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Mosquito diversity (Diptera: Culicidae) and medical importance, in a bird sanctuary inside the flooded forest of Prek Toal, Cambodia

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ABSTRACT

Wild birds have been identified as reservoirs of many pathogens transmissible to humans through bird-biting mosquitoes. Understanding the diversity of vector fauna in bird-conservation areas, is a cornerstone to later develop early detection systems and reduce the risk of emerging disease incidence. Prek Toal is a bird Sanctuary in the Tonle-Sap area in Cambodia hosting the largest migratory bird colony of southeast Asia. The Culicidae fauna was investigated during both dry and rainy seasons. In total, 8,224 mosquitoes from 6 genera and 25 species were collected. The two most represented genera were *Anopheles* (8 species) and *Culex* (11 species), while the dominant species was *Culex vishnui* (58.8%). During the rainy season, 1.6 fold more mosquitoes were caught and the number of species doubled. One of the most notable feature is the absence of any *Aedinii* mosquito which could be attributed to the low human density around the area, and the large populations of migratory birds. A total of 13 species from 3 genera are reported to be of medical importance. 6 medically-important species were collected in all 3 sites, and are known to bite both wild birds and humans: *An. barbirostris*, *An. campestris*, *Cx. bitaeniorhynchus*, *Cx. vishnui*, *Ma. indiana* and *Ma. uniformis*. One of the most important feature is the over-abundance of *Culex vishnui*, largely dominant in Prek Toal throughout the seasons and the sites, and being present everywhere in Cambodia. This finding stresses its potentiality to act as bridge vectors in the case of new disease emergence.

Introduction

Wild and migratory birds have been identified as reservoirs for many pathogens transmissible to humans and other wildlife through bird-biting mosquitoes (Work et al., 1955; Gern et al., 1998). The interactions between birds and the mosquitoes feeding upon them have been extensively studied (Brown and O'Brien, 2011). Migratory birds can play an important role in virus circulation. This observation emerged from the concomitance of large migratory birds' arrivals and outbreaks of virus in new areas, but also from virus isolations from these animals. For instance, outbreaks of the West Nile Virus in temperate regions generally occur during late summer or early fall, coinciding with the arrival of large concentrations of migratory birds (and abundance of mosquitoes) (Work et al., 1955; Taylor et al., 1956; Nir et al., 1967; Brown and O'Brien, 2011). Similarly, in the US, both Eastern and Western equine encephalomyelitis alphaviruses, have been isolated from migrating birds highlighting their role in possible local transmission (Lord & Calisher, 1970). It has therefore been hypothesised that migratory birds can act as introductory host, either by infecting blood-sucking arthropods, by direct transmission, by transporting infected vectors during their migration, or by being infected after their arrival at their final landing point (Brown and O'Brien, 2011). In this realm it is fundamental to study the vector diversity, and latter, the virus prevalence in conserved areas playing a role as resting sites in bird migrations.

Located at the north-west corner of the Tonle Sap Lake, in Cambodia, Prek Toal is a Bird Sanctuary (Ramsar site) since 2001, hosting one of the largest migratory bird population in southeast Asia (Campbell et al., 2006). While several mosquito checklists in South-East Asia have been established (for Thailand see Tsukamoto et al., 1987; Rattanarithikul *et al.*, 2005; for Laos: Motoki et al., 2019, and for Vietnam: Bui & Darsie, 2008), over the past few years, yet only partial informations were available for the Cambodian fauna (Harbach, 2021).

In areas like Prek Toal, hosting a rich avifauna −129 species (MoE, 2010) – comprising both resident and migratory birds- and high mosquito diversity, it provides many opportunities for unique ecological relationships among organisms. Furthermore, there are high risks of

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introduction of new pathogens in bird populations as well as other vertebrates due to migratory birds coming to this area every year. In rapidly changing socio-economic and biophysical environments, particularly in South-East Asia in the context of change in land use, vector-borne diseases can represent a threat for the most vulnerable human populations. Recent pandemic like Zika, Chikungunya, West Nile or Japanese Encephalitis are perfect examples of such recent problems. Yet the Culicidae fauna in conserved areas such as Prek Toal, is still unknown. This knowledge gap is important to fill, considering that change in land use shifts the risk of mosquito-borne disease emergence by changing the relationship between mosquito and host communities both qualitatively and quantitatively (Lourenço-de-Oliveira et al., 2004; Delatte et al., 2010). A modification of the landscape, like deforestation, may facilitate the dispersion of anthropophilic mosquito species into previously unfavorable habitats (Dos Santos et al., 2018) and opportunistic mosquito species may act as bridge vectors. Listing the Culicidae fauna, and its potential vector species is the first step before screening their virome, to be able to properly assess the risk of infectious disease emergence in Prek Toal conserved area.

Materials and methods

Collecting permit

This research was allowed by Cambodian authority, with an authorisation letter from the ministry of Environment, issued on 15th November 2019, (Permit N°1443), and an authorisation letter from the Directorate General of Nature Conservation and Protection, issued on the 14th of February 2019 (Permit N°057).

Study sites

Cambodia is largely covered by the Tonle Sap Lake (TSL), which display a very peculiar hydrological regime due to the bisannual reversal flow of the Tonle Sap river into the Mekong. The TSL can become more than four times larger during the rainy season making it one of the world's largest wetland area (Ringler & Cai, 2006). The TSL area consists of three main habitats: an open lake at the center, seasonally flooded grasslands and rice fields at the margins. The Tonle Sap floodplains are the predominant breeding and feeding areas for many species of migratory waterbirds and more than 129 species have been recorded from Prek Toal bird sanctuary (MoE, 2010; Sokha pers. Com.).

The sampling sites were located in two sites inside the bird sanctuary, and in Prek Toal floating village in Battambang Province (Fig. 1).

The first site was located inside the conserved area in the flooded forest, at close proximity to the birds nests on one of the branch of the Sangker river at 5.9 km 60°SSW of Prek Toal floating village. The second site, still in the conserved area, on a path perpendicular to the Sangker river, was located 7.5 km, 54°SSW from Prek Toal floating village. The last location, site 3, was inside the floating village itself.

Collection methods

Adult mosquitoes were collected using BG-1 Sentinel™ Mosquito Traps, 7.5–12 Volt (BioQuip, CA, U.S.A.), baited with BG-Lure® (BioQuip, CA, U.S.A.) and CDC Mini Light Traps with incandescent light baited with dry ice placed in a dry ice dispenser next to the traps. Eggs were collected using an ovitrap, and immature stages prospection was carried out every time a suitable breeding site containing either temporary or permanent water body was identified. Eggs, larvae and pupae were kept in water bottles on the field, and then –back at the Institut Pasteur du Cambodge- bred in the insectarium using a mosquito breeder® (BioQuip, CA, U.S.A.).

Collection trips took place during both dry (25 February 2019 to 01 March 2019) and rainy seasons (25 to 30 November 2019). The meteorological conditions observed in 2019 did not differ from the previous years. Climate data were obtained from climateengine.org and displayed in the Fig. 2. The temperatures were gathered using Terraclimate and CFSR satellite data, while the precipitation were obtained from CHIRPS satellite datas.

The collection process consisted of 15 traps on the first two sites: 5 ovitraps, 5 CDC Mini Light Trap, 5 BG-1 Sentinel™ Mosquito Trap, 7.5–12 Volt (BioQuip, CA, U.S.A.). Both sites were sampled during 3 consecutive days (n = 72 h) and traps (beside the ovitrap) were harvested daily. Site 3 consisted of 5 lightraps and 5 BG traps. For logistical reasons, this site was sampled for 12 h, during night time.

Morphological identification

The mosquitoes were kept individually in Eppendorf tubes, identified on the field, and kept in an electric tank cooler at 4 °C for the time of the expedition. Specimens were then placed in −80 °C for virus conservation. They were identified using diagnostic morphological characters (Rattanarithikul et al., 2005a, 2005b, 2006a; 2006b; 2007; 2010; Ool et al., 2004; Oo et al., 2006). The identification was confirmed by the examination of the original description of the species.

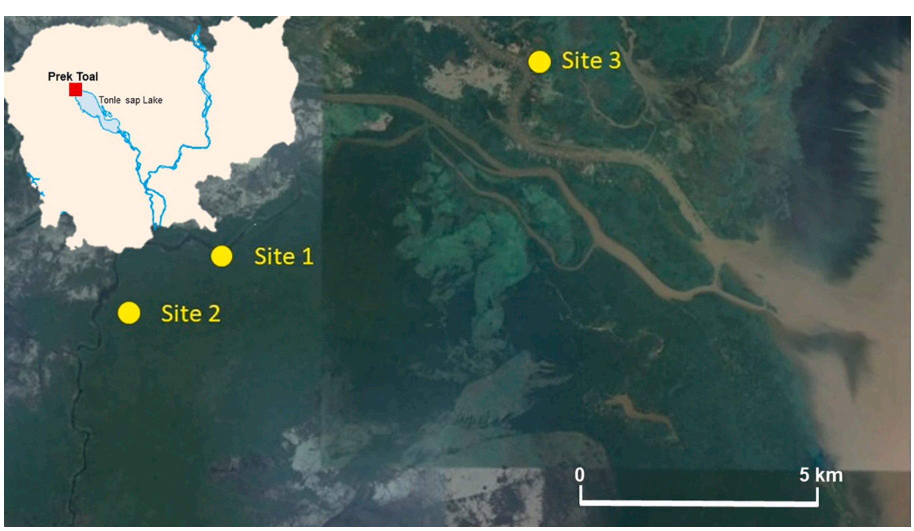

Fig. 1. Location of Prek Toal Bird sanctuary in Cambodia, and location of the three sites in the area of the park. Map generated using Google Earth Pro.

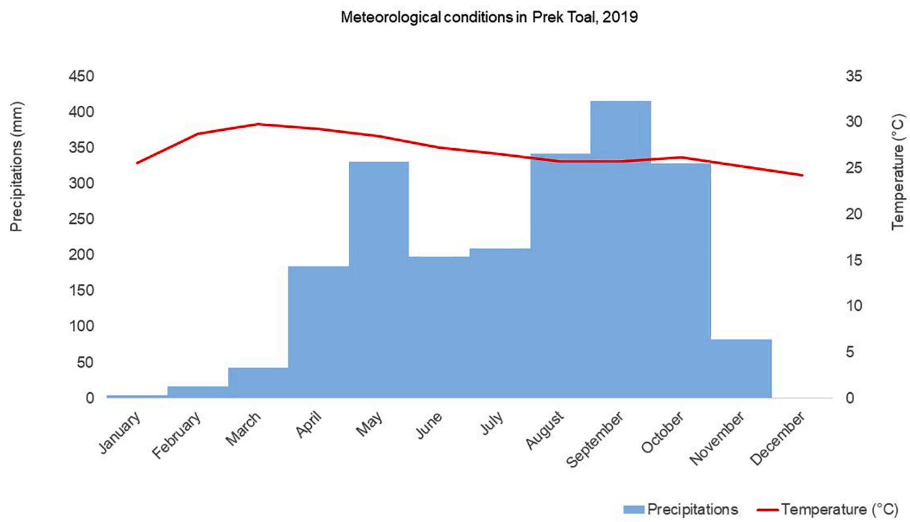

Fig. 2. Meteorological conditions in Prek Toal site in 2019.

Data analysis

The Shannon diversity index (H') and the Simpson diversity index (D) were calculated using the formula provided in Morris et al. (2014), and reported hereafter:

$$H' = -\sum P_i \ln(P_i) \text{ OR } H' = -\Sigma(n_i/N) * \log(n_i/N)$$

$$D = 1 - \sum P_i^2$$

where, P_i is the proportion of specimens belonging to a species and is calculated by dividing "n_i" (the number of specimens belonging to one species) by "N", the total number of specimens from all species in the site.

For the diversity indexes, and the total count of species, only the identified species were taken into account, not the specimens identified only to the genus level unless they were the only recorded species in the genus.

Results

The Culicidae fauna collected during the two expeditions is reported

Table 1
List of mosquito species collected in Prek Toal.

Species	Site 1(along the river)		Site 2(perpendicular to the river)		Site 3 (floating village)		
	Dry season	Raining season	Dry season	Raining season	Dry season	Raining season	**Total**
Anopheles (*Anopheles*) *argyropus* (Swellengrebel, 1914)	–	7	–	–	–	–	**7**
Anopheles (*Anopheles*) *barbirostris* van der Wulp, 1884	–	36	–	33	–	11	**80**
Anopheles (*Anopheles*) *campestris* Reid, 1962	–	70	–	9	7	–	**86**
Anopheles (*Anopheles*) *hodgkini* Reid, 1962	–	25	–	–	–	–	**25**
Anopheles (*Anopheles*) *nigerrimus* Giles, 1900	–	1	–	–	–	–	**1**
Anopheles (*Anopheles*) *nitidus* Harrison, Scanlon & Reid, 1973	–	13	–	–	–	–	**13**
Anopheles (*Anopheles*) *peditaeniatus* (Leicester, 1908)	–	15	–	–	–	–	**15**
Anopheles (*Anopheles*) *sinensis* Wiedemann, 1828	7	–	–	–	–	–	**7**
Anopheles sp	3	53	–	36	11	–	**103**
Culex (*Oculeomyia*) *bitaeniorhynchus* Giles, 1901	18	773	19	377	2	25	**1214**
Culex (*Eumelanomyia*) *brevipalpis* (Giles, 1902)	–	7	–	–	–	–	**7**
Culex (*Culex*) *fuscocephala* Theobald, 1907	–	1	–	–	–	–	**1**
Culex (*Culex*) *gelidus* Theobald, 1901	1	14	1	–	–	–	**16**
Culex (*Culex*) *perplexus* Leicester, 1908	1	33	–	3	–	–	**37**
Culex (*Culex*) *pseudovishnui* Colless, 1957	–	12	–	–	–	–	**12**
Culex (*Oculeomyia*) *sinensis* Theobald, 1903	–	425	–	38	–	–	**463**
Culex (*Culex*) *sitiens* Wiedemann, 1828	–	2	–	–	–	–	**2**
Culex (*Culex*) *quinquefasciatus* Say, 1823	14	–	–	–	2	–	**16**
Culex (*Culex*) *tritaeniorhynchus* Giles, 1901	163	65	–	4	–	–	**232**
Culex (*Culex*) *vishnui* Theobald, 1901	2577	1746	15	347	76	75	**4836**
Culex sp	19	215	1	145	–	–	**380**
Ficalbia (*Ficalbia*) *minima* (Theobald, 1901)	–	4	–	–	–	–	**4**
Mansonia (*Mansonioides*) *annulifera* (Theobald, 1901)	–	13	–	–	–	–	**13**
Mansonia (*Mansonioides*) *indiana* Edwards, 1930	13	84	5	8	2	–	**112**
Mansonia (*Mansonioides*) *uniformis* (Theobald, 1901)	70	249	1	49	–	1	**370**
Mansonia sp	1	127	–	35	1	–	**164**
Mimomyia (*Mimomyia*) *aurea* (Leicester, 1908)	–	4	–	–	–	–	**4**
Mimomyia sp	–	3	–	–	–	–	**3**
Uranotaenia sp	–	–	–	–	1	–	**1**
Total	**2887**	**3997**	**42**	**1084**	**102**	**112**	**8224**
Number of species	9	22	5	9	6	4	**25**
Simpson index (D)	0,81	0,31	0,36	0,38	0,72	0,62	
Shannon index (H')	0,45	1,64	1,16	1,30	0,65	0,85	

in table 1.

In total 8,224 mosquito were collected. About 7.9% (651 specimens) were only identifiable to the genus level. During the study, 25 species were collected, mostly in *Culex* and *Anopheles* genus with respectively 11 and 8 species, followed by *Mansonia* (3 sp), *Mimomyia*, *Ficalbia* and *Uranotaenia* (1 species each). Out of the 260 known species recorded from Cambodia (Harbach, 2021), the sampled mosquitoes in Prek Toal represent 8.4% of the Cambodian fauna.

The Culicidae species number increases during the rainy season where it more than doubled: in the site 1 it went from 9 species to 22, while on the site 2, from 5 to 9 species. This change might be attributed to the increase of favourable breeding places due to the changing rainfall pattern, and an increase in migratory birds, acting as main hosts (Chathuranga et al., 2018). However, in the site 3 (in the floating village) the number of species decreased but the number of specimens collected remained the same between the two seasons.

Among the different genera, *Culex* mosquitoes are the most represented, with 11 species, accounting for 87.74% of all collected mosquitoes. Two dominant species are greatly responsible of this: *Cx. vishnui* accounting for 58.8% of all collected mosquitoes (regardless the season), followed by *Cx. bitaeniorhynchus* (14.76%, mainly present during the rainy season). This observation is translated in biodiversity indices: Shannon index increased during the rainy season. While it is strong for the first site (from 0.45 to 1.64), it is mild in the second site (1.16–1.30), and low for the third site (0.65–0.85). The Simpson diversity index, is high in the first site during the dry season (0.81) showing the dominance of *Cx. vishnui* mosquitoes (representing 89.2% of all caught mosquitoes) and lower during the rainy season (0.31) as the proportion of *Cx. vishnui* gets lower (44.1%) and the relative abundance of other species increase. The Simpson diversity index is similar for both seasons in the site 2 (0.36–0.38 for dry and rainy season respectively), and decrease lightly for the site 3 (0.72–0.62).

During the dry season 3,031 mosquitoes were collected in the three sites, for a total of 10 species, while during the rainy season, a total of 5,193 mosquitoes belonging to 22 species were caught. The number of species changed drastically between the seasons: it doubled between the dry and the rainy season on the first two sites: 9 to 22 in site 1; 5 to 9 in site 2.

The site 1 display the largest diversity of *Anopheles* with 8 species, compared to site 2 where 2 species were only present during the raining season, and site 3 (2 species). The same is observable for the *Culex,* having all the species in the site 1 (11 species) while the site 2 and 3 only display 6 and 3 species respectively.

Surprisingly, no species of *Aedes* nor even *Aedini* members were collected in any of these sites. Similarly, out of the 25 identified species, 6 were collected in all 3 sites: *An. barbirostris*, *An. campestris*, *Cx. bitaeniorhynchus*, *Cx. vishnui*, *Ma. indiana* and *Ma. uniformis*.

Discussion

Prek Toal bird sanctuary is characterised by an abundance of *Culex* mosquitoes throughout the different seasons, accounting for 11 species and 87.74% of collected mosquitoes. The large dominance of *Culex* spp. is related to their ornithophilic preference and the large presence of birds in the park (Molaei et al., 2010; Chathuranga et al., 2018). Similar observations were made in Sri Lanka by Chathuranga et al. (2018) using bird-baited traps where 52% of their collected mosquitoes were *Culex* spp. The two most frequently encountered species were *Culex vishnui* and *Cx. bitaeniorhynchus*, representing respectively 58.8% and 14.7% of all collected mosquitoes. Similarly, *Cx. bitaeniorhynchus*, *Cx. gelidus*, *Cx. fuscocephala*, *Cx. quinquefasciatus, Cx. tritaeniorhynchus,* and *Cx. vishnui* were also reported from resting areas of migratory birds in Thailand (Tiawsirisup & Nuchprayoon, 2010 for Asian open-billed stork resting areas; Changbun jong *et al.*, 2013 for Ardeid birds resting areas) highlighting their ornithophilic behaviour in the region.

Anopheles mosquitoes are the second most diverse genus in Prek Toal (337 mosquitoes, 8 species, 4.1% of all collected Culicidae). This abundance might be due to the suitable breeding place offered by the swamps, and the abundance of potential hosts. In other studies undertaken in bird reserves in Thailand, their diversity and proportion was lower: in Asian open-billed stork nesting areas, *Anopheles* mosquitoes represented 1% of the total culicidae population (Tiawsirisup & Nuchprayoon, 2010) and 0.6% in Ardeid birds nesting areas (Changbunjong et al., 2013). From the study from Changbunjong et al., (2013), three species were recorded, and are all present in Prek Toal: *An. barbirostris* (present in all of our sites), *An. argyropus,* and *An. peditaeniatus* (only present in site 1). In the study from Tiawsirisup & Nuchprayoon (2010), four species were recorded but among them, only *An. barbirostris* was collected in our sampling. Similarly in the study conducted in Sri Lanka (Chathuranga et al., 2018) only one species of Anopheles (absent in Prek Toal) was collected, and represented 0.3% of their total collection. Out of the 8 recorded *Anopheles* species in our study, only 2 were found outside the bird reserve area (site 3).

Mansonia mosquitoes, represented by 3 species (*M. annulifera, M. indiana, M. uniformis*) account for 6% of the collected Culicidae. This is the third most diversified genus in our study. These three species are known to be nocturnal and to feed on birds (Jansen et al., 2009; Molaei et al., 2010; Chathuranga et al., 2018). All these species were also recorded from migratory bird nesting areas in Thailand, at the exception of *M. indiana*, in Tiawsirisup & Nuchprayoon (2010), and at the exception of *M. annulifera*, by Changbunjong et al., (2013). Similar results were gathered in Sri-Lanka in the study of Chathuranga et al. (2018) using bird-baited traps, at the sole exception of *M. uniformis*, absent from their traps. These results confirm their ornithophilic behaviour in Cambodia.

Beside the 3 main collected genera *Anopheles*, *Culex* and *Mansonia*, three genus were collected in low numbers: *Ficalbia*, *Mimomyia* and *Uranotaenia* (representing respectively 0.05, 0.09 and 0.01% of all collected mosquito). The biology of the genus *Ficalbia* is very poorly known: they are uncommon and are not known to bite humans (Rattanarithikul et al., 2006b). The same observations apply for *Mimomyia* mosquitoes, while adults of some species have been reported to bite humans (Rattanarithikul et al., 2006b). The genus *Uranotaenia* contains 25 species in Cambodia (Harbach, 2021). Only one unidentified species was collected in Prek Toal during the dry season. It is surprising, considering that Prek Toal reserve would be an good location since it is a primary forest hosting a large diversity of reptiles and amphibians (MoE, 2010), being their main source of blood meal (Christensen et al., 1996; Toma et al., 2014; Young et al., 2020).

One of the most notable feature in this study is the complete absence of any *Aedinii* representative. A similar observation was done in Thailand, in a nesting colony of ardeid birds, where only one *Aedes aegypti*, out of 20,370 mosquito, was collected (Changbunjong et al., 2013). Similarly it was also reported by Tiawsiriup & Nuchprayoon (2010) from a nesting area of asian open-billed stock, were only 2 species of *Aedes* were found: *Ae. albopictus* and *Ae. lineatopennis*. Both of these species represented an average of only 0.68% of all collected mosquitoes (regardless the season), while *Culex* sp. (6 species) accounted for 93.4%. The same observations were made by Chathruanga *et al.* (2018) in Sri Lanka using bird-baited traps where only 4 species were collected, accounting for 0.4% of all collected mosquitoes in the lowland forests, and 1.5% in the highland forests.

In a study conducted in Malayan Borneo, where blood meals from several female mosquitoes were analysed in swamps forests, humans appeared to be the only host for *Aedini* mosquitoes (Young et al., 2020). *Aedinii* members are well known anthropophilic species (Molaei et al., 2010; Chathuranga et al., 2018) that may feed on bird-blood as an opportunistic behaviour. The absence of human settlement in the core of the conserved area might therefore explain their absence.

A total of 13 species belonging to 3 genera are reported to be of medical importance (table 2). Four species are known vectors of malaria *Plasmodium* spp., 1 for Zika virus (ZIKV), 3 for West Nile virus (WNV)

Table 2
List of medically important mosquito species in Prek Toal.

Species	Parasite/virus	Reference
Anopheles (Anopheles) barbirostris Van der Wulp, 1884	MAL	Sinka et al. (2011)
Anopheles (Anopheles) campestris Reid, 1962	MAL	Suntorn and Bhumiratana (2018)
Anopheles (Anopheles) nigerrimus Giles, 1900	MAL	Gunathilaka et al. (2015)
Anopheles (Anopheles) sinensis Wiedemann, 1828	MAL	Feng et al. (2017)
Culex (Oculeomyia) bitaeniorhynchus Giles, 1901	JEV, RVFV	Boyer et al. (2020), Tantely et al. (2015)
Culex (Culex) fuscocephala Theobald, 1907	JEV	Boyer et al. (2020)
Culex (Culex) gelidus Theobald, 1901	JEV	Boyer et al. (2020)
Culex (Culex) quinquefasciatus Say, 1823	ZIKV, JEV, RVFV, WNV	Boyer et al. (2020), Tantely et al. (2015), Khan et al. (2017)
Culex (Culex) pseudovishnui Colless, 1957	JEV	Khan et al. (2017)
Culex (Culex) sitiens Wiedemann, 1828	JEV, FILBM	Boyer et al. (2020)
Culex (Culex) tritaeniorhynchus Giles, 1901	JEV, RVFV, WNV	Boyer et al. (2020), Tantely et al. (2015), Khan et al. (2017)
Culex (Culex) vishnui Theobald, 1901	JEV	Boyer et al. (2020)
Mansonia (Mansonioides) uniformis (Theobald, 1901)	RVFV, WNV	Tantely et al. (2015), Khan et al. (2017)

ZIKV = Zika virus, JEV = Japanese Encephalitis virus, RVFV = Rift Valley Fever virus, WNV = West Nile Virus, MAL = *Plasmodium spp*.

and 8 for the Japanese Encephalitis virus (JEV). Three species are known to transmit filariosis, like *An. sinensis*, *Cx. sitiens* for transmiting only *Brugia malayi*, while *Cx. bitaeniorhynchus* is known to transmit also *Wucheria bancrofti* (Harbach 1988; Tanaka et al., 1979). However, since these parasites were not recorded yet in Cambodia, this will not be developed in the present work.

Cambodia is a region of endemic malaria, with the presence of *Plasmodium falciparum, P. ovale,* and *P. vivax* (St-Laurent et al., 2017). These parasites are carried by *Anopheles* mosquitoes. In Cambodia, with the available knowledge, *An. barbirostris, An. campestris, An. nigerrimus, An. sinensis* are recorded to be potential vectors (Reid 1968; Harrison and Scanlon 1975; Tanaka et al., 1979; Sallum et al. 2005; St-Laurent et al., 2017).

Arboviruses, like Zika, dengue, yellow fever or chikungunya are undergoing a striking resurgence in this century (Wilder-Smith et al., 2017) and are carried by infected blood-sucking arthropods, mostly by mosquitoes and ticks. ZIKV is mainly transmitted by *Aedes* mosquitoes and is known to induce various and nonspecific symptoms, such as fever, rash, arthralgia, headache, and conjunctivitis (Duong et al., 2017). The virus is known to circulate with low intensity in Cambodia, over the past decade, with limited effect on public health (Heang et al., 2012; Duong et al., 2017). While no *Aedes* species were recorded from Prek Toal, *Cx. quinquefasciatus* can be a vector of this disease. This species was collected inside the bird reserve (site 1) and in the city center (site 3).

JEV is widespread in Asia, having an epidemiological cycle between wild birds, pigs and mosquitoes (Boyer et al., 2021). The actual transmission is due to mosquito from wide genera such as *Aedes, Anopheles, Culex* or *Mansonia*. The two main recognized JEV vector species are *Cx. tritaeniorhynchus* and *Cx. gelidus,* both present in Prek Toal. Four more species in the reserve are confirmed vectors: *Cx. fuscocephala*, *Cx. bitaeniorhynchus*, *Cx. quinquefasciatus*, and *Cx. sitiens* (Boyer et al., 2021).

Even though there is no evidence of intense circulation of West-Nile Virus (WNV) in Cambodia (Auerswald et al., 2020), concerns are arising about the ability for WNV to spread along bird migration routes based on its recent expansion in Eurasia (Petersen and Hayes, 2008; Auerswald et al., 2020). This virus is naturally maintained in an enzootic transmission cycle of mosquitoes, acting as vectors, and birds acting as the amplifying hosts. Humans and other mammals can serve as accidental/dead end hosts (Maquart et al., 2016). In humans, infections can induce symptoms ranging from febrile fever to severe neurological syndromes like encephalitis, meningitis and paralysis (Komar, 2000). In Prek Toal, 4 species are known vectors, namely, *Cx. pseudovishnui*, *Cx. tritaeniorrhynchus, Cx quinquefasciatus* and *Mansonia* (*Mansonioides*) *uniformis* (Khan et al., 2017). All of them are known to be zoo-anthropogenic species (Reuben et al., 1992; Bhattacharyya et al., 1994). Blood meal analysis of *Cx. quinquefasciatus* has demonstrated it to be highly ornithophilic besides feeding on mammals (Garcia-Rejon et al., 2010). It is most likely that this vector acts as an important bridge vector and help in viral amplification (Khan et al., 2017).

On a more general note, out of the 25 identified species, 6 species of medical importance were caught in all 3 sites. Two *Anopheles* (*An. barbirostris*, *An. campestris*) two *Culex* (*Cx. bitaeniorhynchus*, *Cx. vishnui*) and two *Mansonia* (*Ma. indiana*, *Ma. uniformis*) were collected from the core of the reserve area -in close proximity with the birds- to the floating village of Prek Toal. All of these species have been reported to feed on either birds or other vertebrates when available, and are non-exclusive bird-blood feeders (Molaei et al., 2010). Another point of concern is the relative abundance of the two main species: *Cx. vishnui* and *Cx. bitaeniorhynchus* accounting for 58.8 and 14.7% of all mosquitoes. They were collected throughout all seasons, and sampled sites. Several authors considered that these two species displayed a strong ornithophilic behaviour, and were reported to readily bite humans when their main host were unavailable (Bram, 1967; Sirivanakarn, 1976; Harbach, 1988). This is particularly problematic considering their abundance in South-East Asia, and particularly in Cambodia. Between 2017 and 2018, Boyer et al. (2020) surveyed the mosquito population in 24 schools in Kampong Cham and Tboung khmum provinces in Cambodia. Out of 37,725 mosquitoes collected, three species accounted for three-quarters of the relative abundance: *Cx. vishnui*, *An. indefinitus* and *Cx. quinquefasciatus*. Nearly 90% of the mosquitoes caught in the schools were identified as potential vectors of pathogens including Japanese encephalitis, dengue, and malaria parasites. Yet again, *Cx. vishnui* represented 51,8% of all collected mosquito. Therefore, the presence of abundant, widespread zoo-anthropogenic mosquitoes from the bird colony of Prek Toal, to inhabited areas, encompassing transition zones, stresses their potentiality to act as bridge-vectors.

Conclusion: Importance for public health

Our findings provide baseline information about the risk of emergence of new diseases from wild bird populations to other vertebrates and humans in Cambodia. Prek Toal is an attractive site for migratory birds that can carry pathogens from their migratory routes to Cambodia and vice versa. Several species of medical importance were found across all sites, namely *Anopheles barbirostris*, *An. campestris*, *Culex bitaeniorhynchus*, *Cx. vishnui*, *Mansonia indiana* and *Ma. uniformis*. One of the most important feature is the presence of *Cx. vishnui*, having both an ornithophilic and zoo-anthropogenic behavior, and being largely dominant in Prek Toal throughout seasons and sites, and being very common in Cambodia and South-East Asia. If new arboviruses were to be recorded in this area, it would have the potential to be a bridge between sylvatic and anthropogenic arboviruses. Further studies should be conducted using NGS technology to investigate the virus diversity among these vectors in Prek Toal to assess the risk of emergence

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aspen.2021.08.001.

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