia which, however, became very low microfilaraemia after attaining a peak. Nevertheless animals exposed to a large single dose of 500 $L_3$ also revealed similar course of infection. There was no correlation between adult worm recovery, quantum of $L_3$ inoculum administered and the intensity of microfilaraemia developed. Animals during incubation period (<75 days) had atrophied lymph nodes containing live worms whereas at patency lymph nodes revealed worms at various stages ranging from live to dead and calcified conditions. It was also interesting to observe oedematous swelling in the inoculated limb of certain percentage of animals receiving 500 $L_3$.

ATTEMPTS to establish *Brugia malayi* infection in nonhuman primates especially in rhesus monkeys (*Macaca mulatta*) for use in experimental chemotherapy and other pathological studies have met with little success[1-3]. Studies reveal that leaf monkeys (*Presbytis melalophos*, *P. obscura* and *P. cristata*) harbour a natural infection of a subperiodic strain of *B. malayi* in Malaysia[4-6]. However, Indian leaf monkey (*P. entellus*) which has some physiological and phylogenetic resemblances to man has not yet been explored as the possible host for *B. malayi* infection. The present communication reports the results of such a study.

## Methods

Males of *P. entellus* (commonly known as langur in India) weighing 3-4 kg, were obtained from local animal dealers. They were kept in quarentine for 30 days during which they were examined for tuberculosis by Montaux test and chest X-rays. Blood and stool samples were also tested from time to time for parasitic infections. Animals negative for all the above mentioned diseases were included in the study.

Twenty-two male langurs in four groups were infected subcutaneously with infective larvae ($L_3$) obtained from laboratory bred *Aedes aegypti* (mosquitoes) previously exposed to microfilaraemic *Mastomys netalensis*[7]. Each animal of group I (four langurs) received a total of 250 $L_3$ in four divided doses within eight days. The second group consisted of eight langurs and each received a total of 500 $L_3$ in four divided doses within the same span of eight days. Group III consisted of seven langurs and each received 500 $L_3$ in four equal doses administered within 36 days. Each of the rest of three langurs comprising group IV received 500 $L_3$ in single dose.

In case of multiple infections, $L_3$ were introduced in ankles of both the kind limbs alternatively, while in singly inoculated group, $L_3$ were injected at one hind ankle only. Table 1 shows details of animals and their exposures with $L_3$ of *B. malayi*.

Exposed animals were examined for peripheral microfilaraemia using the membrane filtration technique[8]. From each langur, 1 ml of venous blood was collected by femoral vene-puncture between 21.00 and 22.00 h on day 60 post inoculation (p.i.) and thereafter at fortnightly interval till the animals became latent (amicrofilaraemic). Separate investigations[9] revealed maximum level of microfilaraemia (*B. malayi*) in this species of host (langur) between 21.00 and 22.00 h.

Any external manifestations specially swellings etc. in inoculated regions of the body were carefully observed.

Post-mortem examination of killed or recently expired animals from different groups was performed at different time intervals. Lymph glands from different regions (sacral, inguinal, popliteal and other associated regions) were examined for presence and condition of adult worms following the method of Buckley and Edeson[10].

## Results

All surviving-exposed langurs, irrespective of size of inoculum and frequency of exposures, developed microfilaraemia by day 75 p.i. Blood examination on day 60 p.i. however, did not reveal any microfilaraemia (Table 1).

In groups I, II and IV the peak microfilarial level was reached on day 120 p.i. after which it decreased abruptly and a state of low microfilaraemia persisted respectively up to days 240, 360 and 300 of observation periods. However, langurs of group III which received 500 $L_3$ in four equally divided doses, showed peak microfilaraemia around day 150 p.i. which persisted up

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**Table 1.** Exposure of *Presbytis entellus* with $L_3$ of *Brugia malayi*.

| Group | No. of animals At start | No. of animals At end (beyond 60 days) | Frequency of administration | Total $L_3$ inoculated | Number and size of inoculation* | Total time period of inoculations (days) |
|---|---|---|---|---|---|---|
| I | 3 | 3 | Multiple | 250 | 4(25), (50), (75), (100) | 8 |
| II | 8 | 7 | Multiple | 500 | 4(50), (100), (150), (200) | 8 |
| III | 7 | 6 | Multiple | 500 | 4(125 at each occasion) | 36 |
| IV | 4 | 3 | Single | 500 | 1(500) | — |

*Size of each inoculum in parentheses.

to day 210 and thereafter declined and low microfilaraemia persisted up to day 300 (Figure 1).

Post-mortem examinations of animals revealed live/dead disintegrated worms from lymph nodes of all animals. In patent (microfilaraemic) animals, popliteal
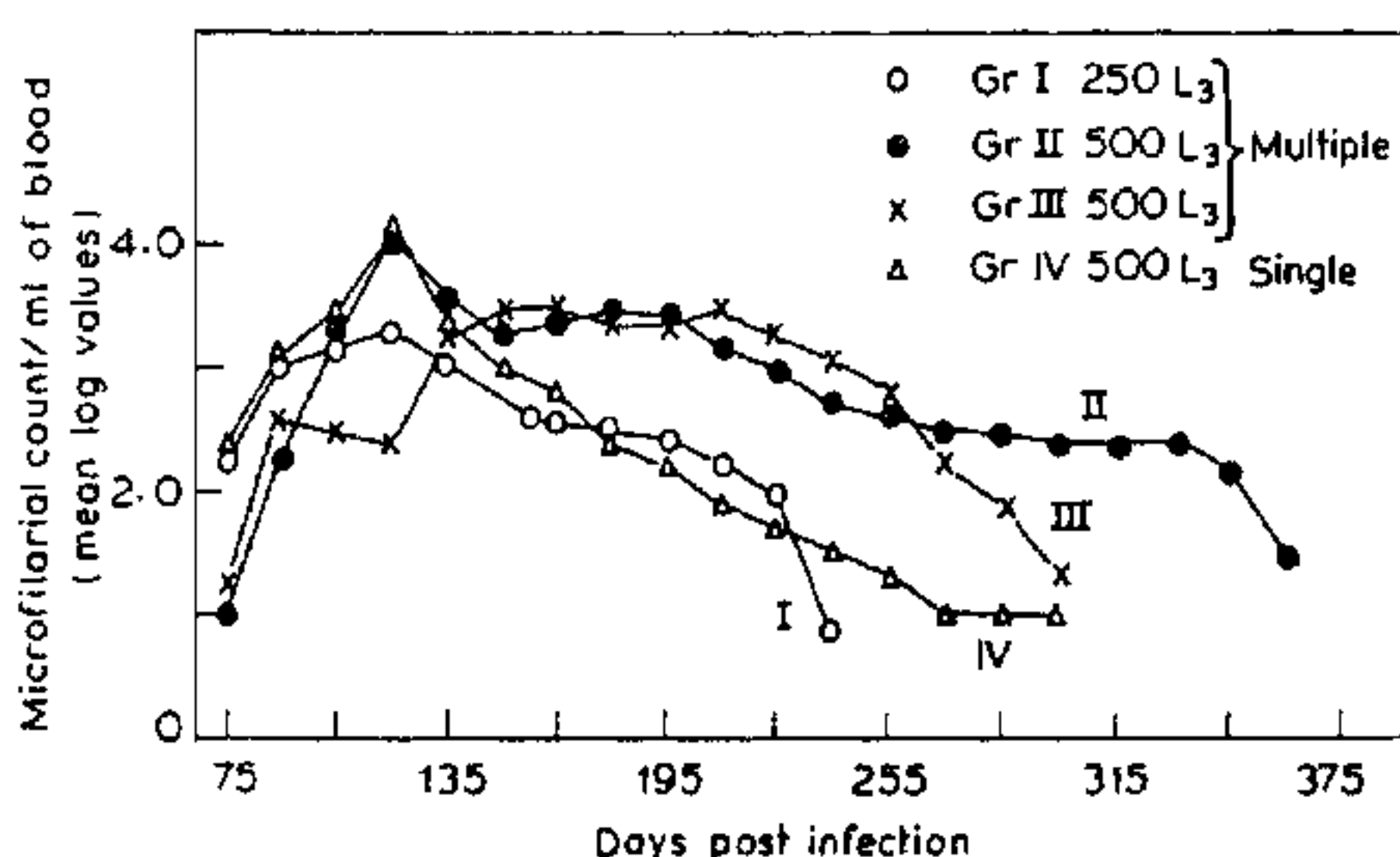


**Figure 1.** Course of microfilaraemia in *Presbytis entellus* infected with *Brugia malayi*.

and inguinal lymph nodes were calcified and contained mostly disintegrated and degenerated worms. On the other hand, the animals at incubation period showed atrophied lymph glands with live worms (Table 2).

Two of the three langurs of group IV (Nos. L-IV-20 & 21) receiving single infective inoculum (500 $L_3$) developed oedematous swelling in the right hind limb (inoculated limbs) during late stage of infection, i.e. around day 210 p.i. and the condition persisted during the next 3 months (Figure 2). Nevertheless, one (No. L-II-7) of seven langurs of group II also developed oedema but at incubation period (around day 60) and persisted up to day 150 p.i. The swelling (oedema) in all the cases was diffuse, soft and pitting type and subsided to a great extent after three to four months.

## Discussion

Need for a suitable primate host with human filarial infection has long been felt. Unlike some of the far

**Table 2.** Recovery of adult worms from *Presbytis entellus* experimentally infected with *Brugia malayi*.

| Animal no. | Period killed/mortality p.i. (days) | Live ♂♂ | Live ♀♀ | Live Total (%) | Dead ♂♂ | Dead ♀♀ | Dead Total (%) | Remarks |
|---|---|---|---|---|---|---|---|---|
| L-II-4 | 315 | 0 | 0 | 0 | 8 | 4 | 2.4 | Calcified lymph glands with remnants of worms |
| L-II-5 | 180 | 0 | 0 | 0 | 1 | 2 | 0.6 | –do– |
| L-II-6 | 170* | — | — | — | — | — | — | — |
| L-II-7 | 266* | — | — | — | — | — | — | — |
| L-II-10 | 360 | 45 | 75 | 24 | 0 | 0 | 0 | Calcified lymph glands with remnants of worms |
| L-II-11 | 60 | 1 | 2 | 0.6 | 0 | 0 | 0 | Glands atrophied with live worms |
| L-II-12 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | Calcified glands |
| L-II-13 | 171 | 0 | 0 | 0 | 0 | 0 | 0 | Calcified glands with fragments of worms |
| L-III-4 | 210 | 4 | 6 | 2 | 0 | 0 | 0 | Calcified glands with degenerated and disintegrated worms |
| L-III-17 | 15* | — | — | — | — | — | — | — |
| L-III-18 | 141* | — | — | — | — | — | — | — |
| L-IV-22 | 42 | 1 | 4 | 1 | 0 | 0 | 0 | Glands atrophied with live worms |

*Post-mortem could not be performed.
Infective dose in all the cases was 500.

**Figure 2.** Swelling in right hind limb of *Presbytis entellus* (L-IV-21) exposed to 500 L$_3$ of *Brugia malayi*.

eastern countries, leaf monkeys with natural filarial infection (*B. malayi*) are not ordinarily encountered in India. Earlier studies with rhesus monkeys did not show encouraging results[3]. It was therefore, worthwhile to evaluate leaf monkey, a widely prevalent species in India as possible experimental host for *B. malayi* infection.

The study demonstrated for the first time that Indian leaf monkey (langur) was highly susceptible for subperiodic strain of *B. malayi*. All the monkeys exposed became patent by day 75 and day 90 of exposure. Nevertheless, it was difficult to compute the optimal dose of L$_3$ to obtain maximum level of infection. If human being in endemic area can provide any indication of infection dynamicity then effect of multiple exposures has to be taken into account. Accordingly, in the present study three multiple and one singly exposed groups were used. Though only four exposures were given to all multiply exposed animals, the essential differences between these groups were the quantum of larvae containing in each inoculum and the time-gap between the exposures. Groups I and II did not show any distinct pattern over their singly exposed

counterparts (group IV) regarding degree and course of microfilaraemia. However, in group III where time-gap between inoculations was the highest (12 days) peak microfilarial count persisted comparatively for longer period suggesting that worms in such cases have developed into adults in batches. Nevertheless, the differences in intensity of microfilaraemia amongst groups were not strictly dependent on quantum of larvae inoculated or the frequency of administration of L$_3$. It was, however, worth evaluating the effect of multiple exposures of low doses of L$_3$ spread over prolonged duration.

A rapid decline in microfilarial count after attainment of peak microfilaraemia was observed in animals of groups I, II and IV. This appeared to be a common feature in nonhuman primates with *B. malayi* infection. Similar decline was earlier reported in rhesus monkey and *P. melalophos*[6,11]. This was in sharp contrast to observation of prolonged microfilaraemia in rodents such as mastomys[7] and gerbil[12,13] infected with subperiodic strain of *B. malayi*.

Difficulty was encountered in the present study to correlate quantum of infective exposure with recovery of adult worms from animals of different groups. Though animals of group I could not be examined owing to certain reasons, thorough examinations of tissues of animals of other groups at different stages of infection (from day 60 to day 360 of exposure) revealed little or no worms (live or dead) in a majority of cases. Recovery of adult worms was not dependent on the age of infection. Thus an animal of group II (L-II-10) receiving 500 L$_3$ when examined for adult worms on day 360 of exposure revealed 120 live worms (24% recovery) all of which were alive. From the same group when another animal (L-II-5) was examined on day 180 showed only three live worms (Table 2). Increased amount of acquired resistance against adult parasites as well as circulating microfilaria might be the reason for recovery of lesser adult worms and development of amicrofilaraemia.

The lymphatic glands adjoining the site of inoculation showed various amount of gross alterations, most common being calcification with parts of degenerated worms. However, atrophied glands with live worms were observed only in early phase of infection (within 60 days of exposures).

It was interesting to note that certain percentage of langurs receiving bigger inoculum size (500 L$_3$) whether administered in single or divided doses, developed oedematous swelling in the area where L$_3$ were inoculated. Thus, 14.2% of group II animals receiving 500 L$_3$ in four divided doses and 66.6% of group IV animals with 500 L$_3$ in single inoculum developed swelling in the area. Though the degree and nature of swelling were more or less similar, the timings of development of such syndrome in the two groups were

different. Whereas in group II, it was detected in late stage of prepatency, the group IV animals developed it at later part of patency. The oedema was invariably reversible though persistence of the condition varied between the groups. It is difficult at this stage to explain the aetiology involved in such conditions. Association of bacterial infections can be ruled out as antibacterial (Terramycin) treatment had no effect on the condition. Involvement of dead or calcified worms may not hold true, as the condition was observed in animals even at early stage of infection. Furthermore, many animals with dead and degenerated worms did not reveal swelling. One of the animals of group III which did not show any manifestations revealed live as well as degenerated worms in lymph glands (popliteal and inguinal glands). Thus mechanical damage or blockade may not be responsible for this reaction. It appears that size of inoculum as well as frequency of exposures might influence the development of manifestations. None of the langurs receiving smaller inoculum size (250 $L_3$) showed external manifestations. Whether such a correlation between size of inoculum and development of manifestations also exists in human filariasis is not known, but certainly not all human beings exposed to filarial infection develop manifestations. It is tempting to speculate that the absence of filarial manifestations in some of infected humans could be due to lower intensity of infective exposure. Nevertheless, correlation between immunological responses and quantum of infection is yet to be found out as it is well established that elephantiasis is the consequences of immunological reactions to parasites[14,15]. Thus langurs with B. malayi can act as a model for investigation on causation of filarial swellings. Though very recently some other models like B. pahangi in dogs[16] have been proposed, langurs being phylogenetically nearer to human beings, would be more suited for this purpose. Nevertheless, due to development of fairly high level of microfilaraemia and shorter incubation period Indian langurs with B. malayi would also be suited for tertiary screening of active antifilarials.

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## RESEARCH COMMUNICATIONS

# Evidence of Chernobyl fallout on a temperate Himalayan glacier

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Several snow/ice samples collected from Chhota Shigri glacier in August 1987 were analysed for total $\beta$ and $\gamma$ activities to see if any fallout from Chernobyl was deposited in the Himalayan region. The activities in snow samples are higher, by a factor of about 15, than those observed in old ice samples. These activities are mainly in the hump region of the glacier located in the altitude band 4150 to 4650 m. These results are the first evidence of Chernobyl fallout deposition on Chhota Shigri glacier, Indian Himalayas.

THE accident in the Chernobyl nuclear reactor on 26 April 1986 caused the release of a lot of radioactivity into the atmosphere. This eventually got deposited in different regions on the earth via precipitation. The radioactivity has been monitored during the last three years in aerosols, rain waters, fresh snow, ocean waters, lake sediments and food products all over the world[1-6], more so by the European scientific community who were concerned about the after-effects of the tragedy on their environment. To see if any fallout from Chernobyl was deposited in the Himalayan region, we collected snow and surface ice samples at different altitudes from the Chhota Shigri glacier, Central Himalaya (32°19'N, 77°31'E; 4000–5660 masl), in August 1987. Most of the snow precipitation over Chhota Shigri takes place generally during the winter months from November to March. The glaciology and hydrology of the glacier and the meteorology of the region have been well studied[7].

We present here the results of measurements of the