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Susceptibility of Species A, B, and C of *Anopheles culicifacies* Complex to *Plasmodium yoelii yoelii* and *Plasmodium vinckei petteri* Infections

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ABSTRACT: The comparative susceptibilities of colonized species A, B, and C of *Anopheles culicifacies* complex and *Anopheles stephensi* were determined for 2 rodent malaria parasites *Plasmodium vinckei petteri* and *Plasmodium yoelii yoelii*. All the 3 members of the complex were found to support complete sporogony with varying success. Controls, *A. stephensi*, become readily infected, with >70% developing oocysts. Of the test groups, species A had the highest percentage of mosquitoes with oocysts (>25%) and sporozoites (>15%). *Anopheles culicifacies* species B were least susceptible; less than 10% had oocysts and sporozoites in the salivary glands. The results demonstrate that *A. culicifacies* species A is most susceptible and species B is least susceptible to infections with both the parasites.

Among the many recognized malaria vectors in India, widely distributed *Anopheles culicifacies* sensu lato is responsible for 60–70% of total malaria cases (Sharma, 1984). Since its recognition as a species complex, many biological differences have been reported among the 4 members (species A, B, C, and D) (Subbarao, 1988). Among these, differential vector potential (Subbarao et al., 1980, 1988, 1992) is probably of greatest epidemiological importance.

Many field studies carried out in India indicate a definite role of species A, C, and D in malaria transmission in different parts of the country. However, species B that has been found in almost all parts of India and is sympatric in certain locations with species A, C, and D is apparently the major vector in southern India (Suguna et al., 1983; Sabesan et al., 1984), whereas its role in northern India appears to be negligible or doubtful (Subbarao et al., 1980, 1988, 1992). The recognition of these sibling species in the *A. culicifacies* complex and their reported variability in malaria transmission warrants their comparative eval-

uation as vectors of the malaria parasite in the laboratory. The present study reports on the comparative susceptibility of species A, B, and C of the *A. culicifacies* complex to 2 rodent malaria parasites *Plasmodium yoelii yoelii* and *Plasmodium vinckei petteri*.

Screening of wild *A. culicifacies* sensu lato populations, the isolation and establishment of cyclic colonies of species A, B, and C of the complex, and their maintenance in the insectary were followed as described by Adak et al. (1999). Mean temperature and humidity of the insectary were 28 ± 1 C and 70 ± 5 % RH, respectively. For feeding experiments, *P. yoelii yoelii* 265BY and *P. vinckei petteri* 279BY parasites supplied by Prof. Irene Landau, Museum national d'histoire Naturelle, Paris (Landau and Boulard, 1978) were cycled in 4–5-wk-old laboratory-bred BALB/c mice either by the inoculation of cryopreserved infected blood samples or by mosquito-sporozoite-mice inoculation of parasite isolates using *Anopheles stephensi* as the vector. Inoculated mice were kept in individual cages. Parasitemia, gametocyte production, and gametocyte sex ratios were determined periodically in the infected animals. The ideal time for mosquito feeding, i.e., 4th and 5th days of infection, was ascertained by the presence of mature gametocytes in the JSB-stained (Singh, 1956) thin blood smears. Four- to 6-day-old mosquitoes starved overnight were used in all feeding experiments. In feeding experiments about 75–100 starved mosquitoes from each test batch (*A. culicifacies*), along with similar number of *A. stephensi* that were used as a positive control, were held in cloth cages and were fed on gametocyte-positive

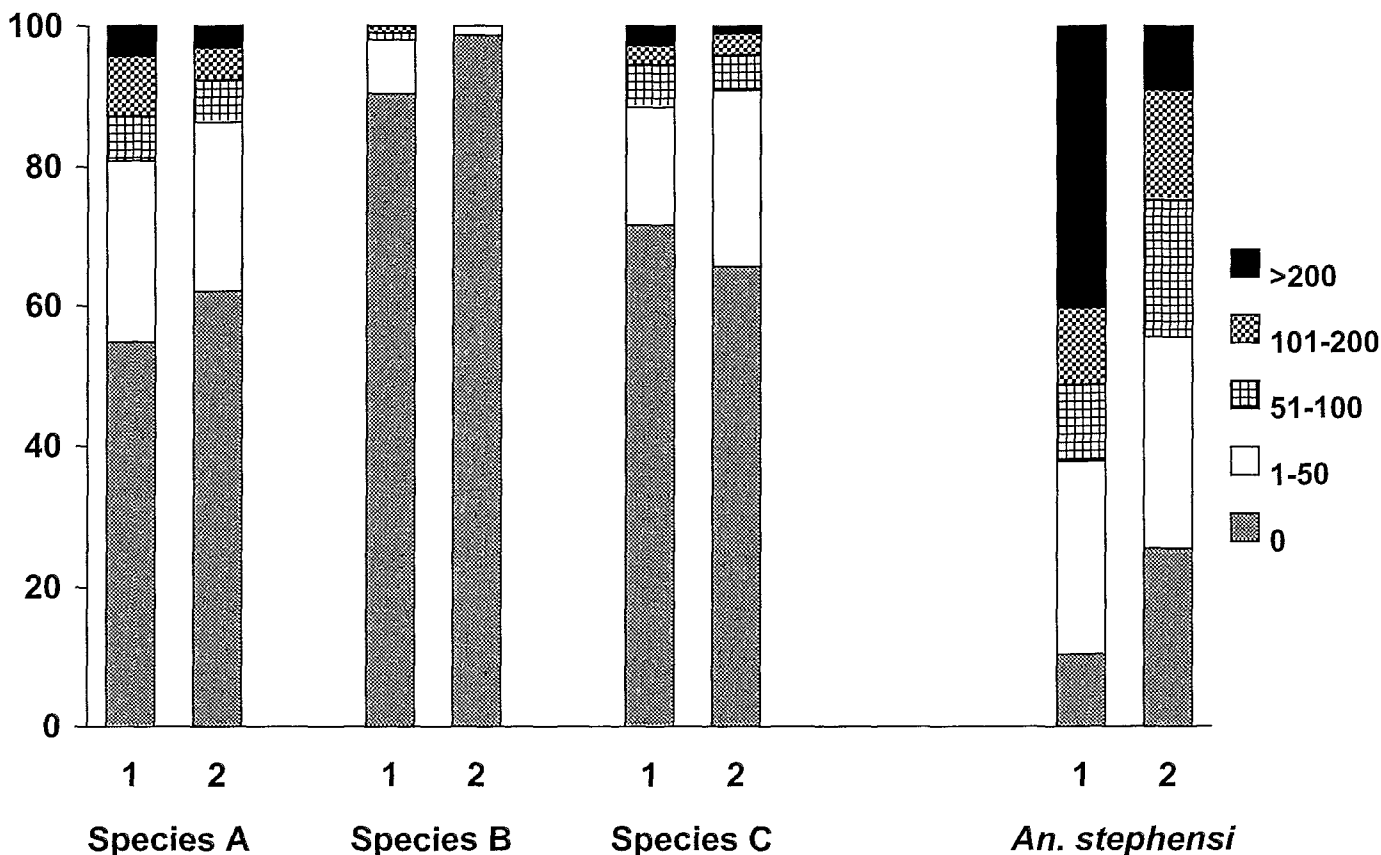


FIGURE 1. Proportion (%) of mosquitoes with different oocyst densities in *Plasmodium yoelii yoelii* (1)- and *Plasmodium vinckei petteri* (2)-infected *Anopheles culicifacies* complex and *Anopheles stephensi*.

mice for 1 hr during the daytime in a room maintained at 23 ± 1 C. Subsequently, fed mosquitoes were separated and then held in a separate insectary maintained at 23 ± 1 C and $70 \pm 5\%$ RH for sporogonic development.

To assess the degree of infection by the presence of oocysts, midguts from 50% of the surviving mosquitoes from each test/control group were dissected in normal saline (0.65% NaCl) on day 6 after infection. Midguts were stained in a drop of 0.5% Mercurochrome (Eyles, 1950) and examined under a $10\times$ objective for the presence of oocysts. To assess the presence of sporozoites, salivary glands were dissected from remaining mosquitoes in normal saline on day 12 after infection and examined using a $40\times$ phase-contrast objective. Oocyst and sporozoite production was assessed by pooling data from all replicates. Data are expressed as the percentage of mosquitoes infected with oocysts or sporozoites. The extent of oocyst infection was estimated by calculating the geometric mean number of oocysts among the infected mosquitoes of all replicates. Replicates in which reference mosquito *A. stephensi* did not acquire infection were excluded from analysis.

Susceptibility was estimated by comparing the differences in oocyst production (percent gut positivity), mean number of oocysts (geometric mean), and percent gland positivity, i.e., sporozoite production, of different members of the *A. culicifacies* complex with *A. stephensi* by pooling results from all replicates. To compare the oocyst and sporozoite production, chi-square tests were performed. The degree of infection was estimated by

calculating the geometric mean number of oocysts among oocyst-positive mosquitoes. To test the differences between mean numbers of oocysts, Student's *t*-tests were performed using log-transformed data.

Anopheles stephensi fed more readily as compared to species A, B, and C of the complex. Among fed mosquitoes, the overall proportions of mosquitoes with oocyst counts of 0, 1–50, 51–100, 101–200, and >200 for each species infected with *P. yoelii yoelii* and *P. vinckei petteri* are given in Figure 1.

The detailed results of the comparative susceptibility of species A, B, and C to *P. yoelii yoelii* and *P. vinckei petteri* are summarized and presented in Tables I and II, respectively. In *A. stephensi*, oocyst production was very high, and oocyst development ranged between 71.3 and 96.8%, whereas oocyst production among different members of the species complex varied widely. The oocyst production in species A was 45.2 and 26.0% for *P. yoelii yoelii* and *P. vinckei petteri*, respectively, as compared to 23.2 and 34.3% in species C, whereas in species B it was only 9.7 and 1.3%. In species B, the mean oocyst number was much lower, i.e., 8.6 and 2.2, as compared to 27.9 and 14.2 in species A, and 20.8 and 14.0 in species C for *P. yoelii yoelii* and *P. vinckei petteri* infections, respectively. The frequency of the infected salivary glands, i.e., sporozoite production was also much lower in species B (5.3 and 0.0%) as compared to 23.1 and 18.3% in species A and 22.7 and 12.9% in species C for *P. yoelii yoelii* and *P. vinckei petteri* infections, respectively.

TABLE I. Comparative susceptibility of species A, B, and C of *Anopheles culicifacies* and *Anopheles stephensi* to *Plasmodium yoelii yoelii*.

Species compared	No. of feed-ings	Oocyst production (n)*	χ^2 and <i>P</i> values	Mean† no. of oocyst (fiducial limits)	<i>t</i> -test (df) and <i>P</i> values	Sporozoite production (n)	χ^2 and <i>P</i> values
Species A vs. <i>A. stephensi</i>	24	45.2% (188)	56.3, <i>P</i> < 0.001	27.9 (23.2–33.6)	3.3 (214) <i>P</i> < 0.01	23.1% (169)	27.3, <i>P</i> < 0.001
		84.5% (155)		61.2 (52.6–71.1)			
Species B vs. <i>A. stephensi</i>	23	9.7% (206)	241.3, <i>P</i> < 0.001	8.6 (5.9–12.5)	8.6 (139) <i>P</i> < 0.001	5.3% (76)	44.7, <i>P</i> < 0.001
		96.8% (125)		170.6 (150.2–193.9)			
Species C vs. <i>A. stephensi</i>	22	23.2% (181)	132.9, <i>P</i> < 0.001	20.8 (16.0–27.1)	4.3 (157) <i>P</i> < 0.001	22.7% (119)	24.8, <i>P</i> < 0.001
		89.3% (131)		70.4 (61.3–80.9)			
Sp. A vs. B			63.4, <i>P</i> < 0.001		2.8 (103) <i>P</i> < 0.01		11.3, <i>P</i> < 0.001
Sp. B vs. C			13.0, <i>P</i> < 0.001		NS‡		10.5, <i>P</i> < 0.01
Sp. A vs. C			19.8, <i>P</i> < 0.001		NS		NS

* n = Number of mosquitoes dissected.

† Geometric mean among oocyst positive mosquitoes.

‡ NS = not significant.

The oocyst/sporozoite production and mean number of oocysts were significantly higher in *A. stephensi* than in all the members of the *A. culicifacies* complex for both the parasite species tested. Among the *A. culicifacies*, oocyst/sporozoite production was significantly lower in species B as compared to species A and C, for both the parasites. There was no significant difference in oocyst/sporozoite production between species A and C for both the parasites, but oocyst production of *P. yoelii yoelii* in species A was significantly higher as compared to species C. No significant differences in mean number of oocysts among oocyst-positive mosquitoes were observed between species A, B, and C, except in species A for *P. yoelii yoelii*, where it was significantly higher.

Analysis of differences in infectivity of *P. yoelii yoelii* and *P. vinckei petteri* in *A. stephensi* revealed significantly higher

oocyst production and mean number of oocysts for *P. yoelii yoelii* as compared to *P. vinckei petteri* (oocyst production, $\chi^2 = 25.8$, *P* < 0.001; mean number of oocyst, *t* = 5.13, *P* < 0.001); however, no significant difference was observed in sporozoite production of either of the parasites. In the *A. culicifacies* complex, all the members exhibited higher oocyst production for *P. yoelii yoelii* as compared to *P. vinckei petteri* (species A, $\chi^2 = 9.8$, *P* < 0.01; species B, $\chi^2 = 11.2$, *P* < 0.001; species C, $\chi^2 = 4.0$, *P* < 0.05), whereas no difference in mean numbers of oocysts and sporozoite production was observed, except in species B where sporozoite production was significantly higher for *P. yoelii yoelii* ($\chi^2 = 4.8$, *P* < 0.05) as compared to *P. vinckei petteri*.

In the present investigation, the relative susceptibility of 3 members of the *A. culicifacies* complex was determined and

TABLE II. Comparative susceptibility of species A, B, and C of *Anopheles culicifacies* and *Anopheles stephensi* to *Plasmodium vinckei petteri*.

Species compared	No. of feed-ings	Oocyst production (n)*	χ^2 and <i>P</i> values	Mean† no. of oocyst (fiducial limits)	<i>t</i> -test (df) and <i>P</i> values	Sporozoite production rate (n)	χ^2 and <i>P</i> values
Species A vs. <i>A. stephensi</i>	16	26.0% (96)	47.2, <i>P</i> < 0.001	14.2 (9.6–20.9)	3.0 (88) <i>P</i> < 0.01	18.3% (71)	7.8, <i>P</i> < 0.01
		77.4% (84)		49.1 (40.0–60.2)			
Species B vs. <i>A. stephensi</i>	14	1.3% (158)	145.3, <i>P</i> < 0.001	2.2 (1.0–5.0)	2.4 (67) <i>P</i> < 0.02	0.0% (90)	70.9, <i>P</i> < 0.001
		71.3% (94)		31.9 (26.4–38.5)			
Species C vs. <i>A. stephensi</i>	12	34.3% (99)	27.7, <i>P</i> < 0.001	14.0 (10.9–18.0)	4.3 (91) <i>P</i> < 0.001	12.9% (70)	25.5, <i>P</i> < 0.001
		76.6% (64)		54.5 (44.9–663)			
Sp. A vs. B			38.6, <i>P</i> < 0.001		NS		17.9, <i>P</i> < 0.001
Sp. B vs. C			55.3, <i>P</i> < 0.001		NS		12.3, <i>P</i> < 0.001
Sp. A vs. C			NS‡		NS		NS

* n = Number of mosquitoes dissected.

† Geometric mean among oocyst positive mosquitoes.

‡ NS = not significant.

compared with reference mosquito species *A. stephensi*. In different feeding experiments, considerable variability was observed in the intensity and level of infection among different replicates, as well as among individual mosquitoes in each replicate. Significantly lower oocyst and sporozoite production was observed in all the members of the *A. culicifacies* complex as compared to *A. stephensi*. Further, among the 3 species, species A was found to be most susceptible and species B was least susceptible. Species B, however, was relatively more susceptible to *P. yoelii yoelii* as compared to *P. vinckei petteri*. Species B of the *A. culicifacies* complex has been reported to be less susceptible to *Plasmodium vivax* infections (Adak et al., 1999). Similar observations were also made from 2 separate vector incrimination studies in the field using species-specific monoclonal antibodies of *P. vivax* and *Plasmodium falciparum* by IRMA (Subbarao et al., 1988, 1992). In the present study, significantly lower numbers of oocysts were observed in species B, but they appeared to develop normally; degenerated or encapsulated oocysts in species B strain were never observed. The evaluation of these mosquito species in relation to their susceptibility to rodent *Plasmodium* species may help in assessing the susceptibility to human *Plasmodium* species, especially where studies on human malaria parasites are not feasible. In view of reduced susceptibility of species B to rodent plasmodia as observed in the present study and to *P. vivax* infection (Adak et al., 1999), it is suggested that *A. culicifacies* species B could be used in the future as an experimental vector model in understanding susceptibility to infection by mosquitoes and may also be promising for selecting plasmodial refractory strains.

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Comparative Biology of *Uncinaria* Spp. in the California Sea Lion (*Zalophus californianus*) and the Northern Fur Seal (*Callorhinus ursinus*) in California

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early winter, revealing that adult *Uncinaria* spp. are spontaneously lost at <3 mo of age of the pups. Sand samples from rookeries, used by both *Z. californianus* and *C. ursinus*, on SMI were negative for free-living, L₃ in summer months but positive in fall and winter months, indicating seasonality occurred.

Taxonomic specificity of the hookworm *Uncinaria* spp. in pinnipeds is uncertain. Two species, *Uncinaria lucasi* and *Uncinaria hamiltoni*, have been described, but types with intermediate measurements have also been reported (Baylis, 1933;

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