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SCIENTIFIC NOTE

ANOPHELES BELENRAE, A POTENTIAL VECTOR OF *PLASMODIUM VIVAX* IN THE REPUBLIC OF KOREA

LEOPOLDO M. RUEDA[1,4] CONG LI,[1] HEUNG CHUL KIM,[2] TERRY A. KLEIN,[3] DESMOND H. FOLEY[1] AND RICHARD C. WILKERSON[1]

ABSTRACT. The malarial parasite, *Plasmodium vivax*, was detected in 4 species of *Anopheles* (Hyrcanus Group) mosquitoes, namely *An. kleini*, *An. pullus*, *An. belenrae*, and *An. sinensis*, from Gyeonggi Province, Republic of Korea (ROK). This study confirmed that *An. belenrae* was infected by *P. vivax*, and implicated this species as a potential vector of *vivax* malaria in the ROK.

KEY WORDS *Anopheles belenrae*, *Plasmodium vivax*, Culicidae, Hyrcanus Group, Korea

Anopheles belenrae Rueda, a member of the *Anopheles* Hyrcanus Group (Culicidae, Diptera), was 1st described in 2005 (Li et al. 2005, Rueda 2005) from specimens collected in the Republic of Korea (ROK). It is presently known to occur in three Asian countries, namely, ROK (Gyeonggi Province; Rueda 2005, Rueda et al. 2006); Democratic People's Republic of Korea (Hwanghaebuk and Pyongyannam Provinces; Rueda and Gao 2008), and China (Liaoning and Shandong Provinces; Rueda et al. 2007).

In the ROK, *An. kleini* Rueda and *An. pullus* Yamada are considered the primary vectors of *Plasmodium vivax* Grassi and Feletti malaria, while *An. sinensis* Wiedemann is a secondary vector (Lee et al. 2007, Klein et al. 2008). Laboratory studies have also shown the potential for *An. lesteri* Baisas and Hu to be an efficient vector (Joshi et al. 2009) and is considered a primary vector (= *An. anthropophagus* Xu and Feng) in parts of China (Rueda et al. 2007). However, since populations of *An. lesteri* are very low, its role in malaria transmission in the ROK is questionable.

We collected about 7,000 adult anopheline mosquitoes by New Jersey light traps (Bioquip, Rancho Dominguez, CA), Mosquito Magnets® (Woodstream Corp., Lititz, PA), and resting catches (mouth aspirator with filter disk; Bioquip) at a variety of locations in the ROK in 2008, to better understand malaria transmission in the field. Each New Jersey light trap was suspended from a wooden pole, near barracks and larval habitats (e.g., rice paddy, irrigation ditch, pond, etc.), about 1.5 m above ground level. New Jersey light traps and Mosquito Magnets were set each afternoon between 1700 and 1730 h, and the collections were picked up the following morning between 0800 and 0830 h. Resting adults were caught using aspirators inside the cattle shed, particularly from the walls, roof, and other parts of the building, between 2000 and 2200 h. Collected specimens were removed, taken to the laboratory, sorted, initially identified morphologically (Tanaka et al. 1979, Rueda 2005), and stored in the freezer prior to polymerase chain reaction (PCR) assay. Only the head and thorax of mosquitoes were assayed by PCR for species identification and confirmation (Wilkerson et al. 2003, Li et al. 2005) and by single step and seminested multiplex-PCR to identify *P. vivax* sporozoite infections (Rubio et al. 1999, Hasan et al. 2009). The abdomen was separated from the mosquito body prior to assay to reduce the probability of detecting DNA from oocysts in the hind gut.

Four *P. vivax*–infected adult females of *An. belenrae* (4/87) were collected from Tongilchon and Ilsan, Gyeonggi Province in 2008. One infected female (1/73) was caught in a New Jersey light trap from Tongilchon (37.90517°N, 126.73367°E), Paju, Gyeonggi Province; with collection/serial no. 8.3435, August 6, 2008. Two infected females (2/9) were collected while resting in a cowshed, using an inhaling type aspirator (with filter disk; Bioquip) from Ilsan (37.67135°N, 126.69677°E), Goyang, Gyeonggi Province; with collection/serial no. 8.0758, July 7, 2008 and serial no. 8.3847, August 10, 2008. One infected female (1/5) was collected in a New Jersey light trap from Camp Casey, Dongduchon

[1] Walter Reed Biosystematics Unit, Division of Entomology, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500.

[2] 5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP 96205-5247.

[3] Force Health Protection and Preventive Medicine, 65th Medical Brigade/USAMEDDAC-Korea, Unit 15281, APO AP 96205-5281.

[4] Walter Reed Biosystematics Unit, Museum Support Center, Smithsonian Institution, 4210 Silver Hill Road, Suitland, MD 20646-2863.

(37.928075°N, 127.05957°E), Gyeonggi Province, with collection/serial no. 8.2789, July 22, 2010. These data implicate *An. belenrae* as a potential vector of *P. vivax*. In addition, Chang et al. (unpublished data) noted that two specimens of *An. belenrae* collected from Paju on May 27, 2009 and Goseong County, ROK on June 22, 2009 were tested positive by PCR for *P. vivax*.

To incriminate a malaria vector in nature, it is necessary to demonstrate: 1) an association between the vector and human malaria cases; 2) evidence of direct human–vector contact; and 3) evidence that the vector harbors sporozoites in the salivary glands (Beier, 2002). In addition to evidence of sporozoites in *An. belenrae*, we should mention that in 2006–2007 human malaria cases had rapidly increased in the area where we collected malaria positive *An. belenrae* (Goyang) (Jun et al. 2009), but the role, if any, of this species in malaria transmission at this location is unknown.

Other *Anopheles* species that were also found positive for *P. vivax* from our collections in Gyeonggi Province, ROK, were *An. kleini*, *An. pullus*, and An. *sinensis*. Six infected females of *An. kleini* (6/199) were collected from the following sites: Warrior Base (37.92444°N, 126.73944°E), $n = 1$ infected female (F) (1/7), August 8, 2008, collection/serial no. 8.3923; Tongilchon, Paju (37.90517°N, 126.738411°E), $n = 1$ F (1/71), July 2, 2008, collection/serial nos. 8.0627, 8.0458; $n = 2$ F (2/119), July 29, 2009, collection/serial nos. 8.2817, 8.2821; and ROK Army Base (37.918832°N, 126.738411°E), $n = 2$ F (2/2), June 13 and July 14, 2008, collection/serial nos. 8.2294, 8.2476. Three infected females of *An. pullus* (3/42) were collected from Tongilchon, Paju, $n = 3$ F, July 2, 2008, collection/serial no. 8.0470, 8.0494, 0508. Fifteen infected females of *An. sinensis* (15/273), were found in Tongilchon, Paju: $n = 5$ F (5/63), July 2, 2008, collection/serial nos. 8.0610, 8.0628, 8.0520, 8.0548, 8.0597; $n = 1$ F (1/60), July 11, 2008, collection/serial no. 8.0966; $n = 3$ F (3/37), July 23, 2008, collection/serial nos. 8.0206, 8.0221, 8.0238; $n = 5$ F (5/107), July 29, 2009; collection/serial nos. 8.2714, 8.2756, 8.2785, 8.2826, 8.2832; and ROK Army Base, $n = 1$ F (1/6), June 13, 2008, collection/serial no. 8.2476.

Furthermore, we are continuing the molecular analysis of several thousand *Anopheles* females from different locations in the ROK, particularly to identify the mosquito species and their associated *Plasmodium* parasites.

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