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Finding *Wolbachia* in Filarial larvae and Culicidae Mosquitoes in Upper Egypt Governorate

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Abstract: *Wolbachia* is an obligatory intracellular endosymbiotic bacterium, present in over 20% of all insects altering insect reproductive capabilities and in a wide range of filarial worms which is essential for worm survival and reproduction. In Egypt, no available data were found about *Wolbachia* searching for it in either mosquitoes or filarial worms. Thus, we aimed to identify the possible concurrent presence of *Wolbachia* within different mosquitoes and filarial parasites, in Assiut Governorate, Egypt using multiplex PCR. Initially, 6 pools were detected positive for *Wolbachia* by single PCR. The simultaneous detection of *Wolbachia* and filarial parasites (*Wuchereria bancrofti, Dirofilaria immitis*, and *Dirofilaria repens*) by multiplex PCR was spotted in 5 out of 6 pools, with an overall estimated rate of infection (ERI) of 0.24%. Unexpectedly, the highest ERI (0.53%) was for *Anopheles pharoensis* with related *Wolbachia* and *W. bancrofti*, followed by *Aedes* (0.42%) and *Culex* (0.26%). We also observed that *Wolbachia* altered *Culex* spp. as a primary vector for *W. bancrofti* to be replaced by *Anopheles* sp. *Wolbachia* within filaria-infected mosquitoes in our locality gives a hope to use bacteria as a new control trend simultaneously targeting the vector and filarial parasites.

Key words: *Wuchereria bancrofti, Dirofilaria immitis*, *Dirofilaria repens*, *Wolbachia*, filaria, Culicidae, single PCR, multiplex PCR

INTRODUCTION

Wolbachia is a confusing α-proteobacterium, first identified in the ovaries of *Culex* mosquitoes [1] and is probably the most common known endosymbiotic microbe in the biosphere [2]. Intracellular *Wolbachia* bacteria are estimated to naturally infect approximately 20% of insect species and up to 28% of surveyed mosquito species [3-5]. Naturally occurring *Wolbachia* has been identified in a range of mosquito species [4-7]. Mosquitoes are infected by "A" and "B" *Wolbachia* super-groups. Single B super-group strain of *Wolbachia* was mostly associated with *Culex pipiens*. The bacteria affect several aspects of insect biology, immunity, ecology, evolution, reproduction including male killing, and parthenogenesis [8]. Cytoplasmic incompatibility (CI) is the most common effect of *Wolbachia* on mosquitoes, as both infected and uninfected males are reproductively compatible with infected females [9], while uninfected female mosquitoes did not result in fertilization [7]. This phenomenon of CI gives infected females a reproductive dvantage and allows *Wolbachia* to drive rapidly through their host [10]. This rapid spread has created an applied interest in the use of this bacterium as a mechanism to drive introduced transgenic traits to spread through wild and naïve mosquito populations to control mosquito-borne diseases [10-13]. Thus identifying naturally occurring *Wolbachia* in mosquitoes is useful as it is technically easier to transfer transgenic traits between closely related mosquito species [14].

The majority of filarial nematodes, including *Dirofilaria immitis, Dirofilaria repens, Onchocerca volvulus, Brugia malayi*, and *Wuchereria bancrofti* harbor the obligate intracellular endosymbiotic bacteria "*Wolbachia*" [15-17]. The effects of the symbiotic bacteria on parasites of public health importance had been studied and had evidenced the obligatory symbiotic relationship between them and *Wolbachia*, indicating a long-term and stable association between the 2 organisms [16]. Most, but not all, species of filariae naturally contain *Wolbachia* of the other 2 groups designated as "C and D" [15], which are required for fertility, molting, development, and survival [8]. *Wolbachia* is present in all developmental stages of filariae: rapidly increasing as the nematode is introduced from the insect to the mammalian host. It increases further as the larvae develop into adults [18], concentrated within the hypodermal lateral cords of both sexes and transmitted through the egg cytoplasm of the female reproductive organs [19]. This ability of *Wolbachia*

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to spread rapidly through mosquitoes and to impair the development of their inside pathogens either virus or metazoan makes it a first-rate candidate for reducing disease transmission by vector species, and novel *Wolbachia* strain for such interventions needs to be carefully considered [20]. Consideration of the mutualistic/symbiotic relationship between the bacteria and their inhabited filariae possibly will sooner or later lead to the discovery of new drug targets.

It is worth to say that lymphatic filariasis is one the most debilitating diseases in tropical medicine [21], in addition to other filarial parasites like *D. immitis* and *D. repens* which are also widely studied due to their zoonotic potential with an increasing number of cases in non-endemic areas [22]. *Wolbachia* rather than the hosted filarial parasites had been considered as the cause of pathogenesis and inflammatory processes in the course of parasitic infections [23,24]. This will allow the development of new therapeutic approaches to filariae [25,26]. Accordingly, *Wolbachia* became a target for filarial nematodes control measures.

Mosquitoes are vectors of filarial nematodes and other vector-transmitted agents, and the current control routine is use of insecticides. However, the presence of mosquito strains resistant with decreased susceptibility has been roused. Likewise, insecticides have negative effects on non-target insect populations, for instance, toxicological effects on humans and environment. Consequently, the implementation of alternative methods to control mosquitoes is needed. *Wolbachia*-based control is low cost, friendly to the environment, and affects indoors vectors [8,18]. It is for that purpose essential to critically assess the incidence of *Wolbachia* in natural populations of mosquitoes in order to better understand how to introduce novel *Wolbachia* strains in the process of vector control. As the release, *Wolbachia*-infected mosquitoes may provide a way to interrupt the transmission cycle of vector-transmitted agents.

As far as we know, there has been no previous study in Upper Egypt conducted to identify the simultaneous presence of naturally infected *Wolbachia* in mosquitoes, humans, and canine filariasis by multiplex-PCR among the studied localities in Assiut Governorate, Assiut, Egypt.

MATERIALS AND METHODS

Mosquito collection and DNA extraction

A total of 2,500 adult engorged female mosquitoes were collected from El-Nikhila, El-Matiaa, Mankabad villages, Sahel Seleem, El-Badary, Dairout and Manfalout districts, Assiut Governorate, Egypt, throughout 22 months using a mechanical aspirator [27]. Collected mosquitoes were identified [28-30]. Female mosquitoes were divided into 100 pools (25 mosquitoes each) according to their genera and collection site. All pool specimens were labeled and maintained refrigerated until being used. Two types of PCR-assays were carried out; firstly single PCR for *Wolbachia* identification followed by multiplex PCR for simultaneous detection of *Wolbachia*, *W. bancrofti*, *D. immitis*, and *D. repens* within the identified mosquito genera pool from their respective collected sites. All used oligonucleotide primers were a pair of forward and reverse primers obtained from Metabion International AG (Martinsried, Germany). Genomic DNA was isolated from mosquitoes using Qiagen tissue kit (QIAamp DNA Minikit, Hilden, Germany), following the manufacturer's instructions.

Single PCR for *Wolbachia* detection

Wolbachia primers used were "*Wolbachia* 16S rDNA, W-Spec forward 5′-CATACCTATTCGAAGGGATAG-3′ and W-Spec reverse 5′-AGCTTCGAGTGAAACCAATTC-3′ [31].

A Perkin Elmer 480 Thermal Cycler (Perkin Elmer Cetus, Norwalk, Connecticut, USA) was used for the PCR amplification, in 25 µl reaction mixtures consisting of 0.5 mM of each primer, 0.6 mM of dNTP, 1 mM $MgCl_2$, 1 µl of the crude DNA extract, 0.2 U of Taq polymerase, 2.5 µl of 10× PCR buffer, and 1 µl of DMSO. Each reaction mixture was overlaid with a drop of mineral oil. PCR was performed with initial denaturation at 94˚C for 2 min, followed by 30 cycles consisting of 94˚C for 30 sec, 50.7˚C for 1 min and 72˚C for 1 min, and a final extension for 4 min at 72˚C. Following PCR, 10 µl of each PCR product was taken underneath the oil and mixed with 3 µl gel loading dye, loaded on a 2% agarose gel, and subjected to electrophoresis. An electric field (70 V) was applied for 45 min to remove the negatively charged DNA molecules through the porous gel. DNA fragments were visualized by staining with ethidium bromide. This fluorescent dye intercalates between bases of DNA. Finally, the PCR-generated fragment sizes were visualized when illuminated with ultraviolet light. DNA bands were compared with the molecular weight marker running alongside (100 bp DNA ladder, Promega, Madison, Wisconsin, USA). The gel was destained in tap water (for 15 min), then photographed.

Multiplex PCR for simultaneous detection of *Wolbachia* and filarial parasites

Positive pools "by single PCR for *Wolbachia*" were examined by multiplex PCR using Qiagen Multiplex PCR kit (Qiagen) following the manufacturer's instructions.

W. bancrofti primers were ispWb12 forward primer 5′-CTGA GTGAAATCAATGAACTGC-3′ and reverse primer pWb12 R 5′-GT CCATCCGATGAAGTTCCACC-3′ primers [32].

The primers used for *D. immitis* were the forward DI COI-F1 5′-AGTGTAGAGGGTCAGCCTGAGTTA-3′ and the reverse DI COI-R1 5′-ACAGGCACTGACAATACCAAT-3′. *D. repens* primers were the forward Dr ITS2-F (5′-CATTGATAGTTTA-CATTC AAATAA-3′) and the reverse Dr ITS2-R (5′-GATTCATTTATTGC ATTA-AGCAAGC-3′) [33].

The procedure of multiplex PCR were as follows. The 2x QIAGEN Multiplex PCR Master Mix, template DNA, RNase-free water, and primer mix were defrosted. The solutions were mixed completely before use. Multiplex PCR components included reaction mix and template DNA. The reaction mix (total volume of 50 µl/reaction) was prepared as follows: 25 µl 2x QIAGEN multiplex PCR Master Mix, 5 µl (10x primer mix, 2 µM of each primer) and variable volumes of RNase-free water. The reaction mix was mixed thoroughly and appropriate volumes were dispensed into PCR tubes. The template DNA (1 µg/50 µl reaction) was added to individual PCR tubes containing the reaction mix. PCR tubes containing the reaction mix were overlaid with approximately 50 µl mineral oil. The PCR tubes were placed in the thermal cycler, and the cycling program was programmed according to the manufacturer's instructions using universal multiplex cycling protocol. Detection of multiplex PCR products were detected by agarose gel electrophoresis, followed by staining with 2% ethidium bromide, and DNA was visualized under ultraviolet illumination compared to 100 bp ladder molecular weight marker (100 bp DNA ladder and negative control (non-blood fed mosquitoes) were obtained from Research Institute of Medical Entomology (Dokki, Egypt) and used in each PCR reaction.

Statistical analysis

The collected data were analyzed by the program SPSS version 20 for Windows (Chicago, Illinois, USA). The rate of infection in mosquitoes was calculated by the estimated rate of infection (ERI) using the following formula: $ERI = 1-(1-X/m)^{1/k}$, where 'X' is the number of positive pools; 'm' is the number of examined pools, and 'k' is the average number of specimens in each pool [34].

RESULTS

Detection of *Wolbachia* by single PCR using *Wolbachia* 16S rDNA

We screened the presence of *Wolbachia* in 2,500 mosquitoes collected from 7 localities. They, representing 5 species belonging to 3 genera of mosquitoes, were identified as *Culex* spp. (*C. pipiens*, *C. antennatus*, *C. pusillus*, and grouped as *Culex* spp. pool), *Anopheles pharoensis*, and *Aedes* caspius.

They were divided into 100 pools. Six pools (6%) were positive for *Wolbachia* in 4 (57%) localities, with an overall ERI of 0.24%. The infection status of each locality and the numbers of screened mosquitoes along with ERI were listed in Table 1 and Fig. 1.

Table 1. Localities monitored by single PCR for *Wolbachia* and number of pools simultanously positive for filaria and *Wolbachia* by multiplex PCR irrespective to mosquitos' genera, along with the estimated rate of infection (ERI; %) for both *Wolbachia* and filarial parasites and their statistical significance

Localities	Infection status by single PCR for *Wolbachia* (No. of mosquitoes)	No. of pools positive for *Wolbachia* and filariae/tested pools (%)	ERI (%)
El-Nikhila	+(575)	3/23 (13.0)	0.557[a]
El-Matiaa	+(325)	1/13 (7.7)	0.319[b]
Sahel Seleem	+(350)	1/14 (7.1)	0.295[c]
Dairout	+(350)	1/14[e] (7.1)	0.295[d]
El-Badary	-(350)	0/14 (0)	0[a,b,c,d]
Mankabad	-(250)	0/10 (0)	0[a,b,c,d]
Manfalout	-(300)	0/12 (0)	0[a,b,c,d]
Total	4-7 (2,500)	6/100 (6.0)	0.24

[a-d]Same letter means significant difference between them.
[e]No filaria was found in Dairout mosquito pools.

Simultaneous detection of *Wolbachia* and filariae by multiplex PCR

Only 64 mosquito pools from the 4 naturally infected *Wolbachia* localities were screened for simultaneous detection of *Wolbachia* and filarial parasites (*W. bancrofti, D. immitis,* and *D.*

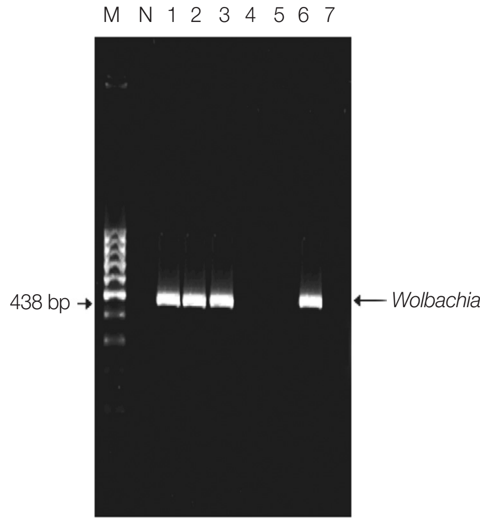

Fig. 1. Detection of *Wolbachia* endosymbiotic bacterial DNA in pools by single PCR of indoor-resting mosquitoes. Lane 1, El-Nikhila village; lane 2, El-Matiaa village; lane 3, Sahel Seleem city; lane 4, El-Badary city; lane 5, Mankabad village; lane 6, Dairout city; lane 7, Manfalout city. M, 100 bp DNA marker; N, negative control (non-blood fed mosquitoes). *Wolbachia* detected at 438 bp.

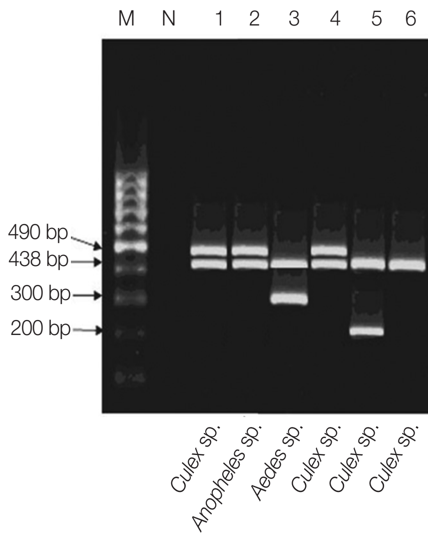

Fig. 2. Multiplex PCR pattern showing association of *Wolbachia* with filarial parasites within their respective vectors in the studied locality. Lanes 1-3, El-Nikhila village; lane 4, El-Matiaa village; lane 5, Sahel Seleem district; lane 6, Dairout district. *Wuchereria bancrofti* at 490 bp, *Wolbachia* at 438 bp, *Dirofilaria repens* at 300 bp, and *Dirofilaria immitis* at 200 bp.

Table 2. Mosquito genera and their positivity for *Wolbachia* and filarial parasites tested by multiplex PCR grouped according to localities along with the percentage of estimated rate of infection (ERI)

Localities	*Culex* spp.						*Anopheles pharoensis*						*Aedes caspius*					
	No. of mosquitoes	No. tested pools	*Wolbachia* positive pools	Filaria (genus) positive pools	ERI (%) *Wolbachia*	ERI (%) Filariae	No. of mosquitoes	No. tested pools	*Wolbachia* positive pools	Filaria (genus) positive pools	ERI (%) *Wolbachia*	ERI (%) Filariae	No. of mosquitoes	No tested pools	*Wolbachia* +ve pool	Filariae +ve pool (filaria genus)	ERI (%) *Wolbachia*	ERI (%) Filariae
El-Nikhila	400	16	1	1 (*W. bancrofti*)	0.25	0.25	75	3	1	1 (*W. bancrofti*)	1.60	1.60	100	4	1	1 (*D. repens*)	1.14	1.14
El-Matiaa	250	10	1	1 (*W. bancrofti*)	0.42	0.42	25	1	0	0	0	0	50	2	0	0	0	0
Sahel Seleem	250	10	1	1 (*D. immitis*)	0.42	0.42	50	2	0	0	0	0	50	2	0	0	0	0
Dairout	250	10	1	0	0.42	0.42	50	2	0	0	0	0	50	2	0	0	0	0
Total	1,150	46	4	3	0.36	0.27	200	8	1	1	0.532	0.532	250	10	1	1	0.42	0.42

Table 3. Number of mosquito specimens and pools tested according to their genera and their positivity for *Wolbachia* and filarial parasites along with the percentage of estimated rate of infection (ERI)

Mosquito genera	Total no. of mosquitoes	No. of tested pools	No. of pools positive for *Wolbachia*/tested (%)	No. of pools positive for filaria/tested (%)	ERI (%)	
					ERI[a]	ERI[b]
Culex spp.	1,150	46	4 (8.7)	3 (6.5)	0.363	0.269
Aedes caspius	250	10	1 (10.0)	1 (10.0)	0.420	0.420
Anopheles pharoensis	200	8	1 (12.5)	1 (10.0)	0.532	0.532
Total	1,600	64	6 (9.4)	5 (7.8)	0.392	0.324

ERI[a] for *Wolbachia*; ERI[b] for filaria.

Table 4. The infection status of the studied mosquito genera by multiplex PCR simultaneously holding *Wolbachia* and filarial parasites

	Culex spp.			*Anopheles pharoensis*			*Aedes caspius*			Mosquito pool positive for *Wolbachia* and filaria		
	No. tested pools	No. of positive pools	ERI (%)	No. tested pools	No. of positive pools	ERI (%)	No. of tested pools	No. of positive pools	ERI (%)	No. tested pools	No. of positive pools	ERI (%)
W. bancrofti	46	2	0.17[a]	3	1	1.6	4	-	-	53	3	0.23[a,b]
D. repens	46	-	-	3	-	-	4	1		53	1	0.07[a]
D. immitis	46	1	0.08[a]	3	-	-	4	-	-	53	1	0.07[b]

Only tested pools proved to be positive for *Wolbachia* were used in this calculation.
[a,b]Same letter means significant difference between them.

repens) in a single pool by multiplex PCR (Table 2). Only 6 pools (9.3%) were positive for *Wolbachia* from which 5 pools (83%) were associated with filarial parasite within their respective vector. Dairout district was filarial parasites free (Tables 1, 2; Fig. 2). Within the tested 4 localities, the genera most commonly represented was *Culex* spp. (n = 1,150; 71%) followed by *A. caspius* (n = 250; 15%) and *A. pharoensis* (n = 200; 12.5%) (Table 3). The 3 mosquito genera were enclosing *Wolbachia* (either within the mosquito itself or the co-infected filarial parasites). Unexpectedly, the overall highest ERI (0.53%) was for *A. pharoensis* and co-infected *W. bancrofti*, followed by *Aedes* (0.42%) co-infected by *D. repens* and *Culex* sp. (0.36%) co-infected by either *W. bancrofti* or *D. immitis* (Table 3).

El-Nikhila village had the highest mosquito rate of infection (ERI 0.55%), where the 3 mosquito genera, *Wolbachia*, and filarial parasites (*W. bancrofti* and *D. repens*) as listed in Tables 1, 2, and Fig. 2 and *A. pharoensis* recorded the highest ERI (1.6%) equally for *Wolbachia* and *W. bancrofti* (Table 2). The rate of infection of mosquitoes co-infected with *Wolbachia* and *W. bancrofti* was of 0.23%; nevertheless, it is less likely to found mosquitoes simultaneously associating *Wolbachia* with either *D. immitis* or *D. repens* as the ERI was 0.07% (Table 4).

DISCUSSION

To our knowledge, investigation on the relation between *Wolbachia*, mosquito genera and filarial parasites in Upper Egypt had not been undertaken so far. For instance, Kassem et al. [35] had studied the relation between *Wolbachia* and *Phlebotomus* sp., and the presence of *Wolbachia* in the Delta region of Egypt had been recorded only within *W. bancrofti* microfilariae [36]. Therefore, it is important to survey the distribution of this bacterium among the mosquitoes population in a given locality and also the harbored filarial parasites in order to plan any *Wolbachia*-based control program. We found out, by single PCR, that just in 2 out of the 7 studied localities "El-Badary and Manfalout" and their collected mosquito pools of "*Culex*, *Aedes*, and *Anopheles* genera" were not only free of filarial parasites but also of endosymbiotic *Wolbachia*. This finding was also reached by Kassem et al. [35] as they failed to detect Wolbachia in 1 out of the 4 colonies of Egyptian sandflies indicating that the inter- and intra-specific spread of *Wolbachia* is discontinuous, signifying the patchy distribution of the bacteria even within a country. We can even add within the same localities, as Mokhtar [37] had screened his mosquito pools for *Wolbachia* prior to his study and were free of it.

Culex spp. are the most abundant species collected from the 4 studied localities (72%) and the most abundant mosquitoes

in Upper Egypt [37-39]. El-Nikhila, El-Matiaa villages, and Sahel Seleem district were positively infected with both filarial parasite and the endosymbiotic bacteria, excluding Dairout where *Wolbachia* inhabits the *Culex* spp. pools; evidencing the invasion to the comprised *Culex* species (*C. pipiens, C. pusillus,* and *C. antennatus*). Osei-Poku [20], from Kenya, found that 26-75% of the collected *Aedes*, *Culex*, and *Mansonia* genera and 42% of *Culex* spp. were infected by *Wolbachia* likewise in India in which Ravikumar et al. [7] found that 50% of collected *Culex* spp. were positive for *Wolbachia* and were of medical importance. *C. pipiens* is described to be infected up to 100% with their respective *Wolbachia* strains throughout the majority of their geographical ranges [8,40,41]. Nonetheless, uninfected *C. pipiens* population were found in many parts of the world [42,43] coming along with the present results in which *C. pipiens* as one of the constituents of the *Culex* pool were free of *Wolbachia*. This finding needs to be investigated by focusing on each species consisting this pool, *C. pusillus, C. antennatus*, and specifically *C. pipiens*.

As the used primer is an all strain "*Wolbachia* 16S rDNA, W-Spec", we cannot know where the *Wolbachia* resides in the mosquito or filarial parasites? Here comes the dilemma, the studied *Culex* pools had the least ERI (0.36% and 0.26% for *Wolbachia* and filariae, respectively) in distinction to *A. pharoensis* with higher ERI (0.53%) and *A. caspius* (0.42%) for either *Wolbachia* or filaria. Despite the fact that *Culex* spp., including *C. antennatus* and specifically *C. pipiens*, are known to be the primary vector of *W. bancrofti* in Egypt [44-46] and reported by authors of the same locality [37,47], where *Culex* spp. had the highest ERI for *W. bancrofti* followed by *Anopheles* sp. The current decline of *Culex* spp. as the primary vector for *W. bancrofti* could be explained by the presence of *Wolbachia* either within the parasite or in the vector. If we are assuming that *Wolbachia* is challenging with the filaria parasite over the vector host; Farid et al. [45] stated that the susceptibility of *Culex* spp. to *W. bancrofti* infection could be altered in different mosquitoe species and even among different geographical strains. *Wolbachia* strains give negative effects by reducing the fitness and shortening the lifespan of their hosts as compared to the uninfected mosquito vectors [18,48-50]. In addition, authors had stated that *Wolbachia* of filarial nematodes (separate clade) could be horizontally transmitted to their enclosed vectors [15,16], consequently adding to, may be present or not, another load of bacteria affecting the vector fitness which could explain this regression in the vector role of *Culex* sp. For that reason, *A. pharoensis* has presently emerged as an alternating vector for *W. bancrofti*, which is known to be a secondary vector for *W. bancrofti* and more focally involved in its transmission [46,51]. It has to be taken into consideration that *Anopheles* sp. are reported to be free of natural *Wolbachia* infection [7,8,20], and the herein detected *Wolbachia* infection is with conviction present within the beholden *W. bancrofti* inside which the bacteria flourish, co-infecting the *A. pharoensis* pool. Therefore, rise up the role of *A. pharoensis* as a secondary vector for *W. bancrofti* which seems refractory to the horizontal transmission of *Wolbachia* and subsequently to the negativity of *Wolbachia* on the mosquito fitness. The ERI of *A. pharoensis* to *W. bancrofti* in El-Nikhila village reached 1.6% in contrast to 0.9% in the same locality by Dyab et al. [47] where it was proved to be free of *Wolbachia*.

The co-infection of mosquito vectors with filaria increases the ability to find *Wolbachia* either in the filarial host or the vector. It may be worth noting that *Culex* pool was also found to be the vector of *D. immitis* and co-infected with *Wolbachia*. Bandi et al. [52] and Pourali et al. [53], used the similar 16S rDNA, W-Spec primer which had proved the *Wolbachia* presence within *D. immitis*. *A. caspius* was found to be co-harbored by *D. repens* and *Wolbachia* with an ERI of 0.42%. It should be noted that more than 1,000 screened *A. caspius* in Italy were negative for *Wolbachia* [5]. Currently, we could not be decisive where does the *Wolbachia* reside? However, many authors had verified its occurrence within *D. repens* [54]. The evident obligatory symbiotic relationship from several reports on filarial worms was that rendering the bacterial depletion kills adult worms and blocks embryogenesis (microfilariae output) [27]. The susceptibility to antibiotic treatment and the development of the host nematode are slowed or prevented by depletion of *Wolbachia* [54]. Since we found that the infection status of the studied mosquito genera simultaneously holding *Wolbachia* and filarial parasites and that *W. bancrofti* had the highest ERI (0.23%) followed by the equal ERI (0.7%) for *D. repens* and *D. immitis*, the assumed presence of *Wolbachia* within *W. bancrofti* and another filarial parasite in our locality should motivate the treatment of patients and control of filariae by the use of specific anti-*Wolbachia* antibiotic in addition to the usual anti-filarial drugs.

In conclusion, our results describes the first molecular detection of *Wolbachia* endobacteria in different genera of mosquito pools co-infected with filarial parasites captured from endemic filaria localities in Assiut Governorate, Egypt. The presence of

Wolbachia in co-existing with *W. bancrofti* within the *Culex* spp. vectors had altered the transmission of lymphatic parasite in favor of the *A. pharoensis* vector. However, this study is still inadequate and needs further works in order to determine the distribution and genotyping of *Wolbachia* endobacteria found in our community mosquito vectors co-infected with filarial parasites.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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