Available online at www.sciencedirect.com
ScienceDirect
Experimental Parasitology 114 (2006) 259–264
Experimental Parasitology
www.elsevier.com/locate/yexpr

Analysis of mosquito bloodmeals using RFLP markers ☆

Mohammad Ali Oshaghi *, Ali Reza Chavshin, Hassan Vatandoost

Department of Medical Entomology, School of Public Health and Institute of Health Researches, Tehran University of Medical Sciences, P.O.Box 14155-6446, Tehran, Iran

Received 7 January 2006; received in revised form 2 April 2006; accepted 3 April 2006
Available online 22 May 2006

Abstract

An important variable in the amplification of arthropod vector-borne diseases is the degree of contact between human hosts and mosquito vectors. To analyze this interaction, a DNA based method was developed to differentiate human bloodmeals from other sources in the mosquito *Anopheles stephensi* (Diptera: Culicidae) Liston. A portion of the host mitochondrial DNA cytochrome *B* genes were PCR amplified and classified to the species level based on their restriction fragment length polymorphism (RFLP). The cytochrome *B* sequences showed sufficient interspecific polymorphism to distinguish between human, cow, sheep, chicken, and guinea pig hosts. *Xho*I could distinguish human from other vertebrates whereas *Taq*I alone could separate the others. The importance of these results in epidemiological studies of malaria and other vector borne diseases is discussed.

Index Descriptors and Abbreviations: Bloodmeal; PCR-RFLP; Mosquito; cytB; Vector borne disease

1. Introduction

One of the most important factors in the dynamics of transmission of malaria or any other arthropod-borne disease is the degree of contact of the vector with the infected host reservoir. Mosquito species are important vectors and differ in their overall preference for different hosts such humans, livestock, birds, and reptiles and in the times of night that they are most actively seeking bloodmeals (Irby and Apperson, 1988; Nanda et al., 1996; Subbarao, 1998). Data regarding the degree of contact between vector and host populations and host preferences under natural conditions can be assayed by identifying the source of bloodmeals in recently blood-fed vector mosquitoes. The anthropophilic index (percentage feeding on humans) is a vital component of vectorial capacity of mosquito species in malaria disease, while knowledge of other hosts reveals the relative importance of reservoirs of vector-borne zoonotic or enzootic infections (Kay et al., 1979).

Contemporary procedures for bloodmeal identification are generally based upon the detection of host antigens by the complement fixation test (Staak et al., 1981) or by enzymelinked immunoabsorbant assays (ELISA) using polyclonal antibodies raised against blood components from potential host vertebrates (Beier et al., 1988; Chow et al., 1993; Mwangangi et al., 2003). This method, however, requires the preparation of immune sera against the blood of each potential host species, a difficult and laborious process. Pre-adsorption steps are also needed to eliminate cross reactions when using this technique (Hunter and Bayly, 1991).

Recent developments in molecular biology have allowed a significant increase in the efficacy and reliability of bloodmeal identification (Gokool et al., 1993; Mukabana et al., 2002a). Today, PCR-based techniques have proved to be highly effective and versatile in recent laboratory trials and are likely to rapidly displace all other approaches. Various DNA based molecular markers have also been used to identify bloodmeals in a limited number of arthropods including *Ixodes ricinus* (Kirstein and Gray, 1996), *Pthirus*

☆ School of Public Health and Institute of Health Researchers, Tehran University of Medical Sciences.

* Corresponding author. Fax: +98 21 6462267.
E-mail address: oshaghima@gmail.com (M.A. Oshaghi).

0014-4894/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2006.04.001

pubis (Replogle et al., 1994, 49; Lord et al., 1998), *Glossina spp* (Kirstein and Gray, 1996; Torr et al., 2001), *Simulium damnosum* (Boakye et al., 1999), *Culex quinquefasciatus* (Michael et al., 2001), *Aedes aegypti* and *Anopheles sinensis* (Sato et al., 1992), and *Anopheles gambiae* (Ansell et al., 2000; Mukabana et al., 2002a).

Boakye et al. (1999) have developed a DNA assay to identify bloodmeals in the blackfly *Simulium damnosum* s.l. by cytochrome *B* (cytB) heteroduplex analysis (HDA). Although HDA is a powerful tool to detect small differences between closely related DNA sequences (Tang and Unnasch, 1995), it is more complicated to apply than the PCR-RFLP assay. Mukabana et al. (2002b) have reviewed the extent of different molecular markers have been used for analysis of mosquito bloodmeals and the potential they might have for the future. PCR followed by restriction fragment length polymorphism (PCR-RFLP) assay has been shown to be an easy, reliable, and fast analysis for DNA identification. Steuber et al. (2005) used PCR-RFLP of the cytB gene for distinguishing 10 species of the family Bovidae. Additionally, terminal-restriction fragment length polymorphism (T-RFLP) analysis of cytB has been used to identify mosquito bloodmeal sources on 123 blood or tissue samples from 55 avian, 13 mammalian, and one amphibian species (Meece et al., 2005). Although T-RFLP is a highly reproducible technique to distinguish DNA sequences, it needs an automated sequence analyzer and is not user = friendly, particularly for people in underdeveloped countries.

Here, we present a method to determine the bloodmeal origin particularly from humans as well as from cattle, sheep, chicken, and guinea pig, based on restriction fragment length polymorphism of cytB. Cattle, sheep, and chickens in descending order are the most abundant animal hosts in malarious regions of Iran and neighboring countries, and guinea pig is the most common blood sources for mosquitoes in insectariums of most research centers and laboratories. Moreover, as the tool could be used for other haematophagous arthropods such sandflies, vectors of leishmaniasis, we included guinea pig as a representative of a rodent group. The utility of this method is demonstrated 73 by the identification of bloodmeals from laboratory mosquitoes of *Anopheles stephensi* (Diptera: Culicidae) and wild caught mosquitoes of *A. sacharovi* (Diptera: Culicidae) Favre, which are amongst the most important malaria vectors in Iran and neighboring countries.

2. Materials and methods

2.1. Specimens

Specimens of *A.stephensi* were obtained from a laboratory strain originating from Kazeron, southern Iran, which has been reared by conventional methods in the insectary of School of Public Health and Institute of Health Researches (SPH & IHR) of Tehran University of Medical Sciences (TUMS). Larvae were reared to adult stage and then were maintained on a 6% glucose solution. Three days after emergence, female mosquitoes were held in cages with males and allowed to take their first and only bloodmeal directly or indirectly from (1) the arm of a human (*Homo sapiens* L.) volunteer, (2) domestic chicken (*Gallus gallus* L.), (3) guinea pig (*Cavia porcellus*), (4) cow (*Bos taurus* L.), and (5) sheep (*Ovis aries* L.). Blood fed females were then killed by freezing at −20 °C and used for DNA extraction.

Field specimens were collected after a bloodmeal on 14 human volunteers, 9 sheep, and 20 domestic cows from Meshkin-shahr, Kazeron, Bandar-Abbass, and Iranshahr, respectively, from northwest, center, south, and southeast of Iran using the night biting method in the. Males and unfed female mosquitoes were used as negative controls and tissue or bloods of the vertebrate samples were positive controls in the experiments.

2.2. Ethical clearance

Informed consent was obtained from all the adult human participants and cow owners. The project was approved by the ethical committee at the Tehran University of Medical Sciences, Tehran, I.R.Iran.

2.3. DNA extraction

DNA extraction from whole blood-fed mosquitoes, male mosquitoes, unfed female mosquitoes, and tissue or blood of the vertebrate samples followed the procedure of Steiner et al. (1995). Samples were disrupted by mechanical homogenization in a buffer containing 10 mM Tris–HCl (pH 8.0) 312.5 mM EDTA, 1% (w/v) sodium lauryl sarcosine, and 1% polyvinylpyrollidone. The homogenates were heated to 90 °C for 20 min and chilled on ice for 5 min. Samples were subjected to centrifugation at 13,000*g* for 5 min at room temperature. The supernatant was removed and diluted 20-fold in 10 mM Tris–HCl (pH 8.0) 1 mM EDTA (TE).

2.4. PCR reactions and sequencing

PCR amplifications were undertaken in 25 μl of a solution containing 10 mM Tris–HCl (pH 8.3 at 25 °C) 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM dATP, dCTP, dGTP, and TTP, 0.5 mM each primer, 1 U of *Taq* DNA polymerase (Cinagene, Tehran, I.R.Iran), and 2.5 μl of the DNA template solution, prepared as described above. We used the primers introduced by Kocher et al. (1989) and Boakye et al. (1999) for amplification of a portion (358 bp) of CytB gene of vertebrate mtDNA. The sequence of the primers was as follows: 5′-ccatccaacatctcagcatgatgaaa-3′ (forward) and 5′-cccctcagaatgatatttgtcctca-3′ (reverse). They correspond to sequences of 14380–14405 and 14711–14737 of human mtDNA (GenBank Accession No. DQ112962). Reactions began by incubation at 95 °C for 3.5 min, followed by 36 cycles comprising 30 s at 95 °C, 50 s at 58 °C, and 40 s at 72 °C. The reaction was completed by incubation at 72 °C for 5 min. To obtain species specific