Host Selection of Field-Collected *Anopheles jeyporiensis* and *Anopheles nivipes* in Bangladesh

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Abstract. *Anopheles jeyporiensis* and *Anopheles nivipes* appear to play an important role in contemporary malaria transmission in Bangladesh. However, very little is known about the natural host selection of these vectors. Therefore, host selection of these two species was investigated in Bandarban, the most malarious region of Bangladesh. A total of 480 engorged mosquitoes were analyzed. The human blood index (HBI) of *An. jeyporiensis* varied from 4.17% in outdoor to 19.17% in indoor collections. Similarly, HBI of *An. nivipes* ranged between 0.83% and 22.50% from outdoor to indoor. For both species, cow blood indices were significantly higher than HBI in both indoor and outdoor collections. These data demonstrate the opportunistic and zoophilic nature of *An. jeyporiensis* and *An. nivipes*, which suggests that approaches such as zooprophyllaxis may prove beneficial as a control strategy.

The abundance of *Anopheles baimaii*, *Anopheles minimus*, and *Anopheles philippinensis*, which are historically recognized as principal vectors of malaria in Bangladesh, has been reported to be relatively low in recent studies. By contrast, species such as *Anopheles jeyporiensis* of the *Myzomyia* series and *Anopheles nivipes* of the *Annularis* group were historically not abundant nor considered to be important for malaria transmission, but are now found in relatively high numbers and are positive for malaria parasites by circumsporozoite protein-ELISA and polymerase chain reaction (PCR) in Bandarban.^{1–3} Historical evidence for *An. jeyporiensis* to be implicated as a vector of human malaria is available since 1944 when it was recorded to be positive for sporozoites in Chittagong near Bandarban district.⁴ *Anopheles nivipes* was not a species of interest in Bangladesh until 2012, when a study in Bandarban molecularly identified specimens that had been designated morphologically as *An. philippinensis* and that were *Plasmodium* positive.¹ In another study, most of the *An. philippinensis* were reidentified as *An. nivipes* after molecular confirmation.³ Despite its zoophilic nature, *An. nivipes* has also been reported positive for *Plasmodium* parasites in neighboring India and other countries.^{5,6}

Generally, animal composition of the mosquito blood meal should reflect the relative abundance of available hosts if they do not discriminate between host species. However, mosquitoes may preferentially select one host over others and the degree of anthropophily strongly affects the vectorial capacity for human pathogens. Such behavior is crucial for incriminating a species to be a human malaria vector.^{7,8} Unfortunately, studies have yet to be conducted regarding the host selection of *An. jeyporiensis* and *An. nivipes* in Bangladesh. Hence, the objective of this study was to identify the host associations of these two critically incriminated vector species in a malaria-endemic setting.

Entomological surveys were conducted between July 2010 and June 2011 in Bandarban, Bangladesh. Detailed description of the study design is provided elsewhere.⁹ Mosquitoes were

collected from both indoor and outdoor sites using CDC miniature light traps, following the collection methods of Alam et al.¹ Mosquitoes were morphologically identified using standard taxonomic keys.¹⁰ The number of blood-engorged *An. jeyporiensis* and *An. nivipes* from these collections was recorded. Because there is no information of host association of these two species in Bangladesh, it was hypothesized that they might not discriminate between human and nonhuman hosts. Therefore, considering 50% expected prevalence for any host with 95% confidence and 0.08 (8%) precision, 120 specimens from each site (indoor/outdoor) and each species were selected by simple random sampling for blood meal identification. Thus, a total of 480 specimens were assessed for this study.

DNA was extracted from mosquito abdomens following Post et al.,¹¹ and a multiplexed blood meal PCR was conducted for domestic mammalian hosts including human, cow, goat, pig, and dog.¹² Data on relative numbers of available hosts including human, cow, goat, pig, and dog were extracted from existing surveillance data.⁹ The number of potential human hosts for *An. jeyporiensis* indoor and outdoor, and *An. nivipes* indoor and outdoor was 340, 401, 261, and 339, respectively; the number of potential cow hosts was 139, 182, 193, and 206, respectively; the number of potential goat hosts was 60, 53, 42, and 67, respectively; the number of potential pig hosts was 27, 35, 46, and 15, respectively; and the number of potential dog hosts was 18, 21, 10, and 22, respectively.

The proportions of blood meals (blood meal index) for each host were calculated for *An. jeyporiensis* and *An. nivipes* by dividing the number of fed mosquitoes by the total number of mosquitoes of that species tested. A two-sample independent proportion test was conducted to compare the difference in blood meal indices between indoor and outdoor specimens. The host selections of mosquitoes among human, cow, and goat were calculated by forage ratio (FR), host selection index, and host feeding index (HFI)—the proportion of feds on one host with respect to another and divided by the comparative proportion of feds on these two hosts. A FR value of < 1.0 indicates the avoidance of host in favor of other available hosts, a value of 1.0 indicates neither a selection nor avoidance for any host, and a value > 1.0 indicates bias to a particular host. A HFI of 1.0 indicated equal

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TABLE 1

Blood meal indices of indoor and outdoor foraging *Anopheles jeyporiensis* and *Anopheles nivipes* from Bandarban, Bangladesh, collected during July 2010–June 2011

Collection site and <i>Anopheles</i> species	Total collected	Number of fed	<i>n</i>	HBI	CBI	GBI	UN
Indoor							
<i>An. jeyporiensis</i>	1,364	465	120	19.17*†‡	40.00*§	5.83†	45.00
<i>An. nivipes</i>	2,373	975	120	22.50	41.67 ¶	0.00	54.17
Outdoor							
<i>An. jeyporiensis</i>	786	441	120	4.17#‡	79.17#§	2.5	15.83
<i>An. nivipes</i>	2,310	1,377	120	0.83	66.67¶	0.8	32.50

CBI = cow blood index in %; GBI = goat blood index in %; HBI = human blood index in %; *n* = number tested; UN = unidentified blood in %.

* CBI of *An. jeyporiensis* is significantly higher than HBI indoor.

† HBI of *An. jeyporiensis* is significantly higher than GBI indoor.

‡ HBI indoor of *An. jeyporiensis* is significantly higher than HBI outdoor.

§ CBI indoor of *An. jeyporiensis* is significantly lower than CBI outdoor.

|| CBI of *An. nivipes* is significantly higher than HBI indoor.

¶ CBI indoor of *An. nivipes* is significantly lower than CBI outdoor.

CBI of *An. jeyporiensis* is significantly higher than HBI outdoor.

feeding and values < 1.0 and > 1.0 indicate decrease or increase.^{13–15}

A total of 8,732 anophelines representing 22 species were collected from indoor and 8,189 anophelines of 23 species were collected from outdoor. *Anopheles nivipes* was the dominant species in both collections, composing 27.18% (*n* = 2,373) of the indoor and 28.21% (*n* = 2,310) of the outdoor collections. The number of fed *An. nivipes* was higher in outdoor (*n* = 1,377) than in indoor (*n* = 975) collections. *Anopheles jeyporiensis* was also abundant in the collections, composing 15.62% (*n* = 1,364) of the indoor and 9.6% (*n* = 786) of the outdoor collections (Table 1).

Cow was the most frequently detected host, irrespective of vector species or collection location (Table 1). Among host blood meals, the cow blood index was 40% for *An. jeyporiensis* and 41.67% for *An. nivipes* collected indoors and 79.17% and 66.67%, respectively, for these species collected outdoors. The human blood index (HBI) values for *An. jeyporiensis* significantly increased from outdoor (4.17%) to indoor (19.17%) collections. Similarly, the HBI value for *An. nivipes* was increased from 0.83% for outdoor collections to 22.5% for indoor collections. Despite these results, the PCR assay was unable to identify the host for a large number of mosquitoes morphologically scored as engorged (45% for *An. jeyporiensis* and 54.17% for *An. nivipes*). This was most likely due to deterioration of the samples during long storage, compromised efficiency of PCR, and limitation of host inclusion in the PCR.

In comparison to cow, the FR for human was low for both species, irrespective of the collection sites (Table 2). The FR for cow was highest for *An. jeyporiensis* (2.21) collected outdoors. Selection of cow as a host was also high for both species. The highest selection index for cow was observed for *An. nivipes* from both indoor (0.92) and outdoor (0.96) collections. By contrast, calculation of the HFI for each pair of hosts revealed that human was less often selected than cow but more frequently selected than goat (Table 3). Overall, this study reveals that cow is the host of choice for *An. jeyporiensis* and *An. nivipes* in Bandarban.

Data obtained from the present study support the endophagic behavior of *An. jeyporiensis*, as reported by previous studies.¹⁶ Whereas a similar number of *An. nivipes* were collected indoors and outdoors, the number of fed mosquitoes was higher in outdoor collections, which support the exophagic behavior of this species as reported from neighboring

northeast India.¹⁷ The human-fed catches indoors, despite the presence of long-lasting insecticidal nets, might be due to indoor early biting behavior for both mosquito species and/or feeding on exposed occupants who sleep on floor mats without mosquito nets.¹⁸ Computation of host selection may be skewed by collection location. Sampling inside human dwellings may overestimate HBI, and similarly, sampling too close to animal enclosures may underestimate HBI. This is reflected in this study as host index varied significantly between collection sites. There is also evidence that mosquito population composition, foraging and resting behavior, and infection status depend on the presence of hosts in a given community.¹⁹ Both species demonstrated feeding on human, cow, goat, and mixed hosts, suggesting that although they may have a host selection preference, they are somewhat opportunistic and responsive to a variety of host cues. Earlier reports from Myanmar documented both anthropophilic and zoophilic behaviors for *An. jeyporiensis*.²⁰ *Anopheles nivipes* is considered to be primarily zoophilic in India and Myanmar, which is concordant with the findings of this study.^{17,20} Field sampling records for this study note that most of the households have domestic animals that are kept in very close proximity to the house. This may mask anthropophilic cues and, therefore, reduce the number of mosquitoes successfully locating a human host or alternatively draw these mosquitoes in proximity with humans to whom they may not usually be attracted. This may also account for the mixed human/nonhuman blood meals. As zooprophyllaxis is a potential control strategy, these results are encouraging.

The presence of human blood in *An. jeyporiensis* and *An. nivipes*, as well as their high abundance, diverse habitats, and infection with malaria sporozoites, indicates their potentiality

TABLE 2

Forage ratios (FRs) and host selection index (HSI) of *Anopheles jeyporiensis* and *Anopheles nivipes* in Bandarban, Bangladesh

Mosquito species	FR			HSI		
	Human	Cow	Goat	Human	Cow	Goat
Indoor						
<i>An. jeyporiensis</i>	0.21	1.33	0.09	0.13	0.81	0.05
<i>An. nivipes</i>	0.07	0.89	0.00	0.08	0.92	0
Outdoor						
<i>An. jeyporiensis</i>	0.05	2.22	0.33	0.02	0.85	0.13
<i>An. nivipes</i>	0	2.07	0.08	0	0.96	0.04

TABLE 3

Host feeding index (HFI) for each pair of mammalian hosts fed by *Anopheles jeyporiensis* and *Anopheles nivipes* in Bandarban, Bangladesh

Host 1 (x)	Host 2 (y)	Indoor		Outdoor	
		<i>An. jeyporiensis</i>	<i>An. nivipes</i>	<i>An. jeyporiensis</i>	<i>An. nivipes</i>
Human*	Cow	0.16	0.08	0.02	0
	Goat	2.76	0	0.16	0
Cow	Human	5.20	12.22	41.92	0
	Goat	17.10	0	6.75	26.75
Goat	Human	0.36	0	6.21	0
	Cow	0.15	0	0.15	0.04

* For example, HFI of human vs. cow was calculated as $(x/y)/(x'/y') = [(0.125/0.317)/(0.55/0.23)]$; the other HFIs were calculated similarly.

for malaria transmission. Additional studies are required to examine the effectiveness of zooprophylaxis and alternative control measures for such emerging malaria vector species in endemic areas of Bangladesh and Southeast Asia.

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