Mosquito Species (Diptera: Culicidae) Reported from Shandong Province, China[1]

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Abstract Field surveys were conducted in 2007 - 2009 to determine the diversity of mosquito species occurring in Shandong Province, China. As a result, 31 species of mosquitoes were identified. *Culex pipiens pallens* Coquillett was the most common species collected. *Aedes albopictus* Skuse, *Anopheles sinensis* Wiedemann, and *Cx. tritaeniorhynchus* Giles also were commonly collected. These 4 species have been documented as the vectors of malaria, dengue, and epidemic encephalitis in China. At least 10 species identified are disease vectors in Shandong Province, and other species could be considered potential vectors of other diseases. Mosquito larvae were routinely collected from natural and artificial structures containing water. The diversity of these water-filled containers might explain the cooccurrence of various culicid species in some localities surveyed. The ecology and breeding behavior of these mosquito species should be further studied to optimize surveillance and management programs and to prevent the emergence of mosquito-transmitted diseases in human and other animal populations in the area.

Key Words mosquitoes, breeding sites, survey, mosquito surveillance

Shandong Province is located on the eastern coastline of China with the Bo Hai Sea to the northeast and the Yellow Sea to the southeast. The Yellow River flows from west to east across the province and into Bo Hai. It has a population of 92.5 million and a total area of 156,700 km^2. The climate is a continental type with an annual mean temperature of 11 - 14°C, periodic monsoons, and an annual precipitation of 790 mm across its peninsular area.

Outbreaks of malaria were reported in the 1960s and 1970s in China involving 6 million people in the 1960s and 4.6 million in the 1970s. Shandong Province was one of the areas in China with the highest prevalence of malaria, but its incidence rate has declined yearly since the 1990s. However, with increased trade and travel, incidences of imported cases of malaria also have increased. Cases of malaria were reported between 1989 and 2008 from several areas in Shandong Province, such as Heze and Jining in the western area of the province and Rizhao on the coast of the Yellow Sea (Gui et al. 2008, Wang and Zhao 2009).

Zhen et al. (1990) reported at least 37 species of mosquitoes from Shandong Province. These included some notorious pest species, e.g., *Aedes albocinctus* Skuse,

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Anopheles lesteri anthropophagus Xu and Feng, *An. Sinensis* Wiedemann, *An. minimus* (Theobald), and others. These and other mosquitoes are important vectors of human diseases such as malaria, dengue fever, yellow fever, and West Nile virus (Foster and Walker 2002).

Arthropod-vectored diseases, both the well-known and relatively rare, impact public health in China and have the potential to increase in importance with changes in demographics, land use, and climate change. However, relatively little is known of their epidemiology and transmission risk in these areas. Therefore, the purpose of this investigation was to catalogue those mosquito species occurring in selected areas of Shandong Province. These results might be used to assess transmission risk of various mosquito-borne diseases and to optimize local mosquito surveillance, prevention, and management programs (Impoinvil et al. 2007).

Materials and Methods

Survey sites. The surveys were conducted in 5 separate districts of Shandong Province (114 - 112°E, 34 - 38°N) in China from 2007 - 2009. The survey areas included Jining, Tai'an, Qingdao, Rizhao, and Heze. Each district survey is composed of cities, counties, and a number of villages. Each year, surveys were conducted during the summer and fall months when mosquitoes are most abundant and diverse. In each of the 5 areas surveyed, at least 2 cities (or counties) and 5 villages were selected to collect mosquitoes. In total, 115 localities (sample areas of 100 × 100 m each) were included in the study.

Larval sampling. The sampling method used was a cluster sampling process established in conjunction with geographical surveys of container profiles according to the methodology described by Troyo et al. (2008a). Upon visiting a sample site, we enlisted residents to show us water bodies in the area. These villages are often segregated from surrounding neighborhoods by large roadways, woodlands, or complexes of buildings that minimize mosquito movement. Due to logistical constraints, we could not comprehensively census all available water bodies, but we identified major water body types and collected mosquito larvae from each subset of water body types identified in each sample area.

The type and number of mosquito-negative and mosquito-positive containers found at each residence sampled were recorded. In large aquatic habitats, a 300-ml dipper was used to collect mosquito larvae (O'Malley 1989). Long-handled dip nets (mesh size = 6 mm, mouth area = 1600 cm^2) also were used to sample vertebrates and aquatic invertebrates large enough to prey upon mosquito larvae (Turner and Trexler 1997). Small water-filled containers were sampled for mosquitoes by pouring all water in the container through a < 200 μm sieve. The contents of each mosquito-positive container were poured into shallow white enamel pans and labeled with the collection date, house identification code, and container type. If the liquid in the container was turbid or dark, tap water was added to the pans so mosquitoes could be seen. Each container, initially identified in the field according to its specific function, was subsequently assigned to a specific category of a classification scheme, i.e., rice field, gutter, lake, sewer, washtub, puddle, tree cavity, rock pool, tire, garbage container, tray/pan, and miscellaneous. To collect larvae of genus *Mansonia*, some lotus stems were cut in each area.

Adult sampling. Female adults were collected using Centers for Disease Control (CDC) miniature light traps, with carbon dioxide from dry ice as an attractant.

The traps were placed in trees approx. 1.5 m above the ground surface. The traps were operated 12 - 14 h from early in the evening until the next morning. Collected mosquitoes were anaesthetized with carbon dioxide, dispensed into plastic ampoules, and immediately killed by freezing on dry ice. They were transported to the laboratory and stored on dry ice and at -70°C until identified.

Mosquito rearing and identification. Mosquito larvae were returned and maintained in an insectary at 26°C, 70% relative humidity, and on a 12:12 h photophase (day:night) cycle with 60 min of simulated dawn and dusk. Larvae were fed 0.03, 0.04, 0.08, 0.16, 0.32 and 0.60 mg of Tetramin per individual on days 1, 2, 3, 4, 5, 6, etc., respectively. Adults were provided an 8% sugar solution except for the 12 h before a blood meal. Mosquitoes were mated in 20-cm^3 plexiglass cages with a mesh window allowing blood feeding. We identified mosquito adults or larvae using a combination of morphological keys and geographic catalogs (Zhen et al. 1990, Lu 1997, Jing and Xin 2005).

Data analysis. Data were grouped and organized according to locality. A database was created in Microsoft Excel© and imported to Statistix 8 software (Analytical Software) for statistical analyses. Chi-square tests of homogeneity were performed to evaluate the distribution of frequencies relating to the mosquito-positive containers per locality with specified taxa (Daniel 2004).

Results

Mosquito occurrence and distribution. Thirty-one mosquito species were identified from surveys of 115 sites in the Jining, Qingdao, Rizhao, Taian, and Heze localities from May through October in 2007, 2008, and 2009 (Table 1). Of those, 18 represented the genus *Culex*, 7 represented *Aedes*, 3 represented *Anopheles*, and 1 each represented the genera *Mansonia*, *Armigeres*, and *Coquillettidia*. In terms of the percentage of sites from which species were collected, the most frequently encountered were *Cx. pipiens pallens* Coquillet (73% of all sites), *Ae. albopictus* (59.1% of all sites), *An. sinensis* (36.5% of all sites), and *Cx. tritaeniorhynchus* Giles (33% of all sites). These 4 species plus *Ar. subalbatus* Coquillet (23.5% of all sites) were the most widely distributed species in that they were collected from all 5 districts included in the survey. And, all 5 are known vectors of malaria, dengue, epidemic encephalitis, and other vertebrate diseases.

The greatest number of mosquito-positive habitats and the greatest diversity of mosquito species (25 total) occurred in the Qingdao area located on Shandong's southern coast on the Yellow Sea (Table 1). Collections from Jining and Tai'an, both located in Shandong's western continental area, yielded 21 species from Jining and 22 species from Tai'an. Fifteen species were collected from the sites surveyed in Rizhao, also located on the southern coastline on the Yellow Seas. The least number of species was from the Heze area, located west of Jining and Tai-an in the western continental area of Shandong.

Compared with the survey of Zhen et al. (1990), our survey indicated that several species, i.e., *An. lindesayi* Giles and *An. pattoni* Christophers, were not as numerous as reported in their survey. In addition, we failed to recover either *Toxorhynchites splendens* Wiedemann or *Ae. scatophagoides* (Theobald), species that they reported from their surveys.

Larval breeding sites. The most common habitats from which we collected mosquito larvae were permanent structures that contained either natural or artificial

Table 1. Species and percentages of total sites yielding of larvae and percentages females of total adults collected by CO_2-baited traps from selected areas in Shandong Province (China), 2007 - 2009.

Genus	Species	Locations Collected	Positive Collection Sites (%)	Adult Females (%)
Culex	*pipiens pallens* Coquillet	Jining, Taian, Rizhao, Heze, Qingdao	73.0	36.0
	tritaeniorhynchus Giles	Jining, Taian, Rizhao, Heze, Qingdao	33.0	10.0
	halifaxia Theobald	Jining, Taian, Heze, Qingdao	7.8	1.2.
	vagans Wiedemann	Jining, Taian, Rizhao, Qingdao	13.9	0.4
	bitaeniorhynchus Giles	Jining, Taian, Rizhao, Heze, Qingdao	6.1	0.09
	fuscanus Wiedemann	Jining, Qingdao	6.9	0.2
	whitmorei (Giles)	Jining, Taian, Rizhao, Qingdao	8.9	0.15
	hayashii Yamada	Jining, Taian, Rizhao	6.0	0.22
	malayi (Leicester)	Jining, Taian, Qingdao	5.1	0.24
	pallidothorax Theobald	Jining, Qingdao	7.4	0.18
	jacksoni Edwards	Taian, Rizhao, Qingdao	5.4	0.12
	sitiens Wiedemann	Rizhao, Qingdao	8.7	0.09
	fuscocephala Theobald	Jining, Rizhao, Qingdao	5.2	0.09
	pseudovishnui Colless	Jining, Taian, Qingdao	6.1	0.06
	fuscifurcatus Edwards	Jining, Rizhao, Qingdao	2.6	0.06
	mimeticus Edwards	Taian, Qingdao	2.6	0
	theleri Theobald	Qingdao	2.6	0.03
	mimulus Edwards	Jining	3.5	0

Table 1. Continued

Genus	Species	Locations Collected	Positive Collection Sites (%)	Adult Females (%)
Aedes	*albopictus* Skuse	Jining, Taian, Rizhao, Heze, Qingdao	59.1	30.0
	vexans Meigen	Jining, Taian, Rizhao, Qingdao	15.7	3.2
	togoi Theobald	Rizhao, Qindao	9.6	1.0
	chemulpoensis Yamada	Jining, Taian, Qingdao	3.5	0.03
	koreicus (Edwards)	Taian, Rizhao, Qingdao	3.5	0.03
	seoulensis Yamada	Jining	4.3	0.03
	dorsalis Meigen	Taian	1.7	0
Anopheles	*sinensis* Wiedemann	Jining, Taian, Rizhao, Heze, Qingdao	36.5	8.0
	lindesayi lindesayi Giles	Taian, Qingdao	4.3	0
	pattoni Christophers	Taian, Qingdao	3.5	0
Armigeres	*subalbatus* Coquillet	Jining, Taian, Rizhao, Heze, Qingdao	23.5	8.4
Mansonia	*uniformis* Theobald	Jining, Taian	13.0	0.18
Coquillettidia	*ochracea* (Theobald)	Taian	2.6	0

Table 2. Containers from which mosquito larvae were collected in Shandong Province (China), 2007 - 2009.

Species	Rice Field	Lake	Gutters	Sewer	Tree cavities	Rock pools	Washtubs	Puddles
An. sinensis	+	+	+			+		+
Ae. chemulpoensis					+		+	
Ae. koreicus						+	+	
Ae. vexans	+							+
Ae. albopictus	+		+		+	+	+	+
Cx. halifaxia				+	+	+	+	
Cx. pipiens pallens	+	+	+	+		+	+	+
Cx. vagans			+			+		
Cx. bitaeniorhynchus		+						+
Cx. tritaeniorhynchus	+	+	+	+		+		+
Cx. fuscanus						+	+	
Cx. whitmorei						+		
Cx. hayashii						+		
Cx. malayi						+		
Cx. pallidothorax						+		
Cx. jacksoni						+		
Cx. fuscocephala						+		
Cx. orientalis						+		
Ar. subalbatus				+			+	

("+" denoted the type of positive habitat for mosquito species)

depositions of water, such as, rice fields, lakes, sewers, tree cavities, puddles, etc. (Table 2). We found that rock pools, gutters, puddles, and washtubs also were frequently infested with larvae. *Culex pipiens pallens, Ae. albopictus,* and *Cx. tritaeniorhynchus* were recovered from the greatest diversity of breeding containers examined in this survey. The greatest number of species were collected from rock pools, where water had seeped from the rock surface or rock aperture.

Discussion

The diversity of mosquito species observed in this survey revealed the coexistence of different mosquitoes in relatively diverse geographic areas of Shandong Province. There are variations in the urban and rural environments among the localities surveyed in the 5 areas included in this study (e.g., Jining, Tai'an, Qingdao, Rizaho, and Heze). These habitat differences are due to urban or rural structural composition, type of vegetation, natural water deposits, and human behavior, all of which can affect the occurrence of and selection of oviposition sites by adult female mosquitoes. Our data further show that the distribution of mosquito infestations in the 5 areas was not homogeneous through the 3 yrs of the study.

The 2 most commonly found species were *Cx. pipiens pallens* and *Ae. albopictus* with the former also a commonly-occurring species in other urban areas of North China (Lu 1999). The peak occurrence of Japanese encephalitis has been found to coincide with the seasonal abundance of *Cx. pipiens pallens* in Shandong Province (Jing et al. 2007, Li and Guo 2004). *Aedes albopictus* also is a vector of dengue fever, which is a prevalent vector-borne disease in the southern provinces of China. *Culex bitaeniorhynchus* Giles, *Cx. tritaeniorhynchus*, *Cx. pipiens pallens*, *Culex pseudovishnui* Colless and *Culex theleri* Theobald are potential vectors of West Nile virus disease in Shandong Province (Yang et al. 2007). Those species are distributed throughout the province are *Cx. pippins pallens* and C*x. tritaenior hynchus*. It is apparent that the risk of mosquito-borne diseases would be increased by immigration of mosquito vectors from other provinces or countries.

Jining, Heze and Taian are located in the western area of Shandong Province and are characterized by open spaces, rice fields, lakes, clusters of houses, and several types of vegetation. Socioeconomic conditions are variable with a few very poor neighborhoods, especially in Jining and Tai'an. High infestations of *Ae. albopictus* and *Cx. pipiens pallens* larvae were reported mainly in miscellaneous containers located outside those houses in those 2 areas. In Heze, characterized mainly of plains, the density of houses is low in comparison with other localities surveyed and probably contributed to low number of mosquito-positive larval habitats. Only 7 mosquito species were collected in this area (Table 1).

Qingdao is a coastal city with a traditional coastal landscape. It is a popular tourist city in China. Yet, some counties and villages in this area are economically underdeveloped. Therefore, the types of housing and construction are quite diverse ranging from beach homes to poor housing for the socioeconomically deprived. Of the 5 areas surveyed, our study showed the highest diversity of mosquitoes in the Qingdoa area (Table 1).

The majority of the mosquito-positive habitats in Shandong Province were nondisposable habitats and miscellaneous containers (Table 2). However, we found public spaces and structures that provided habitats that attracted ovipositing female adults and were conducive to egg, larval and pupal survival and development.

These locations, e.g., schools and streets, are commonly excluded from entomological surveys because of quantity and inconvenience for sampling; yet, they can harbor mosquito-positive water-filled containers that help maintain mosquito populations in endemic areas and can function as a source of mosquitoes that infest nearby areas (Focks et al. 1981, Troyo et al. 2008b).

Many mosquito-positive containers were associated with nonhousehold settings, such as rock pools, puddles, lots, rice fields, and public spaces. Fifteen species were found successfully breeding and developing in rock pools. Similarly, washtubs and water puddles were important larval habitats in western Shandong Province, especially during the dry season. In contrast, most mosquito-positive habitats in eastern areas of Shandong were nondisposable containers. Gutters accumulate water and organic debris creating conditions ideal for mosquito development. And, the height and inaccessibility of roof gutters hampers consistent surveillance by household inhabitants. Together, washtubs and gutters could explain the permanence of mosquitoes during the mosquito season in areas of Shandong Province and, thus, could potentially facilitate and contribute to the outbreak of mosquito-borne diseases.

We found that breeding places for *Ae. albopictus* are usually small water-filled containers, including basins, pots, discarded tires, etc. *Anopheles sinensis* oviposits in outdoor containers filled with clear water. And, the low occurrence of anophelines *An. lindesayi* and *An. pattoni* might be attributed to increased pollution of brook or spring waters in comparison the Zhen et al. (1990) surveys.

Culex pipiens pallens is the dominant culicid species in Shangong Province and possessed the highest diversity index values for nearly all categories of mosquito-breeding containers (Table 2). The treatment or elimination of such water-filled containers should be required to manage this species in infested areas. Other culicids exhibited lower diversity index values for specific types of containers (Table 2). Therefore, having different species of mosquitoes coexisting in the urban or rural environments suggests that different strategies might need to be considered for the control of nuisance mosquitoes and potential vectors of human diseases in Shandong Province in China.

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