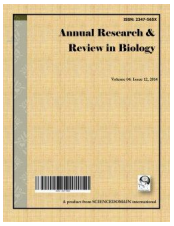

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The Fauna, Habitats and Medically Importance of Mosquito Larvae (Diptera: Culicidae) in Salfit District- Palestinian State

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to discuss the mosquito fauna, breeding sites and its habitat during January 2017 to May 2018 at Salfit district (Northwestern West Bank).
Study Design: A cross-sectional study
Methods: A cross-sectional survey was conducted in West Bank -Salfit District to collect mosquito larvae from January 2017 to May 2018.
Results: Six species of the family Culicidae were collected belonging to four genera *(Anopheles claviger, Aedes albopictus, Culex pipiens, Culex perexiguus*, *Culex laticinictus* and *Culiseta longiareolata*) were recorded from Salfit District. *Cu. longiareolata, Cx. laticinictus* and *Cx. pipiens* were the most collected species. *Anopheles claviger* was reported for the first time from Salfit District.
Limitations of the Study: The study discussed the fauna of mosquito breeding sites and larvae habitats at Salfit District during 2017-2018. Larval habitats were recognised visually and some of the other factors not tested (PH, temperature, turbidity of water and percentage of water pollution).

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Conclusion: The results show the occurrence of six species in the Salfit District during the study period (2017-2018). Three of them are known to be considered as a vector for viral diseases (e.g. West Nile virus). Further studies are needed to address the systematics, ecology, biology and habitat preference of these species.

Keywords: Mosquito; Culicidae; larvae; Salfit district; distribution; Palestinian Territories.

1. INTRODUCTION

Until now about 3,490 species of mosquitoes are known in the world [1]. Beside their nuisance, mosquitoes are considered insects of medical importance since they are capable of transmitting many diseases for human and animals (West Nile, dengue, Yellow fever, Eastern Equine Encephalitis and malaria) [2-7]. Changes in environmental factors, limited water resources for mosquitoes breeding site, and vector control programmes affect the mosquitoes fauna, distributions and habitats [8-14].

In Historical Palestine, many studies discussed the mosquitoes fauna [15-27]. Margalite and Tahori [21] recorded a total of 42 mosquito species in the Historical Palestine]. They found that some of these species have become extinct in the area mainly due to the control measures and changes in their environment resulting from drainage or water pollution.

The fauna and distribution of mosquitoes were affected with different factors such as environmental factors (temperatures, rainwater, humidity, physiochemical properties of water), trading, civilisation, development and environmental manipulation and modification and availability of water resources [8-27].

In the last decades, Palestine was endemic with malaria [27,21]. Now a days, Palestine is free of malaria [27] with exception of imported cases from outside the country. Other mosquitoes became medically important since they transmitted viral diseases [26,28,29]. In last ten years, according to Palestinian annual reports sporadic cases of West Nile Virus were recorded at different districts [30].

In the Palestinian regions, there is a lack of information regarding the ecology of larvae, including their habitats, ecological data (larval habitats, species composition, and active season), this information will play an important role in integrated vector management. These data are used in source reduction through environmental manipulation and modification. An investigation was carried out in Salfit District to study the fauna and some aspects of the ecology of mosquito larvae, including habitats, species composition, association occasions, and percentages. The study is the first of its kind which discusses the larvae fauna of mosquito and highlights and promote other scientists and researchers to study the mosquito fauna and their medical importance in disease transmission in the Palestinian land.

1.1 MATERIALS AND METHODS

1.2 Study Area

Salfit District is located at 32º 7′ 5.5″ N and 35º 5′ 25″ E with a total area around 202 km^2. It is situated in north-western part of the West Bank, bordered by the districts of Ramallah and Al-Bireh to the South, Nablus to the East, and Qalqilya to the North as well as Israel to the West **(**Fig. 1). According to the Palestinian Central Bureau of Statistics [31], the governorate has a population of 72000 inhabitants, most of them work in agriculture and trade. Economic activity depends on agriculture, where olives, almonds, figs, grapes, and apples are the main crops. Climate is hot with dry summers and rainy winters, with an average maximum and minimum temperature of 29ºC and 6ºC, respectively. The average humidity in the region is 62% that may rise upto 67%. Rainfall is concentrated in the winter, with an average value of 660 mm per year [31].

1.3 Study Design

This study was conducted as a cross-sectional survey to collect larvae from various sites in Salfit District during January 2017 to May 2018. Different habitats were investigated for Larvae in their breeding sites (vegetation area, animal area, springs, cistern wells, and discarded tires). Coordinates were recorded by a Global Positioning System (GPS) device and also number of examined larvae, their habitats, date and location of collection were recorded.

1.4 Larva Collection and Species Identification

Mosquito larvae were collected from all available potential breeding sites (Tin cans, discarded tires, plastic and metal container waters, springs, cistern wells, sewage water canals, rainwater pool, and irrigated canals). Breeding sites were visited twice a week. Pipette or standard dipper of 350ml technique depending on the size of the exposed surface was used to collect the larvae [32]. Larval habitats were recorded visually including water condition (stagnant or running) and turbidity, vegetation and sunlight exposure. Larvae were preserved in plastic cups and transported to the laboratory at Salfit Primary Health Center, washed, killed with hot water and kept in 70% alcohol [32].

The third and fourth stage larvae were prepared on microscopic slides using Berlese's medium, morphological characteristics were used to identify the larvae by using identification keys [33-38]. For confirming the species, some of the identified larvae species were reared to become adults and identified.

1.5 Data Analysis

Data Management and Analysis Data were entered in SPSS software 16 and analysed by using simple descriptive statistics.

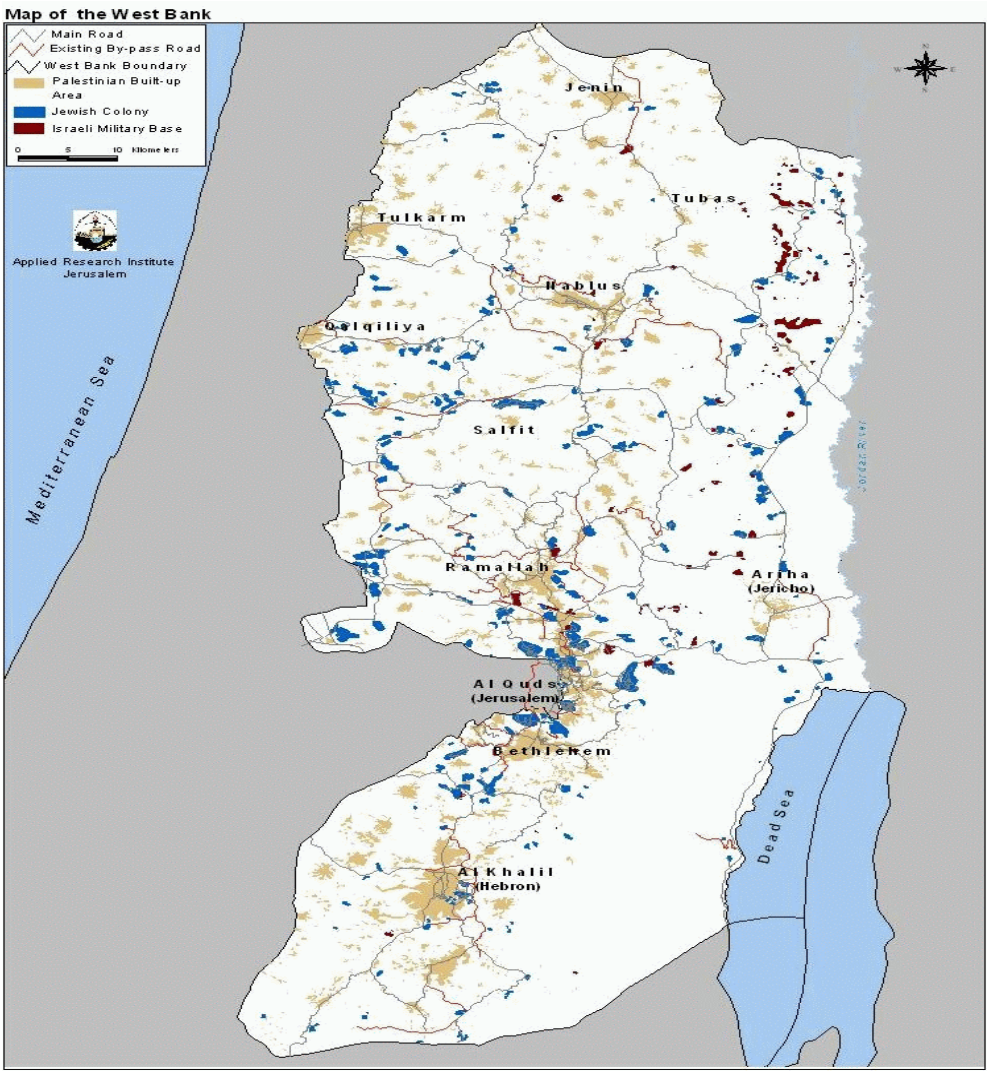

Fig. 1. Map showing the location of Salfit District in the West Bank (Palestine Central Bureau of statisti, 2017)

The Pattern of occurrence for mosquito larvae at Salfit District was estimated using the (C%), according to the method of Rydzanicz and Lonc [39], using the following formula: C=n/N*100.

Where C= distribution, n = number of sites positive for the occurrence of mosquitoes and N = total number of studied sites. According to occurrence value, mosquito species were classified into 5 categories: C= 0–20%; the distribution pattern of the species is sporadic, C= 20.1–40%; the distribution pattern of the species is infrequent, C= 40.1–60%; the distribution pattern of the species is moderate, C= 60.1–80%; the distribution pattern of the species is frequent and C= 80.1–100%; the distribution pattern of the species is constant

Density was expressed as the percentage of specimens for a given species in the whole sample according to the following formula D=I/L*100%

Where D= density, I= number of specimens of each mosquito species, L= total number of specimens, and the following density class were used:

Satellite species {D<1%}
Subdominant species {1<D5%}
Dominant species {D>5%}

2. RESULTS

300 breeding sites were visited at Salfit District. A total of 6120 mosquito larvae were collected and examined. Six species belonging to four genera (*Anopheles claviger, Aedes albopictus, Culex pipiens, Culex perexiguus, Culex lticinictus and Culiseta longiareolata*) were recorded.

2.1 Subfamily Anophelinae

Anopheles (Anopheles) claviger (Meigen, 1804)

Material examined: 200 larvae were collected from (3 spring sites): At Qarwa spring, Salfit spring and Khirbit Qis spring.

Remarks: The larvae found and collected from springs ponds in agricultural areas and partial sunlight. Larvae started to appear from the beginning of April until August, and the peak collection during May and June. They were found a few numbers and associated with *Culex laticinictus and Culiseta longiareolata* (Fig. 2 and Tables 1,2,3).

2.2 Subfamily Culicinae

2.2.1 *Aedes* (Stegomyia) *albopictus* (Skuse, 1894)

Material examined: 400 larvae were collected from end of July until November, and the peak collection was in October while specimens were not collected during the winter and spring of 2017 and 2018.

Remarks: Most of the larvae were collected from large container (80%) with stagnant (100%) and clean water (80%) and collected from vegetation area with partial sunlight. Larvae were collected from different localities and different habitats (vegetation cover such as house gardens, plant farms and nurseries) in Salfit District. *Aedes albopictus* was recorded in a sporadic abundance with dominant distribution at Salfit District. It was recorded that most breeding occurred in clear water and small cans in nurseries. Adwai (2012) recorded *Ae. albopictus* in two localities in Salfit District for the first time, now this species spreads in all Salfit District (Fig. 2 and Tables 1,2,3).

2.2.2 *Culex* (culex) *perexiguus* (Theobald, 1903)

Material examined: 250 larvae were collected from temporary rainy pool found in Deir ballout locality at Salfit District.

Remarks: It was found in a sporadic abundance with dominant distribution. Larvae were collected from annual rain pools water which has stagnant (100%) water and aquatic plant during March. After that no larvae were collected because of dryness of rain pool (Fig. 2 and Tables 1,2,3).

2.2.3 *Culex* (culex) *pipiens* L. 1758

Material examined: 500 larvae were collected mostly from stagnant, turbid, ground water (52%, 80%, 80% respectively) and collected from the different site at Salfit District.

Remarks: In summer season, it was most common in sewage water tank while in winter season, it was collected from discarded tires along with *Culex laticinictus*. It was found in a moderate abundance with dominant distribution. Peak collection was recorded during June to September (Fig. 2 and Tables 1,2,3).

2.2.4 Culex(culex) laticinictus (Edwards, 1913)

Material examined: 1500 larvae were identified and it was found in a moderate abundance with dominant distribution.

Remarks: The species was recorded in large number in the same habitats and breeding sites *of Culiceta longiareolata* and *Anopheles claviger* (water ponds of springs, artificial wells and mostly breed in stagnant clear water in large container of 93%, 90%, 75% respectively). The species was also found only with *Cx. pipiens* in discarded tires. The species was collected from January until December in all localities at Salfit district with the peak of collection were from June to September (Fig. 2 and Tables 1,2,3).

2.2.5 *Culiseta* (Allotheobaldia) longiareolata (Macquart, 1838)

Material examined: 2500 larvae were collected from water springs, artificial wells and large container in Salfit District.

Remarks: The species was recorded in a large number from the breeding sites, and it was the most common species in Salfit District. This species was found in a frequent abundance with dominant distribution from January to December during 2017 and until May 2018. The species was found to be associated with other species (*Cx. laticinictus* and *An. claviger*) and has the same breeding sites of *Culex laticinictus* as shown in Fig. 2 and Tables 1,2,3.

Table 1. Density and distribution status of larvae species in Salfit District during the period of 2017-2018

Species	Number of collected larvae	Number of positive sites	C%	D%	Density	Occurrence
An. claviger	200	3	1	3.26	Dominant	Sporadic
Ae. albopictus	400	50	16.66	6.53	Dominant	Sporadic
Cx. pipiens	1270	130	43.33	20.75	Dominant	Moderate
Cx. perexiguus	250	1	0.33	4.08	Dominant	Sporadic
Cx.laticinictus	1500	150	50	24.50	Dominant	Moderate
Cu. longiareolata	2500	185	61.66	40.84	Dominant	Frequent
Summation	6120					

Total number of visited site= 300

C= Occurrence 0–20% sporadic, C= 20.1–40% infrequent, C= 40.1–60% moderate, C= 60.1–80% the frequent and C= 80.1–100% is constant.

D= density, Satellite species {D<1%}, Subdominant species {1<D5%} and Dominant species {D>5%}

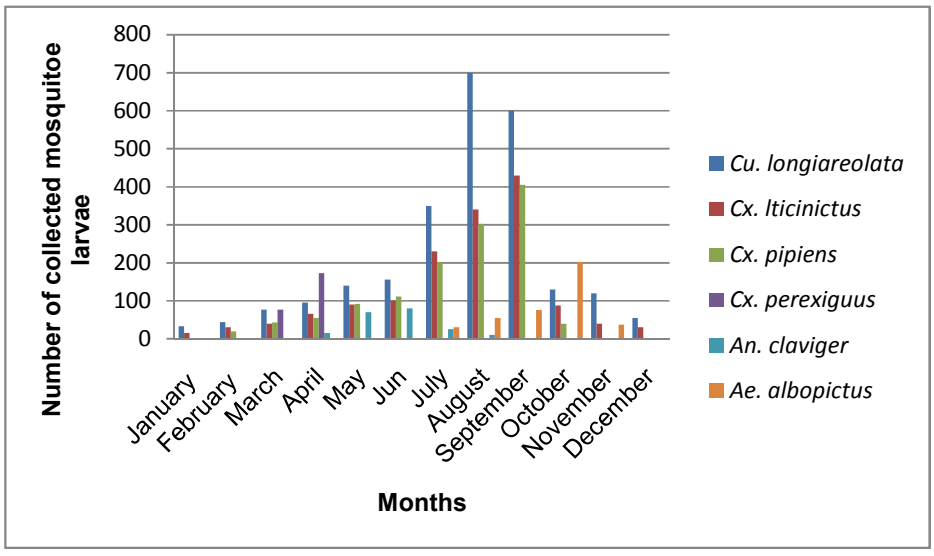

Fig. 2. Number of larvae of Mosquitoes species collected on monthly basis at Salfit Distict during 2017-2018

Table 2. Features of the larval habitat and occurrence percentage of different mosquito larvae in Salfit District during 2017-2018

Larval breeding site: Characteristics and habitats	*An. claviger*	*Ae. albopictus*	*Cx. pipiens*	*Cx. laticinictus*	*Cu. longiareolata*	*Cx. perexiguus*
A) Habitat type						
1. Spring pool	100	5	5	5	10	0
2. Large container	0	90	7	75	80	0
3. Small containers (cans etc.)	0	5	2	1	5	0
4. Tires	0	0	24	15	2	0
5.Rain water collection wells	0	0	4	2	0	0
6. Ground water	0	0	52	2	3	0
7. Collecting sewage thank	0	0	6	0	0	0
B) Water conditions						
1. Stagnant water	100	100	80	93	100	100
2. Slow-running water	0	0	20	7	0	0
3. Clear	100	80	20	90	100	0
4. Turbid	0	20	80	10	0	100
C) Vegetation						
1. With vegetation	100	100	10	75	80	100
2. Without vegetation	0	0	90	25	20	0
D) Sunlight exposure						
1. Full sunlight	0	0	70	70	70	0
2. Partial sunlight	100	100	30	30	30	100
E) Polluted water						
1.Opened Sewage system	0	0	80	0	0	0
2. Collecting sewage tank	0	0	20	0	0	0

Table 3. Shows the association (sharing the same breeding sites and habitats) between larvae species recorded in Salfit District during 2017-2018

Species	Associated with											
	An. claviger		*Ae. albopictus*		*Cx. pipiens*		*Cx. laticinictus*		*Cu. longiareolata*		*Cx. perexiguus*	
An. claviger							•	*	*			
Ae. albopictus												
Cx. pipiens							•	*				
Cx.laticinictus	•	*			•	*			•	*		
Cu. longiareolata	•	*					•	*				
Cx. perexiguus												

(•) and (*) = vertical association and • horizontal association between species*

3. DISCUSSION

Little information was available regarding the mosquitoes fauna and its medical importance in the occupied Palestinian territories (West Bank). So, this study was performed to highlight some of information about the mosquitoes fauna, breeding sites, habitats and their medical importance and possibility of transmitting diseases.

Four genera (*Anopheles, Aedes, Culex* and *Culiseta*) and six species of larvae mosquito (*An. Claviger, Ae. albopictus, Cx. pipiens, Cx. laticinictus, Cu. longiareolata and Cx. perexiguus*) were recorded at Salfit District and their breeding in different habitats was also studied. These results were compatible with other studies done in Historic Palestine [15-26].

In Salfit District, different environmental factors might affect the mosquitoes fauna i.e., breeding sites such as rainwater, temperature, existence of drainage system, availability of water resources. Therefore, most of the breeding site found in Salfit were sewage collection hull which was not completely closed and found closed to houses also open sewage water canals in some locality, even existence of the collected grey or fresh water in house garden which used in plants irrigation. These factors found to be suitable breeding site for mosquitoes especially *Cx. pipience* which mostly breeding in sewage collection hull and open sewage water canals and *Ae. albopictus* in gray or clear water. These results agree with several other studies which discussed the effect of environmental factors, VCP, sanitation, trading, developing, etc. in mosquito fauna, distribution and changing in breeding sites [9 -14].

Anopheles claviger was known to be the most common species in the Middle East countries [40]. In Historic Palestine, this species was recorded from north location [41-43,21-23]. According to Muhlens, 1913, *An. claviger* was an important vector of malaria in Jerusalem. The species was recorded first time at Salfit District, the larvae started to collect from the breeding sites (springs) found in vegetation areas away from houses at the beginning of spring season until the end of summer (March to August). These results agree with different studies discussed the *An. claviger* fauna [21-23,40-42]. Recently, no medicinal importance was recorded for this species in Historic Palestine [43].

Aedes albopictus has a widespread global distribution, and it is now listed as one of the top 100 invasive species by the Invasive Species Specialist Group [13]. It is known as a significant biting nuisance, vector of zoonotic pathogens to humans and a vector of virus diseases [13,44]. In Historic Palestine, *Ae. albopictus* was found to be positive for West Nile virus [26]. It is a new invasive species recorded from the occupied Palestinian territories (West Bank) and has been found to be transmitted especially by trading of tires and Ornamental plants [45]. The larvae collection started from summer season (July) and ends during November. The breeding sites were gray or fresh water collected by small cans and large containers found in house garden. These results agree with different studies discussed the *Ae. albopictus* fauna [45].

According to Harbach [24,26], *Culex perexiguus* and *Culex univittatus* are the similar species. Distribution of both the species could help in identification of *Cx. Perexiguus.* It is restricted in North and South Africa, Middle East, South Asia and India. *Culex. perexiguus* was found as a vector with West Nile, Sindbis and/or Usutu viruses in Historic Palestine, Egypt and Saudi Arabia [24,26]. It is found in one locality of Salfit District (Deir Ballout) and could to be collected during 1st March to the end of April in temporary pond. These results agree with different studies discussed the *Cx. perexiguus* ecology, habitat and medical importance [24,26].

Culex pipiens is a common species found in Salfit District. It is a synanthropic mosquito with a widespread distribution in temperate regions [46]. It was also found to be a vector for West Nile virus in Historic Palestine [29,26]. Results show that Cx. *pipiens* were mostly breeding in wastewater, the larvae started to be found and collected from the beginning of March until the end of October. These results agree with other studies which found Cx. *pipiens* breeding in waste water [9-14,26,29].

Culex laticinictus has a widespread distribution in Southwestern Asia and Egypt [24]. It has been recorded in all regions of Historic Palestine [19, 22,23]. No medically importance was recorded for this species in Historic Palestine. It was a widespread species in Safit District and started to collect the larvae from the beginning of January and to the end of December from large containers and water springs. These results agree with other studies which discussed the *Cx. laticinictus* fauna [19,22,23].

Culiseta longiareolata is found in Spian, Ukrania, Middle East, India, England, and Africa [47-51]. This species was medically important as pest and a vector of virus blood parasites and arboviruses [47-51]. In historic Palestine, no studies were recorded for this species as a vector. This species was found to be the most common species at Salfit District and the larvae was collected from the beginning of January to the end of December. These results agree with other studies which discussed the *Cu. longiareolata* fauna [47-51].

The results show that different species were sharing the same breeding sites as *An. claviger, Cx. laticinictus*, *Cu. longiareolata* were found together in the same habitats and breeding sites. It was also found that *Cx. pipiens* and *Cx. laticinictus* were sharing the same breeding site and habitats (discarded tires). These findings corroborate the results of different studies [21,23].

Ae. albopictus was found with a negative association with other mosquito larvae. Little is known regarding the ecology, habitat distribution, phenology and medical importance of the invasive species *Ae. albopictus* in the West Bank. Other studies discussed the association between Aedes species and other species, and found that Aedes species has negative association. The reason may be that this species was a competitive species, and preferred high saline breeding sites, mainly breeding in fall season, physiochemical water properties and availability of food [23,52-54]. as also shown in the studies which found that *Aedes caspius* avoiding *Cu. longiareolata* breeding sites [55-56].

The results show that all species collected from Salfit District are medically important and this corroborates with other studies [26,29,57-59]. In Palestinian historical land, different studies discussed the possibility of different mosquito species in transmitted disease especially virus disease and found that *Ae. albopictus, Cx. pipiens, and Cx. perexiguus*) were positive for West Nile Virus [28,26] and these species were widespread in Salfit District.

4. CONCLUSION

The study shows that six species belonging to four genera were found in Salfit District during the study period (2017-2018). Three species (*Ae. albopictus, Cx. pipiens, and Cx. perexiguus*) was recorded to be the vectors for viral disease especially for the West Nile virus. More studies were needed to address the ecology, phenology, habitats, breeding sites and possibility to transmitted viral diseases like WNV and others.

ETHICAL APPROVAL

The ethical approval was taken from the Salfit primary health care center for the use of the laboratory and all materials needed for the research. Another approval was taken from the volunteer who is in charge of collecting the larvae of mosquito. Finally an approval was taken from the targeted households, gardens and nurseries owners to collect larvae.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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