Wolbachia Endosymbiant Was Not Detected in Field Collected Population of Head and Body Lice From Iran Using Molecular Markers

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Research note

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Abstract

Objectives

Head lice infection can lead to inferiority, depression, insomnia, and lack of education, loss of social status, secondary infections, hair removal, and allergies. Body lice can carry dangerous diseases such as typhus, trench fever, and recurrent fever. Studies on lice control due to pesticides, inappropriate drug use, pesticide risks, and pesticide resistance in the lice population are needed. The study of insect symbiotic microorganisms such as *Wolbachia* is a new approach to control the vector-borne disease.

Results

The presence of this bacterium was investigated in head and body lice collected from the Iranian lice population. Genomic DNA was extracted from both lice specimens and PCR assay was performed to the detection of *Wolbachia* infection by using *Wsp*, *coxA*, and *gatB* primers. All PCR results were negative for *Wolbachia* in comparison with positive controls. Further studies should be performed using new molecular markers to determine the *Wolbachia* infection of lice.

Introduction

Head lice (*Pediculus capitis*) live on human hair and may be responsible for pruritus that may lead to high irritation and even wound infection [1]. They are transmitted through proximity with infested persons, or the sharing of combs and hair products [2]. Body lice (*Pediculus humanus*) live in clothes and may be responsible for the transmission of epidemic typhus, relapsing, and trench fever. Body lice are transmitted through shared clothing or physical contact with infested persons. Pubic lice (*Pthirus pubis*) are not investigated here, and sections relating to them should be removed [1].

lice are a global problem, whose prevalence remains high due to ineffective control strategies and resistance to common chemical treatments [3]. Resistance to anti-lice so far from several countries including the United States, United Kingdom, Argentina, Australia, Israel, France, and Iran has been reported [3-8]. Due to the sensitivity of the subject, the high prevalence of lice infestation in different parts of the world, spending a lot of money and energy to treat the infection, which has different effects, the study of insect symbiont microorganisms is a new approach in the world [9].

The genus of *Wolbachia* is a group of obligate and intracellular bacteria in the Anaplasmatacea family of the -Proteobacteria that are closely related to the *Rickettsia* species [10]. It is one of the most common parasiticmicrobes and is possibly the most common reproductive parasite in the biosphere [11]. Interactions of *Wolbachia* with its hosts are often complex, and in some cases have evolved to be mutualistic rather than parasitic [12]. Some host species cannot reproduce, or even survive, without *Wolbachia* colonization [13]. It was estimated infection at least 40-66% of arthropod species are infected with *Wolbachia* [2, 14-18]. *Wolbachia* is present in the reproductive tissues, testis, and ovaries, of a wide range of arthropods, and transmitted through the egg cytoplasm to the next generation [19]. It causes cytoplasmic incompatibility between strains and species, parthenogenesis induction, feminization, male-killing, and enhanced fecundity and fertility in their hosts [20]. It is suspected that *Wolbachia* would play a decisive role in causing reproductive manipulations by interacting with the host proteins [21].

The genus *Wolbachia* is genetically very diverse based on the sequencing of *ftsz* and *16S rDNA* genes. While eight “subgroups” (A–H) have been identified to date [22-26], only subgroup F has been reported in lice [27]. The *Wolbachia* surface protein (*wsp*) is often used for detection and phylogenetic analysis of *Wolbachia* [28]. The *wsp* is an eight beta-barrel transmembrane protein that participates in host immune response, cell proliferation, pathogenicity, and controlled cell death program [29]. *Wolbachia* has been detected in the lice populations from Australia, Canada, USA, and Argentina using the *wsp* marker [27].

In the current study, *Wolbachia* has not been detected in lice samples, this could be due to mutations in the Wsp priming sites, hence additional primers should be tried before concluding that *Wolbachia* is absent. Interestingly, lice samples collected from the USA, Madagascar, and Russia was not infected with *Wolbachia* using the same marker [30].

Methods

Overall 123 human head and body lice samples were collected in West Azerbaijan, Khorasan, Qom, and Zanjan from the head and body of volunteers (Figure 1 and Table 1). Head lice samples (n=97) were collected from the screening of 24 school student people who are seeking treatment in health centers and trained health workers. Body lice samples (n=26) were collected from 21 persons living in addiction treatment camps by collecting clothes and exchanging them with new clothes. Written informed consent (in Persian) was obtained from interested individuals, volunteers, and the parents or legal guardians of the children.

Head lice (n=49) and body lice (n=26) specimens were randomly subjected to genomic DNA extraction. Genomic DNA was extracted from the whole body of each louse stored in 70% ethanol using the YTA Genomic DNA Extraction Mini Kit (Yekta Tajhiz Azma, Tehran, Iran). The DNA extraction process was followed based on the manufacturer's instructions with slight modification. Briefly, each louse was frizzed and towed in liquid nitrogen, then homogenized in the 200-µl FATG1 buffer (cell lysis) using a micropestle. After the addition of 20-µl proteinase K, the mixture was incubated at 60 °C for 1 h. The rest of the DNA extraction process was followed based on the YTA protocol.

A nested-PCR assay was performed to the detection of *Wolbachia* infection of the lice by using the *wsp* marker [28]. Initially, 81F and 691R primers were used to amplify 632 bp of the partial sequence of the *Wsp* gene. The PCR product of the first step was applied as a template for the second step using 183F and 691R primers to amplify a 501 bp fragment [28]. *Wolbachia* infection of samples was checked again by using cytochrome oxidase subunit 1 (*coxA*) and Glutamyl-Trna (*Gln*) amidotransferase, subunit B (*gatB*) primers [31]. They could amplify 650 bp and 500 bp fragments, respectively [31].

PCR reactions (25 µl) were performed in thin-walled microcentrifuge tubes. The optimized reaction condition was 1 µl of genomic DNA, 1 µl both forward and reverse primers, 12.5 µl Master Mix (Yekta Tajhiz Azma, Tehran, Iran), and 7.5 µl ddH2O. The details of the amplification profile for each pair of primers have been presented in Table 2. The positive control was used DNA from *Wolbachia* positive *Culex* mosquitoes and 1 µl of distilled deionized water was used as the negative control. The DNA for positive test samples were extracted at the same time and place.

PCR products were visualized on a 1.5% agarose gel containing safe stain (Yekta Tajhiz Azma, Tehran, Iran) and using a UV transilluminator.

Results

Overall 75 lice specimens (n=49 head lice and n=26 body lice) from four different provinces of Iran were tested for *Wolbachia* infection. All nested-PCR and PCR results were negative for *Wolbachia* in comparison with a positive control to *coxA*, *gatB,* and *wsp* gene. *Wolbachia* was not detected in all specimens using all 3 primers.

Discussion

In the class of insects, *Wolbachia* has been identified from some orders, in some others has not been detected and in some others has not been studied [32].

Different studies in Iran show variation in *Wolbachia* infection rates across wild-caught mosquito species, Pederus beetles, fruit fly species, sandfly species [33-36]. Also, there were different unpublished sequences of *Wolbachia* in GenBank submitted by Djadid et al., from *Culex pipiens* species (GenBank IDs: EF539841-52 and DQ900650-54) during 2006-2007. The current study is the first report on the *Wolbachia* investigation in human head and body lice in Iran.

Recently, different studies were undertaken on endosymbionts detection in louse in different geographical regions [37-40]. However, not all of them have succeeded in isolating the bacteria. Veracx et al. (2005) have not been detected with *wsp* the *Wolbachia* bacteria from body lice but have been isolated from head lice [41]. Also, it was not detected using *Wsp* primers in head lice collected from the USA, Russia, and Madagascar [30]. In the current study, *Wolbachia* infection was not detected in head and body lice samples, despite using three different molecular markers, *wsp*, *coxA,* and *gatB*. Since the positive control worked properly, it does not seem to be a technical problem such as incorrect test technique, a problem in connecting primers, problems in the raw material used, and so on.

Conclusion

The failure to detect *Wolbachia* in the previous studies has been expressed due to the inability of insects to support the physiology of *Wolbachia*, changes in tropical *Wolbachia* tissue, and inhibition of native microbiome [42-44]. It is not correct to ignore the non-diagnosis of *Wolbachia* in the population of lice due to their resistance to chemical insecticides. However, further investigations with more sample collection from different parts of the country using *ftsz*, 16 sRNA, and end-point PCR targeting ARM (A supergroup repeat motif) [45] markers, also, Next Generation Sequencing may provide better sensitivity and efficiency of *Wolbachia* detection even in low titer *Wolbachia* infections.

Limitations

- Sample collection restricted from limited to four provinces of the country because of funding limitations.
- Limited molecular markers were used due to student project and limited funding.

Abbreviations

wsp (*Wolbachia* surface protein)

YTA (Yekta Tajhiz Azma)

coxA (cytochrome oxidase subunit 1)

gatB (Glutamyl-Trna amidotransferase, subunit B)

ARM (A supergroup repeat motif)

Declarations

Acknowledgment

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Authors' contributions

Conceived and designed the study: SG and SF. Search and data selection: SG, AS, FGK and SF. analyzed the data: SG, SF, and ML. Wrote the paper: SF, SG, FGK, and MAO. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting this article are included within the article and supplementary file.

Ethics approval and consent to participate

Written informed consent (in Persian) was obtained from interested individuals, volunteers, and the parents or legal guardians of the children. The study was approved in ethics committee in National Institute for Medical Research Development (NIMAD) with ID number IR.NIMAD.REC.1396.108.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Number of lice collected and location of Iranian cities where lice were collected

Provine	District	Latitude and Longitude	Number of head and body Lice	Date of collection
Khorasan-Razavi	Mashhad	36.26 ºN and 59.62 ºE	21	2018
Qom	Qom	34.64 ºN and 50.87 ºE	15	2018
Zanjan	Zanjan	36.68 ºN and 48.51 ºE	9	2017
West Azerbaijan	Salmas	38.20 ºN and 44.77 ºE	19	2017
	Urmia	37.55 ºN and 45.08 ºE	59	2017- 2019

Table 2. Nested-PCR and PCR amplification conditions. Steps two to four were repeated 36 times.

Nested-PCR/ PCR	Primer	Initial denaturation		Denaturation		Annealing		Extension		Final Extension	
		Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
Nested-PCR	*wsp*	95◦c	5 min	95◦c	1min	55◦c	90 sec	72◦c	80 sec	72◦c	10 min
PCR	coxA	94◦c	5 min	94◦c	15 sec	55◦c	15 sec	72◦c	30 sec	72◦c	10 min
PCR	*gatB*	94◦c	2 min	94◦c	30 sec	56◦c	45 sec	72◦c	1.30 min	70◦c	10 min

Figures

Figure 1

Locations of lice specimens' collection in Iran (Google. 2020. Google maps sample collection locations in Iran. Retrieved July 27, 2020, from https://www.google.com/maps/place/Iran).