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Occurrence of *Mycobacterium leprae* in Arthropods*

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Mosquitoes, bed-bugs, head lice and scabies mites were collected from the dwellings of persons suffering from lepromatous leprosy (patient collections) and from those where no known case of leprosy existed (random collections). Suspensions made from pools of these arthropods were used for making smears for acid-fast staining, culture on Löwenstein-Jensen medium and for mouse footpad inoculation. In the patient collections acid-fast bacteria were detected microscopically in 4.1% of *Anopheles*, 3.6% of *Culex*, 22.2% of *Anopheles* and *Culex* (mixed), 4.8% of *Cimex*, 7.4% of *Pediculus* and in a single pool of *Sarcoptes*. In the random collections acid-fast bacteria were found in 7.7% of *Anopheles*, 6.8% of *Culex*, 9.2% of *Cimex*, in none of *Pediculus*, and in 2 out of 3 *Sarcoptes* pools. Footpad multiplication was obtained from 2 *Culex* pools, one collected at random and the other from patients. The findings strongly support the conclusion that the acid-fast bacteria obtained from the two pools of *Culex* were indeed *Myco. leprae*.

Introduction

It is generally believed that the portal of entry of *Myco. leprae* into the human body is through the skin, and since the bacteria by themselves cannot penetrate the skin the view has been put forward that biting arthropods may act as vectors. In support of this assumption several workers have demonstrated acid-fast bacteria (AFB) in arthropods associated with man. Dungal (1960) has discussed the possible rôle played by several arthropods such as lice, scabies mites, fleas, and mosquitoes as vectors. Spickett (1961) incriminated follicular mites also. Until recently it was impossible to establish the identity of these AFB as *Myco. leprae* in such arthropods owing to the lack of a reliable technique, but this handicap has been at least partially removed by the mouse footpad inoculation technique of Shepard (1960). The present study was undertaken to investigate whether mosquitoes, bed-bugs, lice, and scabies mites in the leprosy endemic area of Pondicherry and surrounding places harboured AFB in field conditions. Shepard's mouse footpad technique was used to culture and identify *Myco. leprae*.

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Materials and Methods

The arthropod collections were made from the leprosy endemic area around Pondicherry. Suspensions of the arthropods were prepared for microscopic examination for AFB, for inoculation into Löwenstein-Jensen (L-J) medium for isolating cultivable mycobacteria, and for inoculation into mouse footpads for multiplication. The arthropods were collected early in the morning, the mosquitoes from inside dwellings, bed-bugs from furniture and crevices in the walls and floors of dwellings, and lice and mites from persons. Separate collections were made from leprosy patients and their houses (patient collections), and from houses having no known case of leprosy (random collections). Only female mosquitoes were used for preparation of the suspensions and for convenience arthropods were pooled according to genus and locality, except in some instances when *Anopheles* and *Culex* mosquitoes were pooled together.

For microscopic examination field-collected arthropods were killed by chilling and the wings and legs of the freshly killed arthropods detached, leaving the proboscis intact. All procedures were carried out in a cold sterile Petri dish surrounded by ice. The arthropods were then ground in a sterile mortar or a cooled tissue grinder and suspended in 1 ml of 0.1% bovine albumin in Hanks' balanced salt solution (BSS). Smears were prepared from the homogenate and stained by the Ziehl-Neelsen method. As there were very few AFB in the homogenate it was not possible to concentrate the bacteria for counting. Therefore, 0.03 ml of the material was inoculated into the hind footpads of Swiss albino mice (Rockefeller strain). The remainder of the material was treated with a drop of 4% sodium hydroxide for 30 min at room temperature and then neutralized with 8% hydrochloric acid. Aliquots of this material were cultured on the L-J medium. The culture tubes were incubated at 37° and 25° C for 8 weeks and examined at weekly intervals for growth of mycobacteria.

Footpad harvests were planned to be carried out after 6 months, 1 year, and 1 to 2 years by the method described by Shepard (*loc. cit.*). In some initial cases footpads were harvested after 3 months. The footpad tissue was ground in a tissue grinder, using 0.1% bovine albumin in BSS. The AFB obtained from the footpad were counted by the method of Hanks, Chatterjee and Lechat (1964). Following counting, the footpad homogenates were inoculated into the footpads of new mice. Aliquots of the material were also inoculated on L-J medium.

Results

The results of the microscopical examination and footpad harvests pertaining to arthropod collections from patients' houses and random collections are shown in Tables 1 and 2. Of the 218 *Anopheles* pools from patients' houses 9 were positive microscopically and 71 were inoculated into mouse footpads, but harvests at 3 months, 6 months, and 1 year did not show any acid-fast bacilli. Of 111 *Culex* pools, 4 were positive microscopically, while of 15 inoculated into mouse footpads clear indication of footpad multiplication was obtained in only one instance at 6 months harvest; this inoculum had been microscopically negative. Of 36 mixed pools (*Anopheles* and *Culex*) 8 were positive microscopically and 7 were inoculated into mouse footpads. Acid-fast bacilli were seen in one 3-month harvest, but not in countable numbers; further harvests were negative.

TABLE 1

Incidence of non-culturable acid-fast bacteria (AFB) in arthropod collections from leprosy patients' houses and results of footpad harvests

Arthropods	No. of pools examined	Smears positive for AFB	No. of pools inoculated into mouse footpads	Footpad harvests positive for AFB at: 3 months	6 months	1 year
Anopheles	218	9	71	0	0	0
Culex	111	4	15	0	1 (−) (5.6×10^4)	0
Anopheles and *Culex* mixed	36	8	7	1 (+)[a]	0	0
Cimex	62	3	18	0	0	0
Pediculus	54	4	3	0	–	–
Sarcoptes	1	1	1	1 (+)[a]		

(−), From smear negative pool; (+), From smear positive pool; [a], Not countable.

TABLE 2

Incidence of non-culturable acid-fast bacteria in random arthropod collections and results of footpad harvests

Arthropods	No. of pools examined	Smears positive for AFB	No. of pools inoculated into mouse footpads	Footpad harvests positive for AFB at: 3 months	6 months	1 year
Anopheles	246	19	41	1 (+)[a]	1 (+)[a]	0
Culex	292	20	32	0		1 (−) (1.6×10^6)
Cimex	98	9	18	2 (+) 1 (−)[a]	0	0
Pediculus	44	0	3	–	–	–
Sarcoptes	3	2	2	1 +)[a]		

(−), From smear-negative pool; (+), From smear-positive pool; [a], Not countable.

Thus, out of 62 *Cimex* pools 3 were positive microscopically and 18 were inoculated into mouse footpads, but no indication of multiplication of AFB was seen in footpads up to 1 year. Out of 54 *Pediculus* pools 4 were positive microscopically and 3 of these were inoculated into mouse footpads, but there was no indication of footpad multiplication of AFB. The single pool of *Sarcoptes* was positive microscopically and AFB were detected in the footpad at 3 months' harvest, but not at subsequent harvests.

Microscopically, acid-fast bacilli were more common in random collections. Of the 246 *Anopheles* pools, 19 were positive and 41 were inoculated into mouse footpads. AFB were seen in smears made from footpad material in two instances (at 3 months and 6 months), but there were not enough bacteria to make a count. Of the 292 *Culex* pools, 20 were positive microscopically and 32 were inoculated into mouse footpads. Some indication of multiplication in the mouse footpad was seen in one 1 year harvest; the original inoculum of this was negative microscopically. Of the 98 *Cimex* pools, 9 were positive microscopically and 18 were inoculated into mouse footpads. In 3 cases AFB were seen in 3-month footpad harvests, but not subsequently. All 44 *Pediculus* pools were negative

microscopically, and 3 of these when inoculated into footpads gave negative results. Of the 3 *Sarcoptes* pools 2 were positive microscopically, 2 were inoculated into mouse footpads and AFB were seen in one 3-month harvest, but not subsequently.

The incidence of culturable mycobacteria was low in field collected arthropods—as can be seen in Tables 3 and 4.

TABLE 3

Results of culture on L-J of arthropod material from patients' houses

Arthropods	No. of pools cultured	Smears positive for AFB	No. of pools positive on culture
Anopheles	197	8	3
Culex	85	1	0
Anopheles and *Culex* mixed	35	8	0
Cimex	51	3	2
Pediculus	15	2	1
Sarcoptes	1	1	0
Total			

TABLE 4

Results of L-J culture of arthropod material from random collections

Arthropods	No. of pools cultured	Smears positive for AFB	No. of pools positive on culture
Anopheles	215	19	1
Culex	265	21	3
Cimex	84	9	1
Pediculus	18	0	0
Sarcoptes	2	1	0
Total			

As mentioned above, footpad multiplication was obtained from 2 pools of *Culex* mosquitoes, one in a patient collection and the other in a random collection. The patients' pool suspension inoculated into footpads yielded 5.6×10^4 acid-fast bacilli at 6 months. The material from the footpads was re-inoculated into a second batch of mice in order to study the pattern of multiplication. In about 7 months a 200-fold increase was observed. The random *Culex* pool inoculated into footpads yielded 1.6×10^6 acid-fast bacilli in 1 year.

Discussion

That *Culex* mosquitoes and bed-bugs can take up *Myco. leprae* from leprosy patients, and that the number of bacilli taken up depends on the degree of bacteraemia of the patient, has been demonstrated in our laboratory and is being reported separately (Narayanan *et al.* 1972). The microscopical detection of

non-culturable AFB in field-collected arthropods will therefore depend on whether the arthropods have actually fed on patients with a sufficiently high level of bacteraemia. In order to produce multiplication in mouse footpads, the arthropods should have ingested a sufficient number of viable *Myco. leprae.* In observations made in this laboratory *Myco. leprae* has been found to retain its viability in *Culex* mosquitoes for at least 48 h.

This evidence, together with the isolation of non-culturable mycobacteria from *Culex* mosquitoes in the footpads of mice, strongly suggests that at least a few *Culex* mosquitoes in this endemic area carry viable *Myco. leprae.* The 200-fold increase of acid-fast bacilli in footpads in one case and the ceiling number of over one million AFB obtained in another is typical of footpad multiplication of *Myco. leprae.* It is likely that the randomly collected *Culex* might have taken up *Myco. leprae* from leprosy patients and come to rest in a house having no leprosy patient.

With regard to *Anopheles* mosquitoes, bed-bugs, head lice and scabies mites, in the absence of footpad multiplication it is not possible to draw any conclusions at present. Microscopical detection of acid-fast bacteria by itself is inconclusive because they might not be leprosy bacilli, or even if they were, they might be non-viable. On the other hand, samples found to be negative microscopically might nevertheless contain viable *Myco. leprae,* as shown in two instances where mouse footpad multiplication took place.

When suspensions of arthropod material were inoculated into mouse footpads, inflammatory reactions were observed. This problem is being overcome with the use of anti-inflammatory drugs and the results are expected to show whether the inflammatory reaction can interfere with the proper multiplication of *Myco. leprae* in mouse footpads.

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