PD-ABG-101

VBC PROJECT

Tropical Disease Control for Development

Trip Report: Sand Fly and *Leishmania* Surveillance Training: Phase III, Training Reinforcement and Augmentation

August 31 - September 29, 1992

by

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VBC Report No. 81324

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Table of Contents

1. Executive Summary 1

2. Introduction 4

3. Methods and Materials 6

Training activities 6
Logistics 6

4. Results and Discussion 8

Sand fly surveys 8

5. Recommendations 24

6. Itinerary and People Contacted 25

Tables

Table 1. Summary of Sand Fly Collections from Gaur Area. 9
Table 2. Summary of Sand Fly Collections in Biratnagar Area . . . 16

Figures

Figure 1: Mean Number of Female Sand flies — Gaur 10
Figure 2: *P. argentipes* — Gaur 11
Figure 3: *P. papatasi* — Gaur 12
Figure 4: *S. babu* — Gaur 13
Figure 5: Species Densities - Sprayed Versus Unsprayed, Gaur . . . 15
Figure 6: Mean Number of Female Sand flies — Biratnagar 17
Figure 7: *P. argentipes* — Biratnagar 18
Figure 8: *P. papatasi* — Biratnagar 19
Figure 9: Species Densities - Sprayed Versus Unsprayed, Biratnagar 20

Annexes

Annex A. Training Reinforcement and Augmentation Participants . 28

Annex B. Training Schedule 29

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Acknowledgements

The author wishes to thank Dr. Richard Andre, VBC, and Dr. Jerald Sadoff, Director, Division of Communicable Diseases and Immunology, WRAIR, for the opportunity of continued participation in this training activity. Thanks also go to Dr. B.L. Shrestha, Chief, Division of Malaria Control; Mr. Riddhi G. Vaidya, Chief Entomology Section, Division of Malaria Control; Mr. B.B. Chand, Public Health Officer, Rautahat District; and Mr. Janak Das Shrestha, Public Health Officer, Morang District, all of Nepal's Ministry of Health; and Dr. David Oot, Chief, Office of Health and Family Planning, USAID/Kathmandu, for their cooperation and in-country support. Finally, special thanks go to Mr. Shreedhar P. Pradhan, Medical Entomologist/Malaria Advisor, Office of Health and Family Planning, USAID/Kathmandu, whose coordination, organization, management, and translation skills insured the success of the course.

Preparation of this document was sponsored by the Vector Biology and Control Project under Contract No. DPE-5948-C-00-9030-00 to Medical Service Corporation International, Arlington, Virginia, USA, for the Agency for International Development, Office of Health, Bureau for Research and Development.

1. Executive Summary

Through the Ministry of Health's Malaria Control Division, His Majesty's Government of Nepal requested assistance from USAID/Kathmandu to provide training in sand fly and *Leishmania* surveillance for control of kala-azar transmission. In response to this request, the VBC Project asked the author to organize and present a training course. The first training visit, in March — April of 1992, consisted of two phases: Phase I included intensive classroom instruction and laboratory and field demonstrations; Phase II consisted of practical field experience in designing, conducting and evaluating the results of sand fly surveys (see VBC Report No. 81324, Trip Report: Training Course on Sand Fly and *Leishmania* Surveillance in Nepal, March 20 - April 19, 1992). The follow-up activity, or Phase III, detailed here was planned as an extension of Phases I and II. The goal was to reinforce and augment previous training and to conduct preliminary entomological surveys in kala-azar-affected areas in preparation for a national *Leishmania* surveillance and control program.

The author arrived in Nepal on September 2, 1992, three days prior to commencing the training, to permit initial briefings and logistical preparation. Training activities lasted from September 5 - 23, and the author departed the country on September 28, following appropriate debriefings (see pages 25 - 27 for detailed itinerary). Phase III consisted of two similar eight to nine day sessions, one conducted at Gaur, Rautahat District, and the other at Biratnagar, Morang District. These locations were selected by Mr. Pradhan in consultation with staff of the Malaria Control Division and with the respective District Public Health Officers. Ten students were included in each session, four who had attended the March-April course in Janakpur, and six who had not.

The sessions began with a full day's review of material presented in Phases I and II, followed by a one-week field experience. At each study site, two sprayed and two unsprayed villages were selected in which parallel, comparative sand fly surveys were conducted. On the last day of each session, the results of the surveys were reviewed and discussed, and a short, oral final examination was administered. As of this writing, a total of 37 Ministry of Health staff have received the training necessary to conduct sand fly and *Leishmania* surveillance in Nepal.

The sand fly fauna encountered was comprised of the same three species collected during the first field training in Janakpur, namely, *Phlebotomus argentipes* (the confirmed vector of kala-azar in Nepal), *P. papatasi* and *Sergentomyia babu*. The relative density of the kala-azar vector, *P. argentipes*, was lower at Biratnagar than at Gaur. The relative density of the vector in each area was much lower than that observed earlier in Janakpur, although the incidence of kala-azar appeared to be about the same in all three places. Seasonal and climatic conditions may have contributed to the difference in vector density, as well as differences in human-constructed dwellings. Pre-dawn collections produced the most *P. argentipes,* indicating that resting *P. argentipes* are highly sensitive to light and disturbances caused by routine human activities.

Judging from the reduced population of sand flies in sprayed villages in Biratnagar compared to sprayed villages in Gaur (82% and 69%, respectively), it appears that spraying operations in Morang District were more effective and perhaps better supervised than in Rautahat District. Since the critical density of the vector required to maintain endemic transmission is unknown, the impact that current spraying operations will have on kala-azar incidence is also unknown.

A combined total of 2,567 female sand flies (318 *P. argentipes*; 2,246 *P. papatasi*; 3 *S. babu*), from both sites, were dissected and examined for *Leishmania* flagellates. None from either site was found infected with human leishmaniasis. Bloodmeals of 521 sand flies were preserved on filter paper for later analysis and identification of their source.

Based on the combined results of this and the previous training visit, the following actions are recommended:

1. Draft a detailed proposal and seek financial and administrative support for a two-year study of the natural history of kala-azar transmission in Nepal.

2. Expand efforts to delineate specific breeding sites of *Phlebotomus argentipes* using sticky paper traps, emergence traps, mark-release-recapture techniques, and other suitable means to locate immature stages and newly emerged adults.

3. During future survey operations, conduct resting-site collections during pre-dawn hours.

4. Continue and intensify the existing program of residual spraying of human dwellings in affected areas and also include spraying of mixed and animal dwellings. Encourage closer supervision of residual spraying operations to insure that all dwellings (human, animal, mixed) within affected villages are sprayed inside and out.

5. Explore the possibilities of using permethrin-impregnated bed nets and/or curtains to help reduce human-sand fly contact.

6. Conduct laboratory experiments to investigate the vector competence of *P. argentipes* and *P. papatasi* for *L. donovani*.

2. Introduction

Kala-azar continues to be a major public health threat in the Terai Plain of Eastern Nepal, where case incidence occurs in epidemic proportions. Limited resources and countermeasures, as well as a general lack of technical and professional expertise, have hampered efforts to bring the epidemic under control and prompted a request for assistance from His Majesty's Government of Nepal to USAID/Kathmandu. In response to this request, the Vector Biology and Control Project (VBC) asked the author to serve as a consultant to organize and present a training course on sand fly and *Leishmania* surveillance as part of a national effort to curb kala-azar transmission.

During an initial visit in March - April of 1992, training was conducted at Janakpur Dham, Danusha District, in the Eastern Terai plain, about six km from the Indian border. Training was presented in two phases: Phase I included intensive classroom instruction and laboratory and field demonstrations; Phase II consisted of a 15-day practical field experience in designing, conducting and evaluating the results of sand fly surveys (see VBC Report No. 81324, Trip Report: Training Course on Sand Fly and *Leishmania* Surveillance in Nepal, March 20 - April 19, 1992).

The follow-up activity, or Phase III, detailed here was a planned extension of training phases I and II and a continuation of assistance to the Nepal Ministry of Health (MOH) in investigating kala-azar transmission in the eastern Terai Plain. The specific objectives were to:

1. Reinforce and augment previous training, thereby increasing the students' competence and confidence in sand fly and *Leishmania* surveillance procedures.

2. Conduct preliminary entomological surveys in kala-azar-affected areas and relate the results to control efforts.

3. Plan and organize a longitudinal sand fly and *Leishmania* survey and draft a supporting research proposal.

4. Recommend practical prevention and control measures that can be implemented by local MOH staff to reduce human-sand fly contact and thus the incidence of kala-azar.

The visit was planned to coincide with the end of the rainy season. The author arrived in Nepal on September 2, three days before actual training began, to permit initial briefings and logistical preparations. The course ran from September 5-23, and the author departed the country on September 28, following appropriate debriefings (see itinerary). The scope of the work conducted included the following:

1. obtaining and preparing training materials;

2. re-enforcing and augmenting training on identification of vector sand flies in Nepal and on epidemiological survey methods for leishmaniasis, including laboratory and field techniques;

3. expanding a reference teaching collection of the sand flies of Nepal to be used at the Research and Training Center in Hetauda;

4. assisting the MOH in establishing a program for monitoring leishmaniasis transmission risk by determining vector parameters (e.g., species abundance, host preference, resting and oviposition behavior, and natural longevity), infection rates in sand flies, and human prevalence of leishmaniasis in selected villages;

5. providing information to the MOH on possible diagnostic procedures for identifying *Leishmania* in sand flies and mammalian hosts; and

6. assisting the MOH in developing possible leishmaniasis control strategies.

3. Methods and Materials

Training activities

The course was presented in English with simultaneous translation provided, as needed, by Mr. Pradhan. Phase III consisted of two similar eight — nine day sessions, one at Gaur, Rautahat District (September 6 - 13), and the other at Biratnagar, Morang District (September 15 - 24). Training sites were preselected by Mr. Pradhan after consultation with staff of the MOH's Malaria Control Division and the respective District Public Health Officers. MOH staff from only the Central and Eastern Regions of the country participated in the training. Ten students were included in each session, four who had attended the March-April course in Janakpur, and six who were untrained (Annex A).

Each session commenced with a pretest and a full day's review of previous classroom instruction. Then the class was divided into two teams. At both training sites, two sprayed and two unsprayed villages (one for each team) were selected to conduct parallel, comparative surveys. Sand fly collections were made in sprayed and unsprayed villages on alternate days. Survey methods included early morning resting-site collections (using mouth aspirator) from human, animal and mixed-use dwellings, light trap collections, sticky paper collections, and bite-landing collections. Substrate samples from human and mixed-use dwellings were also screened for the presence of immature stages. In addition, sand flies with fresh bloodmeals were squashed on filter paper for later bloodmeal identification to establish a human biting index. On the last day of each session, the results of the survey were reviewed and discussed, and a short, oral final exam was administered. A detailed training schedule is included as Annex B.

Logistics

Classroom facilities were rented from the respective District Public Health Offices in Gaur and Biratnagar. Five compound and five stereoscopic dissecting microscopes were provided by the Research and Training Center, Hetauda. A portable, gasoline-powered electric generator was also provided and proved invaluable for operating the

microscopes during the frequent daily power outages. A USAID vehicle, with driver, was provided for use during the course, and another vehicle, with driver, was rented from a private source, making vehicles available to both student teams. Training was somewhat hampered by the temporary loss of the author's three equipment chests, which should have arrived as his baggage. Halfway through the first session, two of the chests were recovered; the third remained lost until the author departed Kathmandu. Although some augmentative training was cancelled, the bulk of activities proceeded as planned with locally purchased supplies and items left over from the previous training visit.

4. Results and Discussion

As observed during the March-April training visit, all students worked very diligently during the course. Those who had attended previous training remembered well what they had learned and served as mentors for the newcomers, thus reinforcing their own training and making that of the others more efficient. There are now 37 people who have received the training necessary to conduct sand fly and *Leishmania* surveillance in Nepal.

Sand fly surveys

Following are the results of surveys conducted at Gaur and Biratnagar during the field portion of the training.

A. Gaur, Rautahat District

1. Sand fly surveys: A total of 1,839 sand flies were collected by a variety of methods in the four villages surveyed. The vast majority of flies were collected in resting-site collections made with mouth aspirators. As before three species were collected, with relative abundance as follows: *Phlebotomus argentipes* (the confirmed vector of kala-azar in Nepal), 23% of all females, 12% of all males; *Phlebotomus papatasi*, 71% of all females, 75% of all males; *Sergentomyia babu*, 6% of all females, 13% of all males (Table 1). The distributions of species among dwelling types are compared in Figures 1 — 4 and discussed below.

    a. *P. argentipes* were found most commonly in mixed-use dwellings (45% of females; 44% of males), where humans sleep in the same room as their animals, followed by human dwellings (30% of females; 27% of males), followed by animal dwellings (26% of females; 29% of males) (Figure 2). In contrast, in Janakpur, only 11% of females were found in human dwellings. Furthermore, *P. argentipes* did not predominate in any of the three dwelling types.

Table 1
Summary of Sand Fly Collections from Gaur Area.

Species	No. Females	% of Total Females	No. Males	% of Total Males
P. argentipes	297	23	63	12
P. papatasi	939	71	390	75
S. babu	84	6	66	13
Total	1,320		519	

Blood-fed females were easily collected with mouth aspirators in the early morning while resting on the mud-plastered interior walls of all three dwelling types, supporting the suggestion that this species is an opportunistic feeder. The density of flies in exterior resting-site collections was very low (less than 1 fly/hr). In all-night bite-landing collections in human dwellings, the biting density was also low (about 1 fly/hr), however, 48% of the flies collected in this manner were *P. argentipes.* This indicates that the relative distribution of this species at rest in various dwelling types does not reflect actual exposure experienced by human inhabitants.

b. *P. papatasi* predominated in all three dwelling types and were found in greatest abundance in human dwellings (68% of females; 72% of males), followed by mixed-use (24% of females; 21% of males), followed by animal dwellings (9% of females; 7% of males) (Figure 3). This trend reflects their anthropophilic tendency.

c. *S. babu* females were found in low numbers and were most abundant in human dwellings (77% of females; 77% of males), followed by mixed-use (15% of females; 21% of males), followed by animal dwellings (8% of females; 2% of males), (Figure 4).

Figure 1. Mean number of female sand flies in resting-site collections in unsprayed dwellings at Gaur.

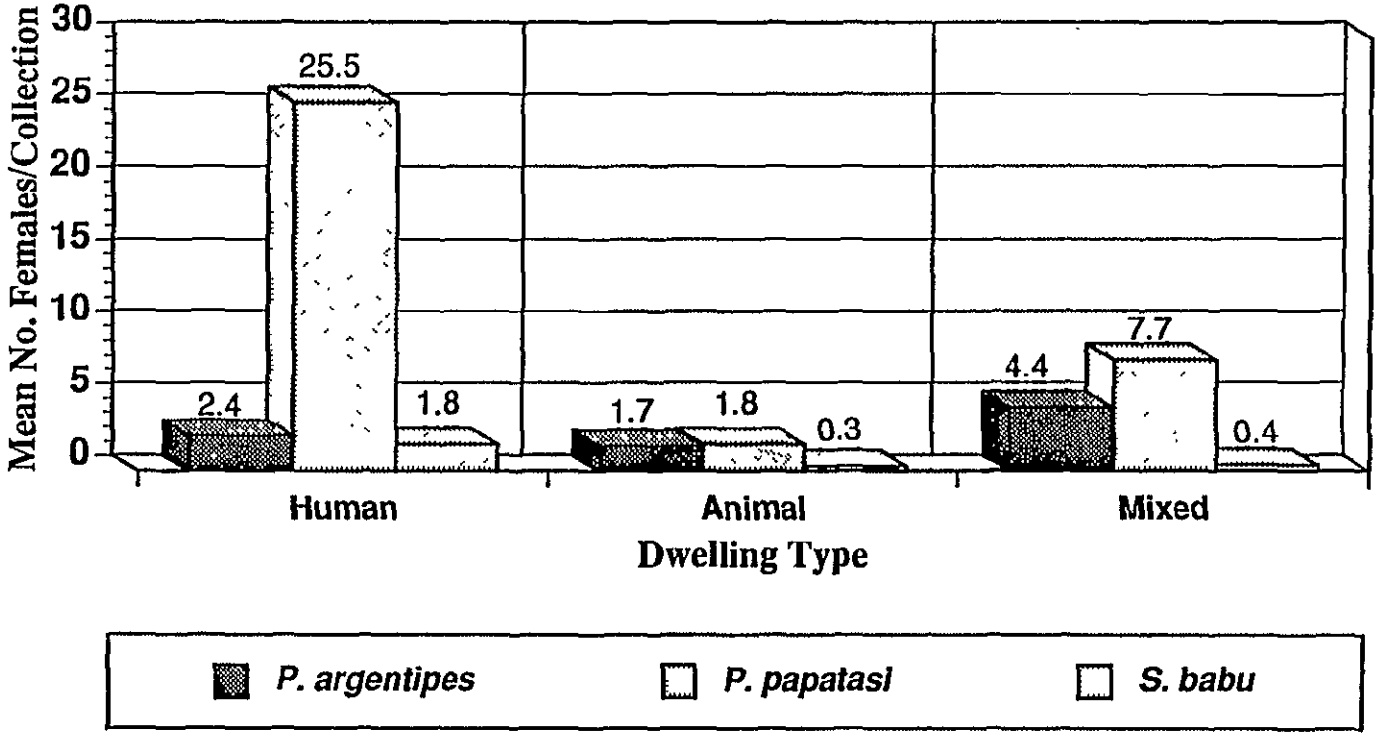


Figure 2. Numbers of *Phlebotomus argentipes* collected during a 7-day period from resting sites in three types of dwelling at Gaur.

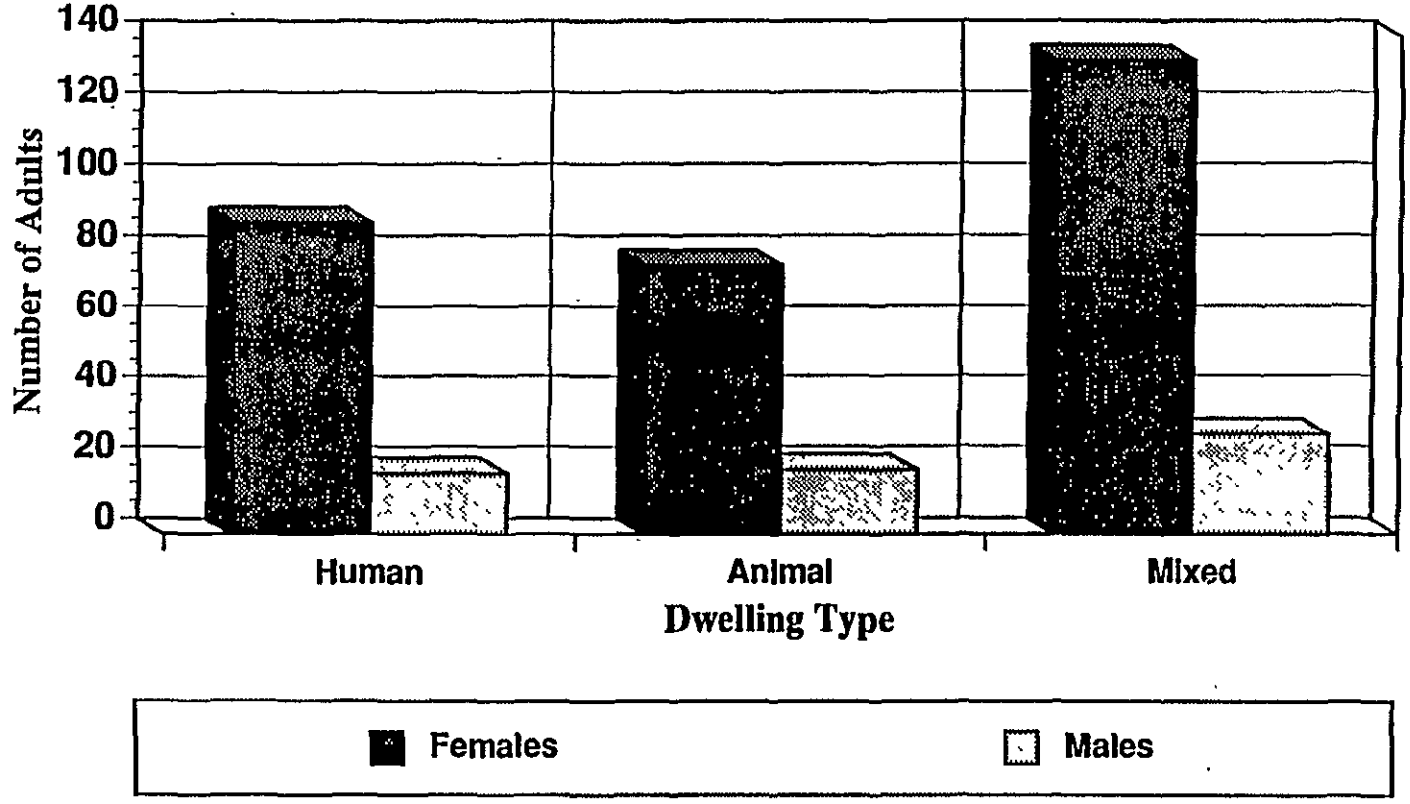


Figure 3. Numbers of *Phlebotomus papatasi* collected during a 7-day period from resting sites in three types of dwelling at Gaur.

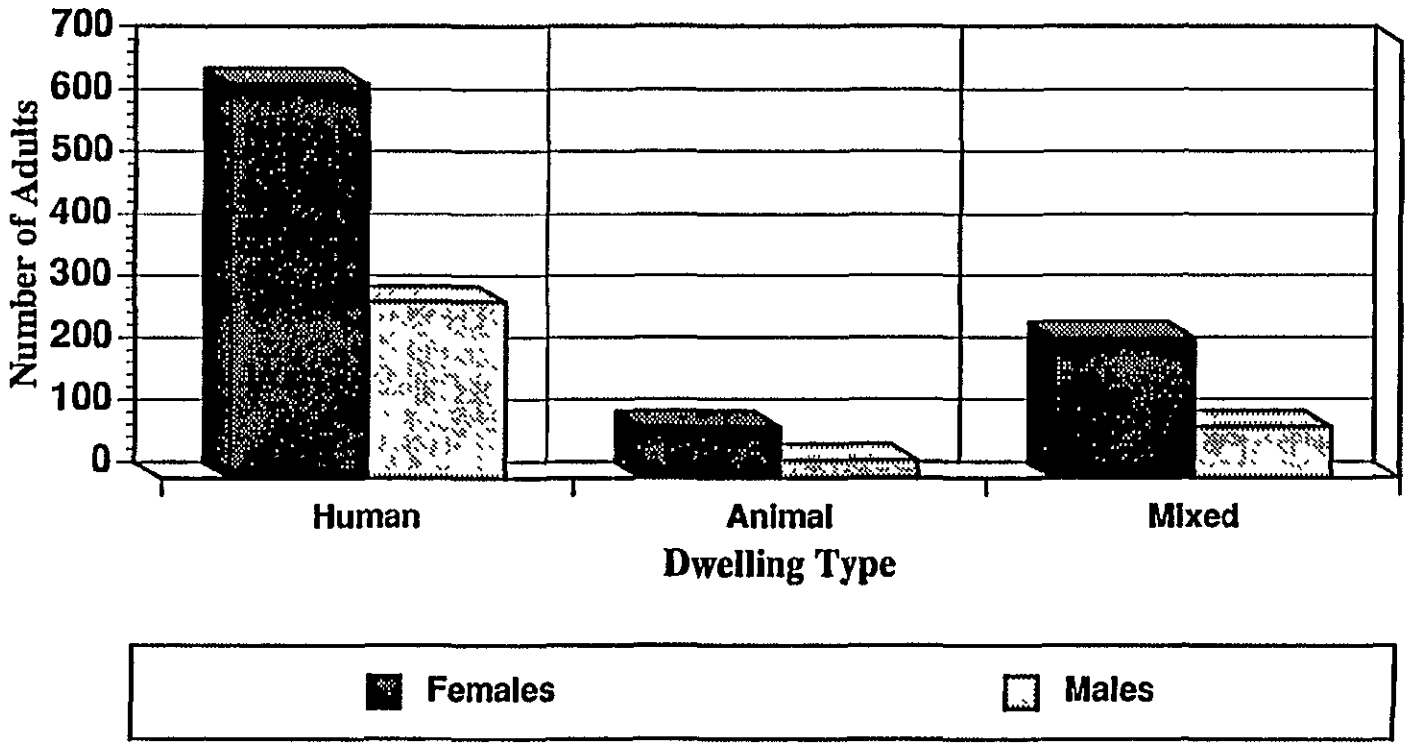
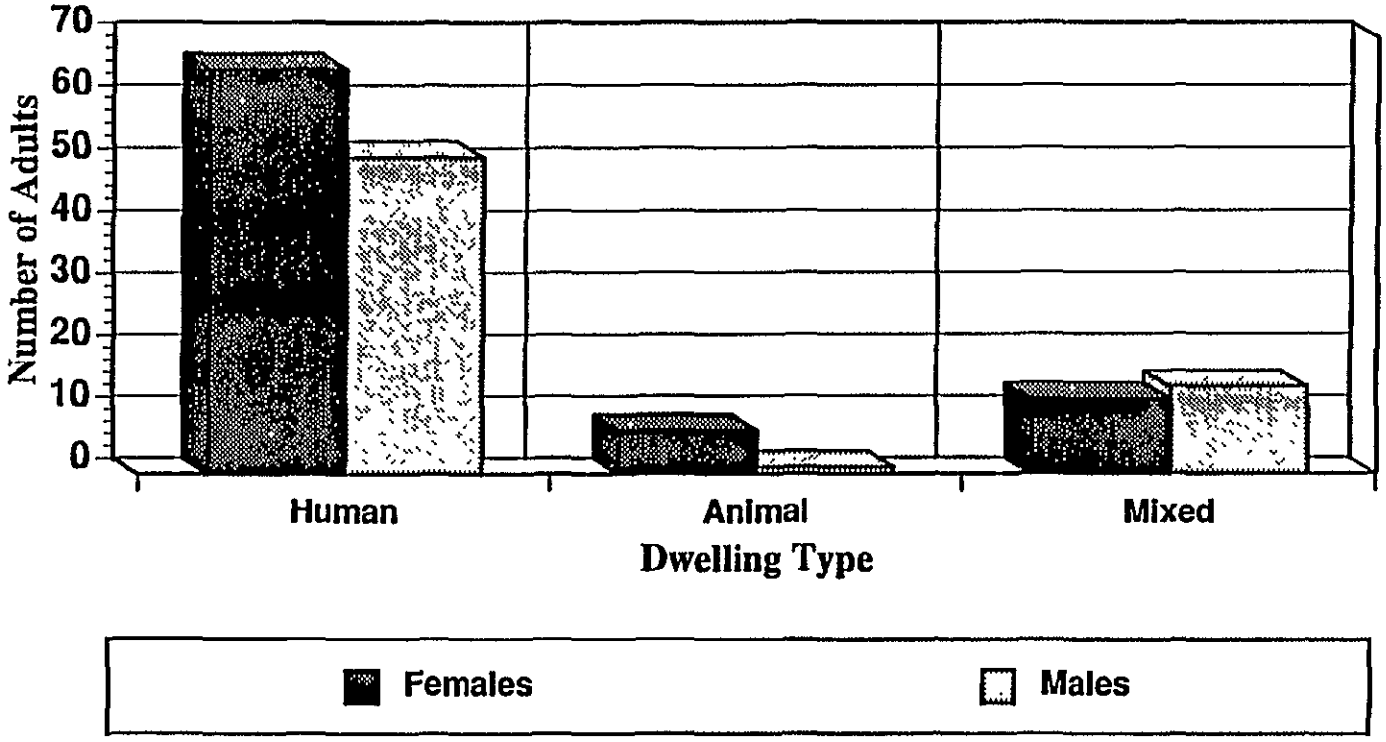


Figure 4. Numbers of *Sergentomyia babu* collected during a 7-day period from resting sites in three types of dwelling at Gaur.



2. Sand fly dissections:

A total of 1,069 female sand flies were dissected and examined for *Leishmania* flagellates (272 *P. argentipes;* 794 *P. papatasi;* and 3 *S. babu*). One fly, a *S. babu*, had a heavy hindgut infection of *Leishmania*-like flagellates. Because the parasites were attached in the hindgut of this non-vector (hypopylarian development), it is assumed that they were *Sauroleishmania* and not human pathogens.

3. Host preference study/bloodmeal analysis: Bloodmeals of 171 sand flies (25 *P. argentipes,* 145 *P. papatasi,* 1 *S. babu*) were squashed on filter paper to preserve them for later analysis and identification of their source.

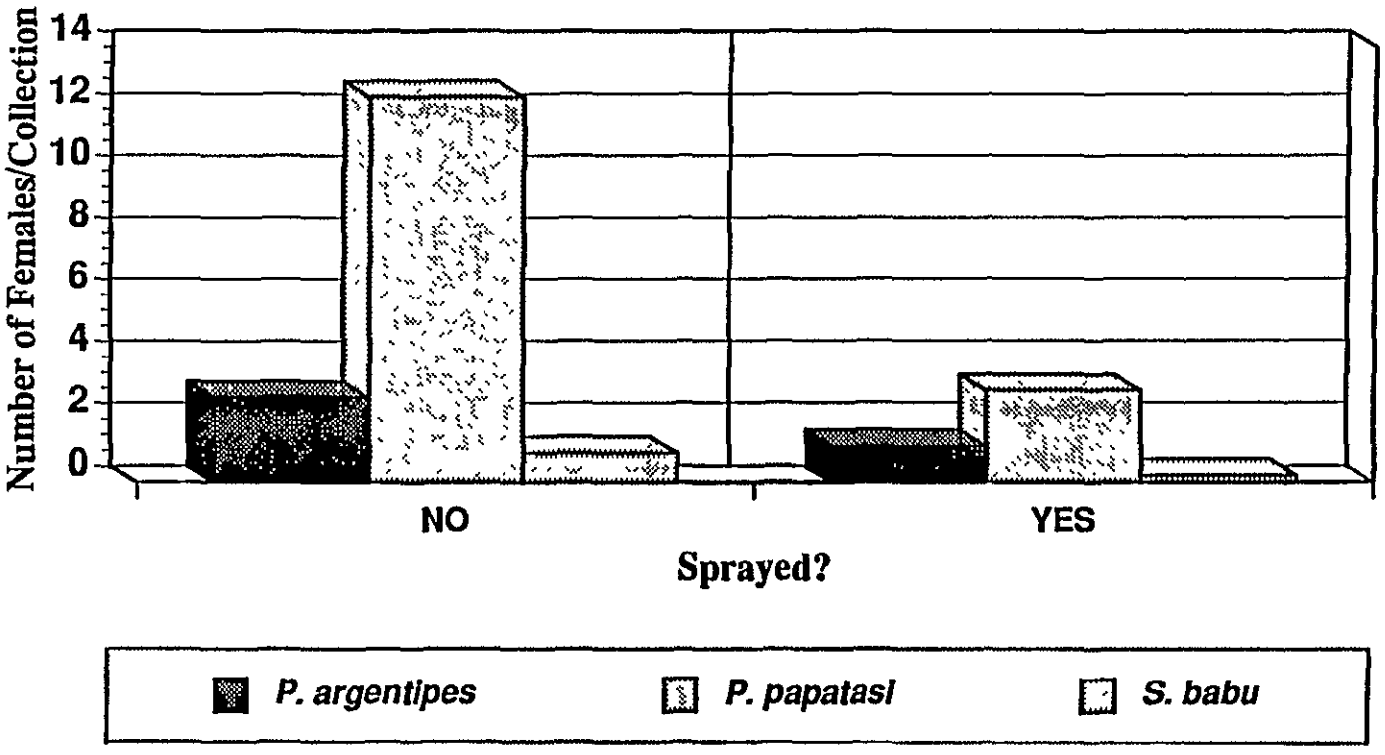
4. Unsprayed vs. sprayed villages: In April of 1993, the MOH began a residual spraying program in kala-azar-affected villages. Under this program, the interior walls of homes of kala-azar patients and those of their adjacent neighbors are sprayed quarterly with Icon[1], a pyrethroid insecticide that provides an estimated 16-week residual. The unsprayed villages surveyed had not yet been included in the program. Sprayed villages selected for the survey had been treated within the previous 30 days. The density of female sand flies collected in unsprayed villages averaged 16 females per 20-minute resting-site collection. In sprayed villages the density was 5 females per collection, suggesting a 69% reduction in the female sand fly population. Species-specific reduction was 84% for *P. argentipes*, 69% for *P. papatasi,* and virtually 100% for *S. babu* (see also Figure 5).

B. Biratnagar, Morang District

1. Sand fly surveys: A total of 2,319 sand flies were collected by a variety of methods in the four villages surveyed in the vicinity of Biratnagar. Again, the vast majority of flies were collected in resting-site collections made with mouth aspirators. The relative abundance of each species collected was as follows: *P. argentipes,* 5% of all females, 1% of all males; *P. papatasi,* 94% of all

1. Icon is a registered trademark of the Imperial Chemical Company, England.

Figure 5. Comparison of species densities in unsprayed versus sprayed villages at Gaur.



females, 99% of males; *S. babu*, <1% of all females and males (Table 2). The distribution of species among dwelling types are compared in Figures 6 - 9 and discussed below.

Table 2
Summary of Sand Fly Collections in Biratnagar Area

Species	No. Female	% of Total Females	No. Males	% of Total Males
P. argentipes	99	5	4	1
P. papatasi	1,802	94	404	99
S. babu	8	<1	2	<1
Total	1,909		410	

a. *P. argentipes* were found in much lower densities in Biratnagar than in other areas surveyed. Unlike in Gaur and Janakpur, this vector species was most commonly found in human dwellings (91% of females; 100% of males), followed by mixed and then animal dwellings (Figure 7). *P. argentipes* were most easily collected with mouth aspirators in human dwellings around 0500 hrs, before daylight and before human activity drove them from their resting places. They were frequently collected in upper-level sleeping quarters of two-story houses and on the exterior walls of human dwellings where people were sleeping on the varandas. No *P. argentipes* were collected in all-night bite-landing collections, quite different from the picture seen at Gaur. Sticky paper collections were generally unsuccessful except in one unsprayed village where three male *P. argentipes* were collected on a paper inserted into a rodent hole in the wall of a human dwelling. These males had unrotated or partially rotated genitalia, indicating that they had emerged within the preceding 12-18 hours. This suggests that *P. argentipes* may breed in rodent burrows and galleries excavated between interior and exterior wall surfaces. Time did not permit further study of this hypothesis.

Figure 6. Mean number of female sand flies in resting-site collections in unsprayed dwellings at Biratnagar.

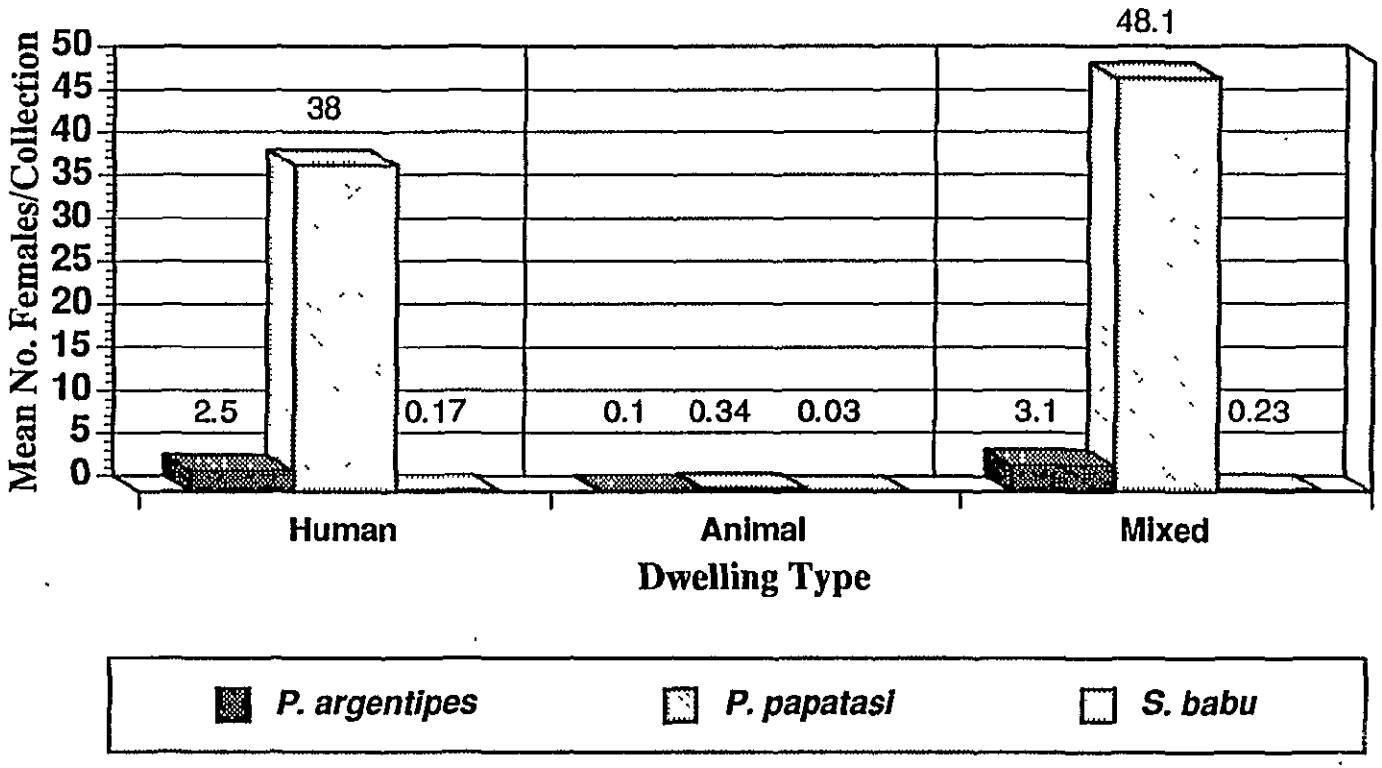


Figure 7. Numbers of *Phlebotomus argentipes* collected during an 8-day period from resting sites in three types of dwelling at Biratnagar.

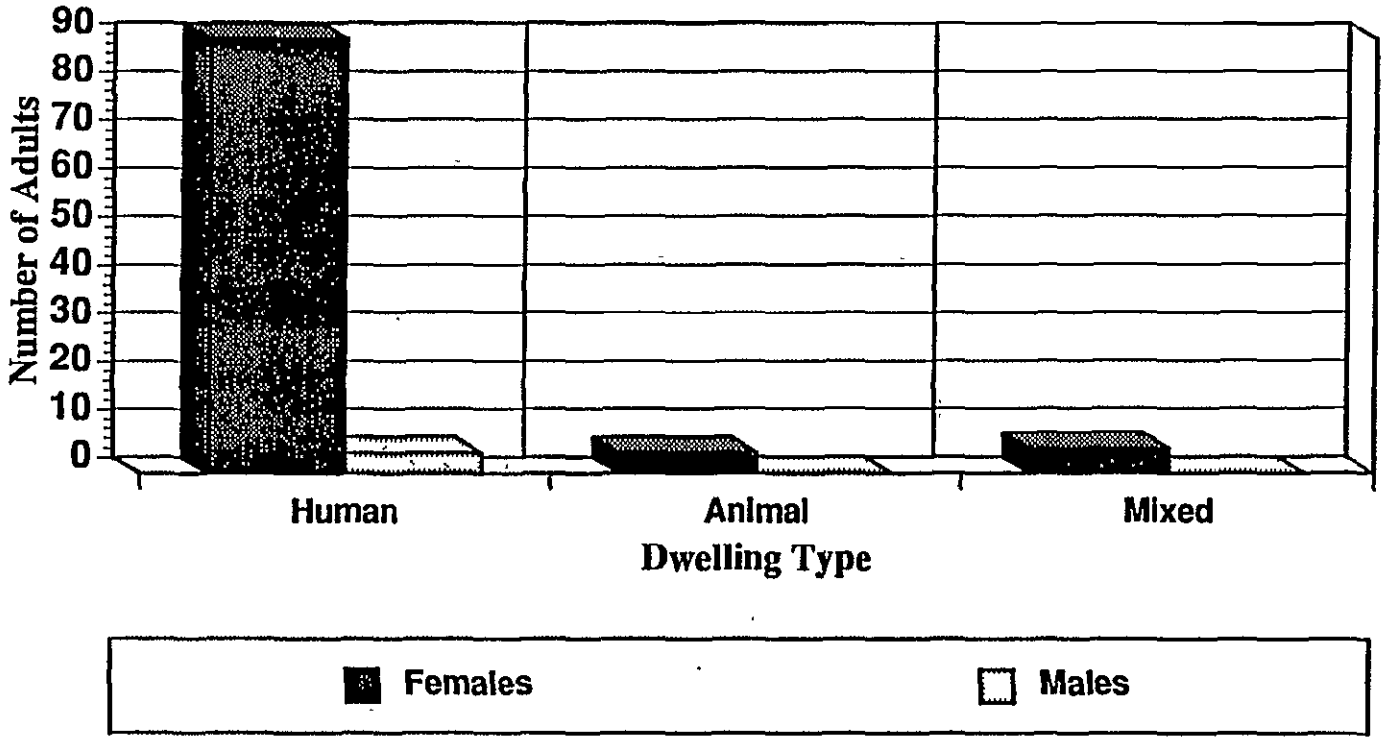


Figure 8. Numbers of *Phlebotomus papatasi* collected during an 8-day period from resting sites in three types of dwelling at Biratnagar.

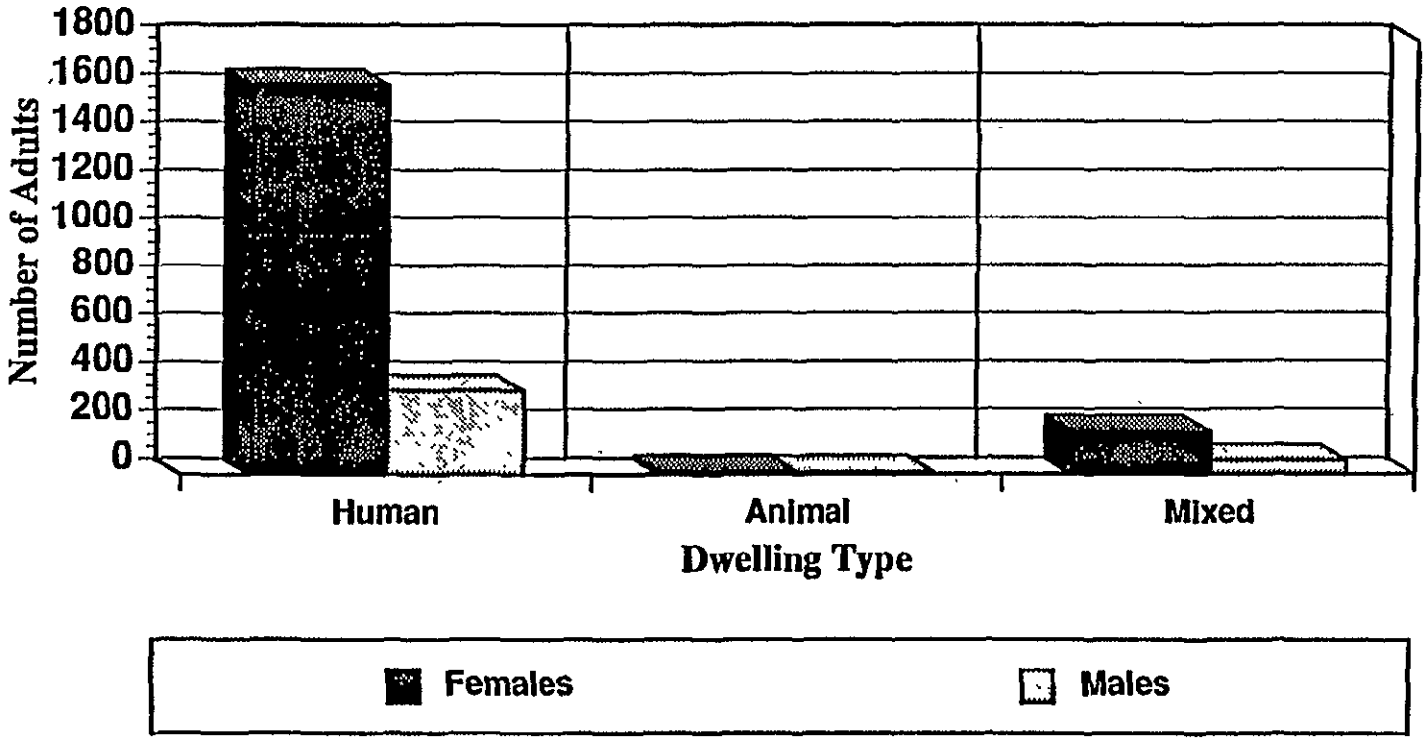
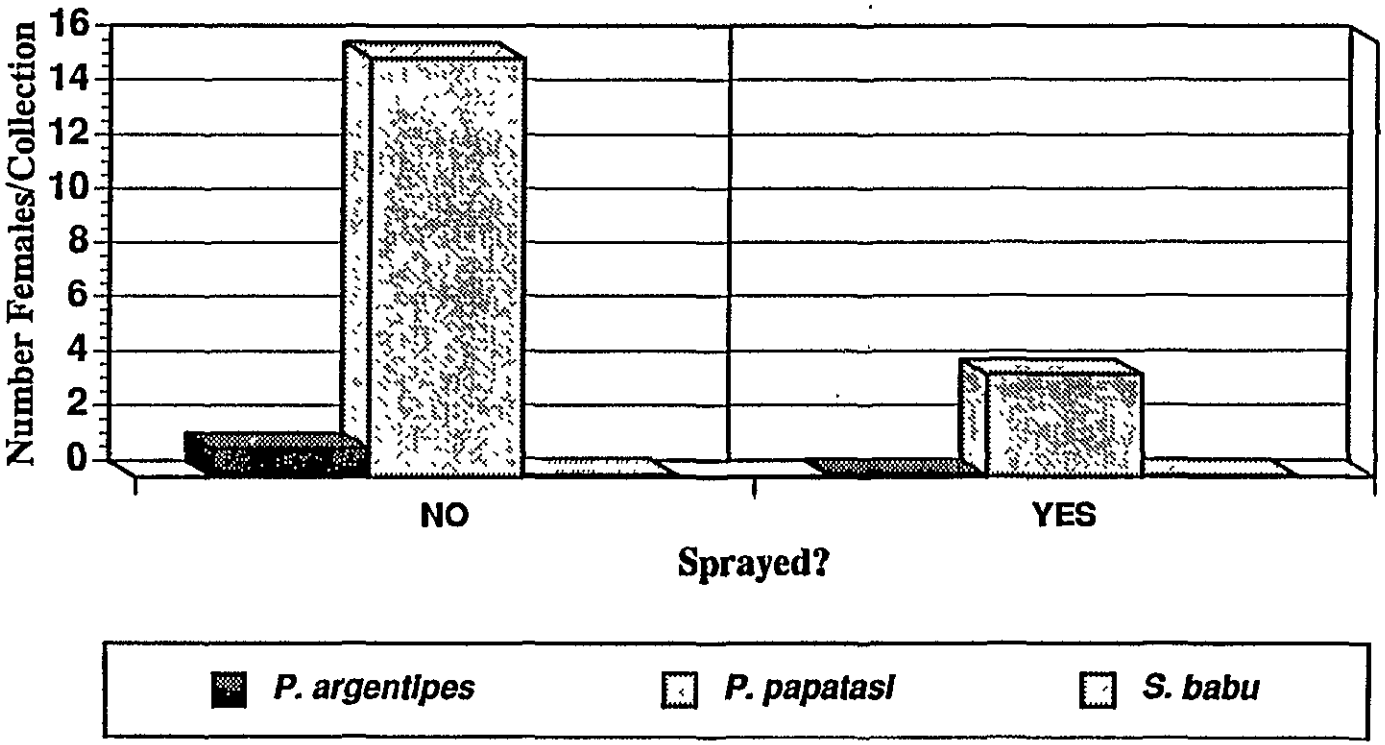


Figure 9. Comparison of species densities in unsprayed versus sprayed villages at Biratnagar.



b. *P. papatasi* predominated in all collections in Biratnagar and were found in greatest abundance in human dwellings (90% of females; 85% of males), followed by mixed dwellings (0.5% of females; 10% of males), followed by animal dwellings (0.1%) (Figure 8). As with *P. argentipes,* they were most easily collected with mouth aspirators from resting sites inside human dwellings before daylight and before the occupants stirred.

c. *S. babu* were found only in negligible numbers, mainly in human dwellings (88% of females; 50% of males), followed by animal dwellings (13% of females; 50% of males). None were collected in mixed dwellings.

2. Sand fly dissections: A total of 1,498 female sand flies were dissected and examined for *Leishmania* flagellates (46 *P. argentipes*; 1,452 *P. papatasi*). No infected sand flies were found.

3. Host preference study/bloodmeal analysis: Bloodmeals of 350 *P. papatasi* were squashed on filter paper to preserve them for later analysis and identification of the bloodmeal source. No *P. argentipes* or *S. babu* were collected for bloodmeal analysis in Biratnagar.

4. Unsprayed vs. sprayed villages: The density of female sand flies collected in unsprayed villages averaged 16.5 females per 20-minute resting-site collection. In sprayed villages the density was three females per collection, suggesting a reduction of 82% in the female sand fly population. Species-specific reduction was 68% for *P. argentipes*, 80% for *P. papatasi*, and virtually 100% for *S. babu.* In Biratnagar, no *P. argentipes* were collected in bite-landing collections (Figure 9). In contrast, the density of biting *P. papatasi* was relatively high in unsprayed villages (up to 16 flies per hour) and low in sprayed villages (about 2 per hour).

Discussion

In unsprayed villages, the relative density of the kala-azar vector, *P. argentipes*, was much lower at Biratnagar than at Gaur. The relative density of the vector in each area was much lower than that observed in Janakpur during March and April. Seasonal and climatic conditions

undoubtedly contributed to this difference. By the time this training activity began, both study sites had experienced two to three months of frequent rains. Differences in dwelling construction and behavioral differences in the vector populations may also have affected the results of our surveys. In Gaur and Janakpur, animal, human, and mixed-use dwellings were usually fully enclosed with four mud-plastered walls and, except for the entrance, had no major openings to the outside. Such dwellings provided abundant dark, protected resting sites for sand flies. In Biratnagar, mixed and animal dwellings were usually open on one side, and the walls on the remaining three sides were usually constructed of interwoven sticks and bamboo. Rarely were walls mud plastered. Such dwellings afforded few protected resting sites for sand flies, and this was reflected in our collection results. Human dwellings in Biratnagar, however, afforded good resting sites for sand flies, except two-storied houses, in which part of the upper level was typically constructed of interwoven bamboo, letting in light and discouraging sand flies from resting there after sunrise. Nonetheless, some *P. argentipes* were obtained in resting-site collections in second- story sleeping rooms. Pre-dawn collections were the most productive, indicating that resting *P. argentipes* are highly sensitive to light and to disturbances caused by routine human activities.

Although some reports mention kala-azar control as a collateral benefit of DDT spraying for malaria control[2], there is no literature on control efforts directed specifically against kala-azar vectors in Nepal. A renewed kala-azar vector control effort began in April 1992, and was targeted at rural villages in the Eastern Terai. Judging from our observation of greater reduction in sand fly population density in sprayed villages in Biratnagar compared to Gaur (82% and 69%, respectively), it seems reasonable to conclude that spraying operations using Icon in Morang District were more effective and perhaps better supervised than in Rautahat District. Since the critical density of the vector required to maintain endemic transmission is unknown, the impact that current spraying operations will have on kala-azar incidence is also unknown.

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2. Mukhopadhyay AK, and AK Chakravarty, 1992. Studies on the development of *Leishmania donovani* in *Phlebotomus argentipes* and *P. papatasi* after blood meals on kala-azar patients in Bihar, India. *WHO/CTD/VBC 92:* 996; WHO/Leish 92:32.

Kala-azar in Nepal is presumed to be anthroponotic, but no reservoir studies have been conducted in the country (see footnote 2). Results of resting collections, and bloodmeal ELISAs on material obtained during the author's previous trip, indicate that *P. argentipes*, the confirmed vector, is rather zoophilic and opportunistic. Although there is good evidence of man-to-man transmission from the focal distribution of human cases, Killick-Kendrick[3] suggested the ox as a probable host of kala-azar based on reports of the fly's feeding behavior and a convincing account of bovine cutaneous leishmaniasis from an endemic center of human kala-azar.

The surveys reported here were pilot studies and the by-products of a training effort. The results are incomplete and inadequate but provide valuable insights into areas worthy of further investigation. A proper understanding of the natural history of kala-azar transmission in Nepal can come only through a one to two year multi-faceted longitudinal study that focuses on parasite-vector-host relationships. Such a study would yield a more complete picture of the spatial and temporal dynamics of a kala-azar transmission site. Such an understanding would enable MOH personnel to determine when and where kala-azar transmission is likely to occur and where and how to implement countermeasures.

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3. Killick-Kendrick, R: 1990. Are cattle a reservoir host of kala-azar in India? *Trans R Soc Trop Med Hyg 84*:754.

5. Recommendations

Based on the combined results of this and the previous training visit the following actions are recommended:

1. Draft a detailed proposal and seek financial and administrative support for a two-year study of the natural history of kala-azar transmission in Nepal.

2. Expand efforts to delineate specific breeding sites of *P. argentipes* using sticky paper traps, emergence traps, mark-release-recapture techniques and other suitable means to locate oviposition sites, immature stages, and newly emerged adults.

3. During future survey operations, conduct resting site collections during pre-dawn hours.

4. Continue and intensify the existing program of residual spraying of human dwellings in affected areas and include also spraying of mixed and strictly animal dwellings. Encourage closer supervision of residual spraying operations to insure that all dwellings (human, animal, mixed) within affected villages are sprayed inside and out.

5. Explore the possibilities of using permethrin impregnated bed nets and/or curtains to help reduce human-sand fly contact.

6. Conduct laboratory experiments to investigate the vector competence of *P. argentipes* and *P. papatasi* for *L. donovani.*

6. Itinerary and People Contacted

August 31 - September 29, 1992

Date	Time	Particular
08/31/92	1730	Departed Balto/Wash Int. Airport for Nepal.
09/02/92	0850	Arrived in Kathmandu and was met by Shreedhar P. Pradhan, Medical Entomologist/ Malaria Advisor, Division of Health and Family Planning, USAID/Kathmandu, and taken to Soaltee Oberoi Hotel.
09/02/92	1300	Discussed logistical arrangements and training schedule with Shreedhar Pradhan.
09/03/92	0830	Met and briefed Dr. David Oot, Chief, and Mrs. Molly Gingerich, Health Development Officer, Office of Health and Family Planning, USAID/Kathmandu: they were accompanied by S.P. Pradhan.
	1000	Briefed Ms. Theodora Wood-Stervinou, Deputy Director, USAID/Kathmandu, accompanied by S.P. Pradhan.
	1100	Met with Dr. Badri L. Shrestha, Chief, Malaria Control Division, MOH, and his staff, accompanied by S.P. Pradhan, to discuss day-to-day training schedule and to arrange for equipment and supplies.
	PM	Checked and assembled equipment; made local purchases of supplies.

09/04/92	0930	Met with Dr. M.P. Shrestha at Teku Hospital, Kathmandu, accompanied by S.P. Pradhan, and discussed the kala-azar situation in the country and potential for collaboration.
09/04/92	1100-1700	Completed logistical preparations.
09/05/92	0830	Departed Kathmandu for Gaur, Rautahat District, in USAID vehicle, accompanied by S.P. Pradhan.
09/05/92	2000	Arrived in Hetauda and stayed the night; met students from Central Region.
09/06/92	0530	Departed Hetauda for Gaur.
09/06/92	1000	Arrived at Gaur and met with District Public Health Office staff to discuss the training schedule and to review the proposed study sites. Met later with Mr. Bal B. Chand, District Public Health Officer, Rautahat District.
09/06/92 — 09/13/92		Conducted training at Gaur (see Annex B).
09/14/92	0600	Departed Gaur for Biratnagar, Morang District.
09/14/92	1500	Arrived in Biratnagar and met with Mr. Janak Das Shrestha, District Public Health Officer, and his staff to discuss the training and to review the proposed study sites.
09/14/92		Met with Dr. G.R. Karki, Medical Superintendent, and Dr. Shekha Koirala, Physician, Koshi Zonal Hospital, Biratnagar, to discuss the current kala-azar situation and potential collaborations.
09/15/92 — 09/23/92		Conducted training at Biratnagar (see Annex B).

09/24/92	1400	Returned to Kathmandu by plane.
09/25/92	0900	Debriefed with Dr. David Oot and staff, Office of Health and Family Planning, USAID/Kathmandu.
	1100	Debriefed with Ms. Theodora Wood-Stervinou, Deputy Director, USAID/Kathmandu.
	1200	Debriefed with Dr. Badri L. Shrestha, Chief, and Jitendra P.B. Shrestha, Deputy Chief, Malaria Control Division, MOH and their senior technical staff and discussed further activities and research plans for leishmaniasis prevention and control.
	PM	Worked on preliminary trip report.
09/26/92		Worked on preliminary trip report.
09/27/92		Worked on preliminary trip report; attended social function sponsored by Mrs. Theodora Wood-Stervinou, Deputy Director, USAID/Kathmandu.
09/28/92	2230	Departed Kathmandu for Washington, D.C.

Annex A

Nepal Leishmaniasis Project
Sand Fly and *Leishmania* Surveillance Training Phase III: Training Reinforcement and Augmentation

List of Participants

Gaur, Rautahat District, 6-13 September

1. Mr. Bal B. Chand, DPHO, Rautahat District*
2. Mr. Murari L. Das, Entomologist
3. Mr. Ganga L. Shrestha, Assistant Entomologist
4. Mr. Promod Shrestha, Malaria Inspector
5. Mr. Sambhu B. Shrestha, Malaria Inspector*
6. Mr. Ram Sinhasan Shah, Malaria Inspector
7. Mr. Upendra Singh, Malaria Inspector*
8. Mr. Mohan Singh, Insect Collector*
9. Mr. Manoj Banskota, Insect Collector*
10. Mr. Harischandra Chaudhari, Insect Collector*

Biratnagar, Morang District, 15-23 September

1. Mr. Janak D. Shrestha, DPHO, Morang District*
2. Mr. Baber B. Pradhan, Assistant Entomologist*
3. Mr. Udhab Sharma, Malaria Assistant*
4. Mr. Bhanu Prasad Aryal, Malaria Inspector
5. Mr. Shyam Lama, Malaria Inspector
6. Mr. Bishnu B. Shrestha, Malaria Inspector
7. Mr. Badri B. Thapa, Malaria Inspector
8. Mr. Khagendra Shrestha, Malaria Inspector*
9. Mr. Indra K. Shrestha, Insect Collector*
10. Mr. Rabin Nagarkoti, Insect Collector*

* New student

Annex B

Nepal Leishmaniasis Project
Sand Fly and *Leishmania* Surveillance Training Phase III: Reinforcement and Augmentation Training Schedule

Gaur, Rautahat District, September 6-13, 1992

Sunday, September 6

0800-0830 Welcome/Orientation

0830-0900 Pretest

0900-1000 Sand fly population dynamics

a. assessing population densities
b. age structure
c. effects of extrinsic factors

1000-1130 Factors affecting transmission rates

a. vectorial capacity
b. transmission season
c. transmission site
d. human biting index (HBI)
e. entomological inoculation rate (EIR)

1130-1140 Break

1140-1300 Criteria for incriminating a vector

1300-1400 Sand fly surveillance techniques

a. species diversity and habitats/sticky traps
b. adult dispersion behavior and flight range mark-release-recapture
c. breeding sites-emergence traps

Sunday, September 6 (cont.)

1400-until finished	Site selection and setting traps

Monday, September 7

0600-0800	Sand Fly Surveillance Methods
0800-0830	Breakfast
0830-until finished	Processing collections/Sand fly dissection and examination/Sand fly taxonomy/record keeping.
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Tuesday, September 8

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Wednesday, September 9

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast

Wednesday, September 9 (cont.)

0830-until finished	Processing collection/sand fly dissection and examination/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Thursday, September 10

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/ sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Friday, September 11

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Saturday, September 12

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Biratnagar, Morang District, September 15-23, 1992

Monday, September 14

0600	Proceed to Biratnagar, Morang District, and meet with Dr. Janak Das Shrestha, DPHO, to discuss study sites

Tuesday, September 15

0800-0830	Welcome/Orientation
0830-0900	Pretest
0900-1000	Sand fly population dynamics

a. assessing population densities
b. age structure
c. effects of extrinsic factors

Tuesday, September 15 (cont.)

1000-1130 Factors affecting transmission rates

a. vectorial capacity
b. transmission season
c. transmission site
d. human biting index (HBI)
e. entomological inoculation rate (EIR)

1130-1140 Break

1140-1400 Criteria for incriminating a vector

1300-1400 Sand fly surveillance techniques

a. species diversity and habitats/sticky traps
b. adult dispersion behavior and flight range mark-release-recapture
c. breeding sites-emergence traps

1400-until finished Site selection and setting traps

Wednesday, September 17

0600-0800 Sand fly surveillance methods

0800-0830 Breakfast

0830-until finished Processing collection/Sand fly dissection and examination/Sand fly taxonomy/record keeping

1300-1400 Lunch

1600-until finished Sand fly surveillance methods

Thursday, September 18

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Friday, September 19

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Saturday, September 20

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/Sand fly dissection and examination/sand fly taxonomy/record keeping

Saturday, September 20 (cont.)

1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Sunday, September 21

0600-0800	Sand fly surveillance methods
0800-830	Breakfast
0830-until finished	Processing collection/Sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Monday, September 22

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Tuesday, September 23

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/sand fly dissection and examination/sand fly taxonomy/record keeping
1300-1400	Lunch
1600-until finished	Sand fly surveillance methods

Wednesday, September 24

0600-0800	Sand fly surveillance methods
0800-0830	Breakfast
0830-until finished	Processing collection/ sand fly dissection and examination/sand fly taxonomy/record keeping
1200-1300	Review of survey results and oral final exam
1300-until finished	Service and pack supplies and equipment

Thursday, September 25

Return to Kathmandu by Air