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Vector Competence and the Susceptibility of *Anopheles pullus* and *Anopheles belenrae* to *Plasmodium vivax*-Infected Blood from Thai Patients

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Introduction:

As the geographical distribution of *Anopheles spp.* varies in the Republic of Korea (ROK), determining the relative vector competence of the *Anopheles* spp. provides a basis for delineating malaria risks to Korean populations and US military/civilian populations deployed to the ROK. Our understanding of the *Anopheles spp.* in the transmission of *P. vivax* in the ROK was based solely on their distribution and being positive for *P. vivax* using PCR techniques. Therefore, several comparative susceptibility studies using artificial membrane feeding were inevitably conducted to demonstrate the susceptibility studies of the potential Anopheles vectors in the ROK to *P. vivax* infections. Studies characterizing the malaria epidemiology and transmission of *P. vivax* in the ROK often described vector competence of three members of the *Anopheles* Hyrcanus Group, *An. kleini, An. lesteri,* and *An. sinensis*, but did not address the vector competence of *An. pullus* and *An. belenrae*. In this study, we proposed to determine the vector competence and the susceptibility of *An. pullus* and *An. belenrae* to *P. vivax* infection.

Purpose:

To provide understanding of the potential transmission dynamics of *P. vivax* in the ROK by determining the vector competence of *An. pullus* and *An. belenrae*, both of which were implicated as vectors of *P. vivax* in the ROK.

Methods:

- Mosquito Colonization and Rearing:
  - Blood-fed *Anopheles spp.* females were collected at a cattle farm (37^0 54'32.18" N, 126^0 44'01.88" E) located approximately 3 km. from the DMZ, Tongilchon, Gyeonggi Province, ROK.
  - The mosquitoes were shipped from ROK to Entomology Department, AFRIMS and maintained at the insectary.
  - Gravid females were allowed to oviposit. The females were identified to species using PCR.
  - After species identification, *An. pullus* and *An. belenrae* were placed separately and reared at AFRIMS insectary at 25^{0}C ± 1^{0}C, 80% RH. A 5% (v/v) solution of multivitamin syrup was provided as a source of energy.
  - For mosquito colonization, adult mosquitoes were provided blood meals via artificial membrane feeding. Five-seven day old (F27-37) mosquitoes were used to determine the susceptibility to *P. vivax* infection.
  - *An. dirus* Peyton and Harrison, colonized for >25 years at AFRIMS insectary and highly susceptible to *Plasmodium spp.* infection, was used as a positive control (F204-214).
- Blood Sample Collection & Mosquito blood feeding:
  - Total 6 ml blood samples were collected from Thai *P. vivax*-infected patients (x 10 replicates).
  - Blood samples were fed to a total of 100 5-7 day-old female *An. belenrae*, *An. pullus*, and *An.dirus* (control) by artificial membrane feeding.
- Determination of Infection:
  - On day 8-9 post-feed, up to 25% of the blood fed mosquitoes were dissected and the number of oocysts in the midgut were counted.
  - On day 14-15 post-feed, the salivary glands of the remaining mosquitoes were dissected and the number of sporozoites were determined.

Figure 1: Mosquito collection area, *Anopheles* mosquito species, *P. vivax*-infected blood samples collection, artificial membrane feeding and the determination of *P. vivax* infection.

Results:

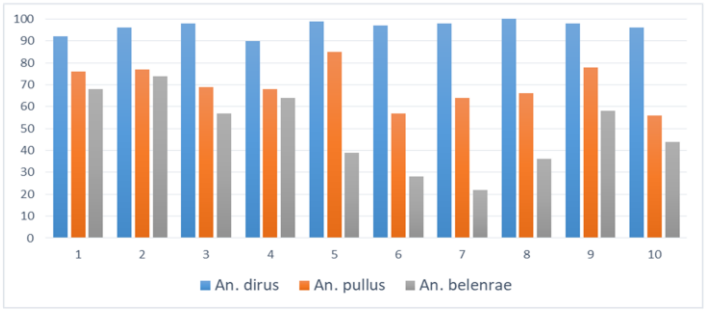

Figure 2: The number of blood fed mosquitoes (100 mosquitoes each per replicate x 10) include *An.dirus, An.pullus,* and *An.belenrae* that were provided blood meals on blood obtained from *P. vivax*-positive Thai patients.

Table 1: Number and percentage of engorged mosquitoes after membrane feeding (100 mosquitoes per replicate x 10) with blood obtained from *P. vivax*-positive Thai patients.

Anopheles Species	Number of replicates	Number Engorged (%)[a]	Number of Engorged Mosquitoes	
			Min	Max
An. dirus (control)	10	963 (96.3)[b]	90	100
An. pullus	10	684 (68.4)[c]	53	85
An. belenrae	10	480 (48.0)[d]	22	72

[a] The chi-square test was used to determine significant differences in the percentage of engorged mosquitoes. Different letters for engorged mosquitoes denote significant differences among each of the species (p-value <0.05).

Discussion & Conclusion:

- In this present study, blood-feeding rates among *An. pullus* (68%) and *An. belenrae* (48%) was significantly lower than that of *An. dirus* (96.3%). This may be due to the recent colonization of the species from Korea compare to the long-term adaptation of *An. dirus*.
- Although *An. pullus* and *An. belenrae* are susceptible to *P. vivax* midgut infections, that either there is a salivary gland barrier or that there is incomplete oocyst development, or a combination of both that inhibits large numbers of sporozoites from entering the salivary glands for transmission to occur.
- These data indicate that both *An. belenrae* and *An. pullus* are very poor vectors of *P. vivax*.

Table 2: Number and percentage of mosquitoes with oocysts, by species, dissected on days 8-9 post-feed on membrane feeders with blood from *P. vivax*-positive Thai patients, mean and range number of oocysts, minimum and maximum diameter of oocysts.

Anopheles Species	Number Dissected[a]	Number (%) with Oocysts[b]	Mean (Range) Number Oocyst[c]	Oocyst Size (µm)	
				Min	Max
An. dirus (control)	250	161 (64.4)[d]	28.7 (1-169)[f]	19.0	32.0
An. pullus	250	30 (12.0)[e]	2.0 (1-8)[f]	17.9	20.7
An. belenrae	250	29 (11.6)[e]	2.9 (1-10)[f]	19.1	24.5

[a] In total, 10 replicates of female mosquitoes that fed on blood from P. vivax infected patients were positive for oocysts
[b] The chi-square test was used to determine significant differences in the percentage of mosquitoes positive for oocysts (p-value < 0.05). The same letter denotes no significant difference.
[c] The Mann-Whitney U test was used to determine significant differences in the mean numbers of oocysts among the mosquito species. There is no significant difference in the mean number of oocysts (p-value > 0.05). The same letter denotes no significant difference.

Table 3: Number (%) of mosquitoes dissected, by species, on days 14-15 post-feed that fed on membrane feeders with blood obtained from local Thai patients positive for *P. vivax*, and number (%) positive for sporozoite indices

Anopheles Species	Number Trials	Number Dissected	No. (%) with Sporozoites[a]	Number (%) Positive by Sporozoite Index[d]			
				1	2	3	4
An. dirus (control)	7	174	147 (84.5)[b]	5 (3.4)[e]	12 (8.2)[g]	23 (15.6)	107 (72.8)
An. pullus	7	175	6 (3.4)[c]	5 (83.3)[f]	1 (16.7)[g]	0	0
An. belenrae	6	137	7 (5.1)[c]	5 (71.4)[f]	2 (28.6)[g]	0	0

[a] Number and percentage of sporozoites positive mosquitoes. The chi-square test was used to determine significant differences in the percentage of mosquitoes positive for sporozoites (p-value <0.05). The same letter denotes no significant difference.
[d] Number of positive mosquitoes by sporozoite indices (Estimated sporozoite number): +1 (1-10), +2 (11-100), +3 (101-1,000), +4 (>1,000). The chi-square test was used to determine significant differences in the number of mosquitoes positive sporozoite indices +1 (p-value <0.05). Different letters denote significant difference between apecies (p-value <0.05), while the same letter denotes no significant difference. There were no significant differences in the number of mosquito positive indices for +2 salivary glands (p-value >0.05).

Disclaimer

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