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Original Article

Distribution of vectors and arboviruses, and healthcare workers' knowledge of vector-borne diseases in Armenia

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Abstract

Introduction: Armenia's favorable geographical and climatic conditions support mosquitoes, sandflies, and ticks that can transmit various diseases. This study aimed to determine the prevalence of these vectors and circulating arboviruses in Armenia and assess healthcare workers' knowledge of arboviral diseases.

Methodology: In 2021, we conducted fieldwork, combining morphological identification of vectors with polymerase chain reaction (PCR) analysis of pathogens to map the distribution of potential arbovirus vectors across Armenia.

Results: Our entomological surveys identified four mosquito genera—*Anopheles*, *Aedes*, *Culex*, and *Culiseta*—comprising 20 species; and 11 species of Ixodidae ticks. *Culex pipiens* was found in all 11 regions, while *Culiseta* spp. was absent in Ararat Province. PCR testing of mosquito and tick samples revealed Crimean-Congo hemorrhagic fever virus (CCHFV) in 13 tick samples, but West Nile virus (WNV) was not detected in mosquitoes. Specifically, 13 out of 525 *Ixodes* tick pools tested positive for CCHFV; the positive samples originated from *Hyalomma marginatum* ticks in Syunik's Sisian region. None of the 11 pools that contained 473 *Cx. pipiens* mosquitoes tested positive for WNV. Analysis of questionnaires from 499 healthcare workers showed that epidemiologists, infectious disease specialists, and family doctors had greater awareness of arboviral diseases than other specialists. However, there was a low rate of sample submission for laboratory diagnosis and confirmation.

Conclusions: The extensive presence of vectors combined with limited knowledge of arboviral diseases complicates disease understanding in Armenia. Strengthening the surveillance system through training and improved sample collection is essential for disease monitoring and public health interventions.

Key words: vectors; arbovirus; mosquitoes; Armenia.

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Introduction

The climatic conditions in the Republic of Armenia (Armenia) provide a favorable environment for various arthropods which can carry and transmit numerous dangerous pathogens. Historically, a number of vector-borne diseases have been, or currently are circulating in Armenia including Crimean Congo hemorrhagic fever (CCHF), West Nile fever (WNV), malaria, leishmaniasis, pappataci fever, relapsing fever, rickettsiosis, Q fever, plague, and tularemia [1–3].

Previous studies in Armenia in 2016 identified 29 different species of mosquitoes, including the invasive *Aedes* (*Ae*) *albopictus* for the first time, showing further expansion of its host range. The presence of *Ae. albopictus* was recorded in one locality of Armenia on the border with Georgia in 2016, and was again recorded in 2017 and 2018 [4]. This demonstrates the establishment and spread of this invasive species in northern Armenia and suggests that there should be concern for pathogens like Zika, dengue, and chikungunya viruses that this species can carry and transmit. With the increase of vectors and their identification in new areas, healthcare workers need to be aware of the additional diseases that can occur when these species are present. Along with disease awareness and disease reporting, it is imperative to be able to identify, track and address disease outbreaks. Cases and outbreaks of arboviral diseases caused by Crimean-Congo hemorrhagic fever virus (CCHF) and West Nile virus (WNV) have been recorded in the countries bordering Armenia and their expansion is expected.

The transmission cycle of WNV includes avian species as the primary reservoirs, mosquitoes as vectors, and humans, horses, and mammals as dead-end hosts [5]. WNV is transmitted through the bite of infected mosquitoes, primarily *Culex* (*Cx*), and usually causes no symptoms. However, approximately 20% of patients present with a febrile illness and the disease can become severe with cases of meningitis and encephalitis, and can result in death in an estimated 1 out of 150 patients [6]. Despite the presence of the common vector *Culex* in the region, there is limited information about the existence of WNV in mosquito vectors in neighboring countries [7]. A previous study from Iran identified 4 genera and 6 species of mosquitoes: *Anopheles maculipennis* sensu lato (s.l.), *Cx. hortensis*, *Cx. pipiens* s.l., *Cx. theileri*, *Culiseta longiareolata*, and *Ae. ochlerotatus* (*Och*) *caspius*. Among these identified species, WNV was detected by polymerase chain reaction (PCR) and sequencing in *Ae. Och. caspius*, from Sangar, Makoo County in Iran [8]. This study highlights the importance of identifying the presence of WNV in Armenia because there are wetlands and significant populations of migratory birds that can carry the disease across borders.

In 2014, Georgia's National Centers for Disease Control and Public Health detected 22 human cases of CCHF in the country. CCHF is caused by infection with a tickborne virus of the Bunyaviridae family. Transmission can occur following the bite of an infected tick or from contact with infected bodily fluids or tissues from both animals and humans [9]. CCHF begins as a nonspecific febrile illness followed by a hemorrhagic phase, often with a rapid decline in severe cases that can lead to multi-organ failure, shock, and death [10]. Due to the clinical severity and high transmissibility of the disease caused by CCHF, this viral hemorrhagic disease is considered a category A priority pathogen [11].

In Armenia, the only case of CCHF was registered in 1974 [12]. During the 1986-1996 entomological survey, as well as in 2016, CCHF virus (CCHFV) was detected in ticks in Armenia [13]. In 2006, a large entomological survey identified 125 distinct strains of arboviruses, including WNV [1].

Currently, healthcare facilities do not report suspect cases of arboviral diseases and do not routinely send the specimen for laboratory investigations. In addition, complete epidemiological data on the prevalence of arboviral diseases in Armenia does not exist. As temperatures rise and the human population grows and expands into new areas, habitats for vectors of infectious diseases continue to expand. It is important to continue to perform surveillance on the full range of invasive vectors throughout their habitat to understand their potential to carry and transmit infectious diseases and implement measures to combat them. Ongoing research is needed to identify the presence of vectors, the pathogens they are carrying, and their impact on the human healthcare system. This includes the need to continually educate healthcare workers on the characteristics of diseases to be aware of and the importance of referring these specimens for laboratory confirmation. Here, we report on the continued surveillance of arthropods and the potential presence of CCHFV and WNV in certain vectors of Armenia.

Methodology

Study area

Entomological studies were conducted on mosquitos and ticks collected by the National Centers for Disease Control (NCDC) throughout all 10 regions of Armenia (Aragatsotn, Ararat, Armavir, Gegharkunik, Kotayk, Lori, Shirak, Syunik, Tavush, Vayotz Dzor) and Yerevan city. Fieldwork observations were combined with the morphological identification of arthropods and results of PCR investigations of mosquito- and tick-borne pathogens, to map the distribution of arthropods, which are potential vectors of arboviruses throughout Armenia.

Collection of adult mosquitoes

Mosquitoes were collected throughout Armenia starting from the end of April to the end of October 2021 by classical entomological methods from shelters (endophilic species) to open areas (exophilic species). We used light traps and aspirators to sample mosquitoes both outside of animal shelters and inside animal shelters as previously described [4]. Traps were set in the evening and collected the next morning; and the adult mosquitoes were transported to the laboratory in an isothermal box.

Collection of mosquito larvae

Mosquito larvae were collected from ponds using the application technique. Briefly, a 400 mL white plastic cup with an extended handle, or bucket, was partially immersed into the pond to collect 3-12 larvae from the pond habitat. We collected samples from several types of insect habitats: stagnant temporary water bodies (crevices, ponds, forest holes, valleys, flooded meadows, or forests), semi-permanent water bodies with vegetation (ponds with vegetation, swamps, canals), semi-aquatic bodies without vegetation (ponds, road tracks, new ditches, etc.), running water (rivers, streams, ditches, drainage), natural containers (rock pools, tree holes, other plant soils), and man-made containers (artificial containers, hunting pools, holes). The larvae were collected in a vial with 70% ethanol, transported to the laboratory, and labeled according to our labeling scheme (Sampling ID or LHU_ID_date).

Collection of ticks

In order to determine the species and the seasonal density of ticks, they were collected during the spring starting from the end of April to the end of June 2021. As for the summer–autumn period, the most optimal months of August and September were selected for collection of ticks. These time frames provide the ideal temperature for tick activity in Armenia. Locations and time periods of collection of ticks were determined based on the climate of the given area. The ticks were collected in the morning and evening hours from livestock, as well as from the soil surface using the flag method. Different methods of collecting ticks were used depending on the nature and ecological characteristics of the study area.

Collection of ticks using the flag method

In steppe areas (all Armenian regions) ticks were collected using a wide piece of fabric—a 1.50 × 2 m, solid light-colored, fuzzy cloth (flannel) —with wooden rods attached to its opposite narrow ends.

In meadows with tall grass and forests (all Armenian regions), ticks were collected using a flag. The flag was dragged over grass, bushes or the ground/soil (early in the season), to the right or left side of the researcher (but never from behind). Inspection of the flag, the wide piece of fabric, and the clothing of the person collecting ticks was carried out after every 30–50 steps, depending on the abundance of ticks. Ticks on the grass stuck to the flannel cloth and clothing, from which they were collected with forceps and placed in a tube. The tubes were closed with a cork or a lid, and notes were made (on the tube or on paper) about the collection (date of collection, location including the coordinates if possible). The tubes with ticks were transported in a tightly closed metal box. To keep ticks alive, a green plant was placed in the tubes and the tubes were kept in a cool place.

Collecting ticks from animals

Ticks are mainly concentrated on the animal's neck, earlobes, eyelids, armpits, anus region, tail base and tip, and sub-thoracic and inguinal areas. The ticks were removed from the host's body by field technicians wearing rubber gloves and using special forceps or thread, tying the latter around the parasite's head. The ticks were removed gently by swinging and twisting, without any sharp movements, so as not to damage the gnathosoma of the tick and to prevent crushing of the tick.

Collecting ticks from rodent nests

To collect ticks from rodent nests in the field, we utilized a tool with a flexible metal cable and a square (15–20 cm) flannel fabric attached to the end. This tool was inserted into the nest as deep as possible, held there for about 30 seconds, and then carefully rotated. Then the sampling tool was placed in a sealed plastic bag and examined for ticks.

Mapping

Mapping was performed using the program ArcGIS.10. Individual maps of mosquitoes and ticks collected between 2016 and 2021 were created based on species distribution.

Questionnaire

We assessed the knowledge of arboviral diseases among 499 epidemiologists and clinicians of different specialties (family doctors, physicians, surgeons, otorhinolaryngologists, oculists, dermatologists, and infectious diseases specialists) in all 10 study regions and the capital city of Yerevan. We evaluated the responses based on age, gender, medical specialty, and their distance from Yerevan. The structured pre-tested questionnaire included 5 questions on epidemiology, distribution, etiology, and clinical signs and symptoms (Supplementary document 1). Every question was assessed by assigning a score based on the answer, with the number of correct answers identifying the level of knowledge on arboviral diseases. Participants with a higher score had a higher disease awareness of arboviral infections.

Laboratory investigation

After transporting the ticks and mosquitoes to the laboratory, morphological identification of the genus and species was carried out by microscopic examination. Once identified and separated by species, the ticks and mosquitos were washed in 96% alcohol, followed by physiological saline solution to remove any external contamination prior to RNA extraction. Then, a homogenous suspension was made by grinding the ticks or mosquitos in physiological saline.

There were 1,659 *Ixodes* ticks that were divided into 525 pools based on their volume of blood feed, location, and species. In the case of mosquitoes, we identified 473 *Cx. pipiens* that were sorted into 11 pools. We focused on *Cx. pipiens* as they are considered to be the main vectors of WNV, and are highly abundant in Armenia. In addition, we had limited resources and therefore could not evaluate all species. RNA was extracted from mosquito or tick pool homogenates utilizing the AmpliSense®, RIBO-PREP extraction kit (Moscow, Russia) according to the manufacturer's instructions. Extraction of RNA from biological material was carried out in the presence of an internal control sample to assess the performance of each sample. Reverse transcriptase PCR (RT-PCR) investigations were conducted on ticks for CCHFV, and on mosquitoes for WNV, utilizing the AmpliSense®, CCHF-FL (Moscow, Russia) and AmpliSense®, WNV-FL (Moscow, Russia) according to the manufacturer's instructions.

Results

Location and identification of mosquito and tick species

We performed entomological investigations in all regions of Armenia, over different landscape and ecological zones in 2021. A total of 2,372 mosquitoes and larvae were collected from live traps and 618 ponds. In addition, 2,750 ticks were collected from fields, animals, and over 1,000 rodent nests. All specimens were identified based on standard morphological keys. We identified 4 different genera of mosquitoes belonging to *Anopheles* (Figures 1 and 2), *Aedes* (Figures 1 and 3), *Culex* (Figures 1 and 4), and *Culiseta* (Figures 1 and 5). Mosquitoes of the genus *Anopheles* were found in Ararat, Aragatsotn, Vayots Dzor, Tavush, and Kotayk regions. *Culex* mosquitoes were found in Ararat, Aragatsotn, Vayots Dzor, Tavush, Kotayk, Armavir, Lori, and Syunik regions; and Yerevan city. Mosquitoes of the genus *Aedes* were found in Ararat, Aragatsotn, Armavir, Lori, Tavush, and Kotayk regions; and Yerevan city. Mosquitoes of the genus *Culiseta* were found in Aragatsotn, Armavir, Lori, Vayots Dzor, Tavush, and Kotayk regions; and Yerevan city.

We identified 20 individual species belonging to the 4 genera in 2021, which included: *An. claviger*, *An. hyrcanus*, *An. maculipennis*, *An. plumbeus*, *An. sacharovi*, *Ae. albopictus*, *Ae. annulipes*, *Ae. caspius*, *Ae. geniculatus*, *Ae. vexans*, *Cx. hortensis*, *Cx. martinii*, *Cx. modestus*, *Cx. pipiens*, *Cx. territans*, *Cx. theileri*, *Cx. torrentium*, *Cs. annulata*, *Cs. longiareolata*, and *Cs.*

Figure 1. Distribution of *Anopheles*, *Aedes*, *Culex*, *Culiseta* mosquitoes in Armenia in 2021.

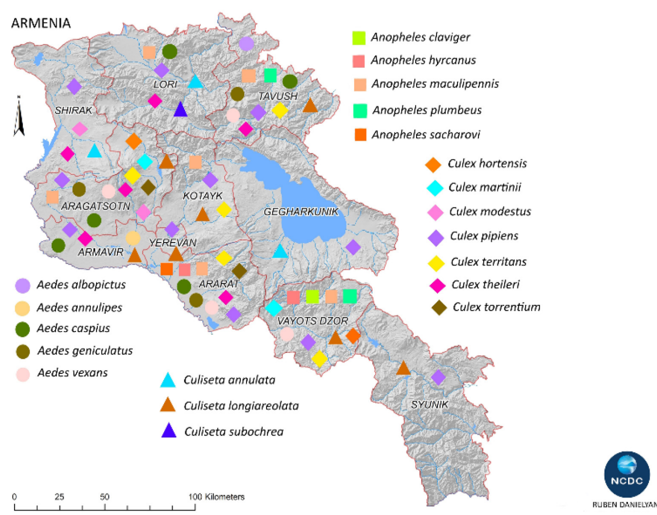


Table 1. Location of mosquito species recorded in Armenia in 2021 in the 11 regions studied.

	Aragatsotn	Ararat	Armavir	Gegharkunik	Lori	Kotayk	Shirak	Syunik	Vayots Dzor	Tavush	Yerevan
An. claviger									■		
An. hyrcanus		■							■		
An. maculipennis	■	■			■	■			■	■	
An. plumbeus									■	■	
An. sacharovi		■									
Ae. albopictus										■	
Ae. annulipes			■								
Ae. caspius	■	■	■		■					■	
Ae. geniculatus	■	■								■	
Ae. vexans	■	■							■	■	
Cx. hortensis	■								■		
Cx. martinii	■								■		
Cx. modestus	■						■				
Cx. pipiens	■	■	■	■	■	■	■	■	■	■	■
Cx. territans	■	■				■			■	■	
Cx. theileri	■	■	■		■		■			■	
Cx. torrentium	■	■									
Cs. annulata				■	■		■				
Cs. longiareolata	■		■			■		■	■	■	■
Cs. subochrea					■						

An: *Anopheles;* Ae: *Aedes*; Cx: *Culex*; Cs: *Culiseta*.

subochrea (Figure 1, Table 1). The cumulative distribution of individual mosquito species identified in Armenia from 2016–2021 is shown in Figures 2–5.

Among the ticks collected, 11 different species from 5 genera in the Ixodidae family were identified in 2021: *Haemaphysalis* (*H. bursa*, *H. punctata*), *Hyalomma* (*H. marginatum*, *H. plumbeum plumbeum*, *H. anatolicum anatolicum*), *Dermacentor* (*D. niveus*, *D. marginatus*, *D. reticulatus*), *Ixodes* (*I. ricinus*), and *Rhipicephalus* (*R. annulatus*, *R. sanguineus*). The species composition throughout their zones was determined (Figure 6).

Figure 2. Cumulative distribution of *Anopheles* mosquitoes in Armenia from 2016–2021.

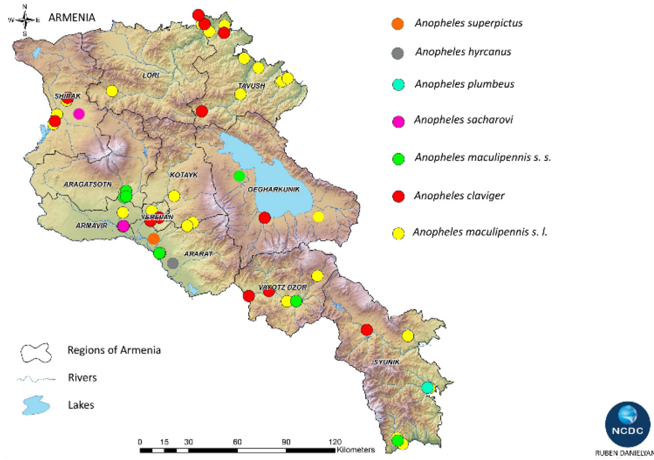


Figure 3. Cumulative distribution of *Aedes* mosquitoes in Armenia from 2016–2021.

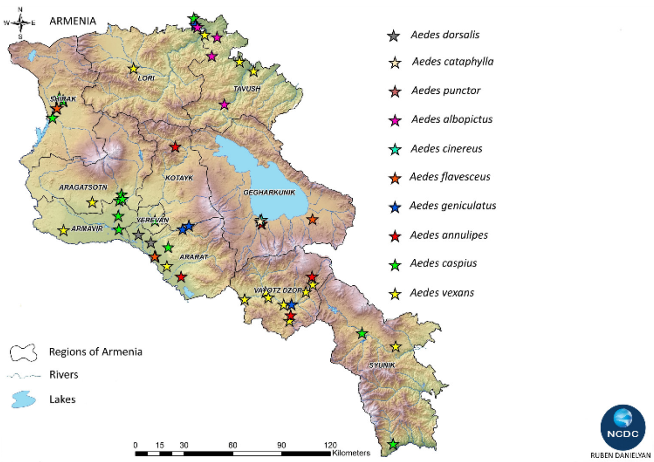


Figure 4. Cumulative distribution of *Culex* mosquitoes in Armenia from 2016–2021.

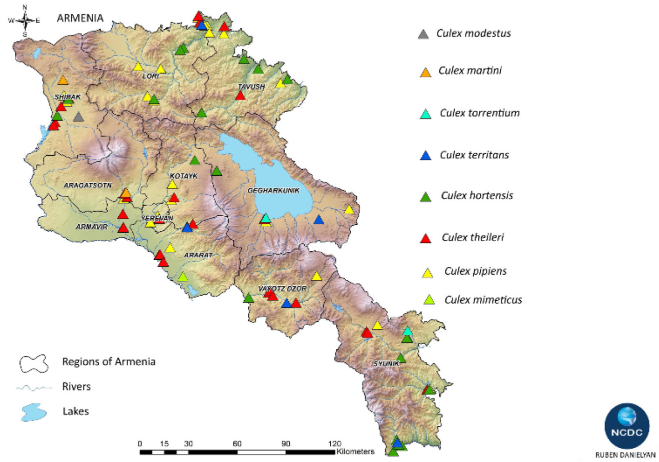


Figure 5. Cumulative distribution of *Culiseta*, *Uranotaenia*, *Coquilletitidia* mosquitoes in Armenia from 2016–2021.

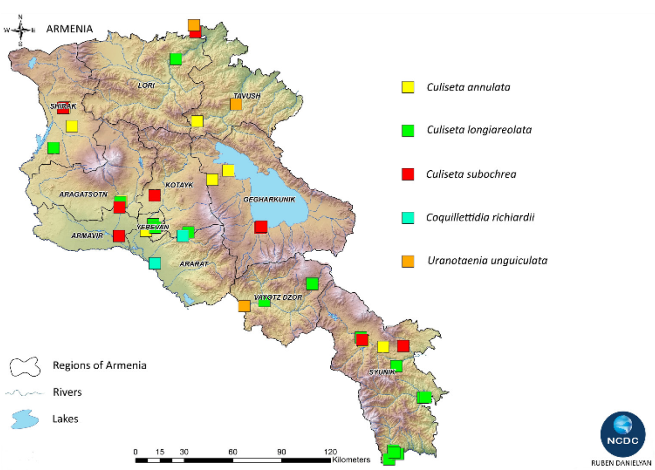
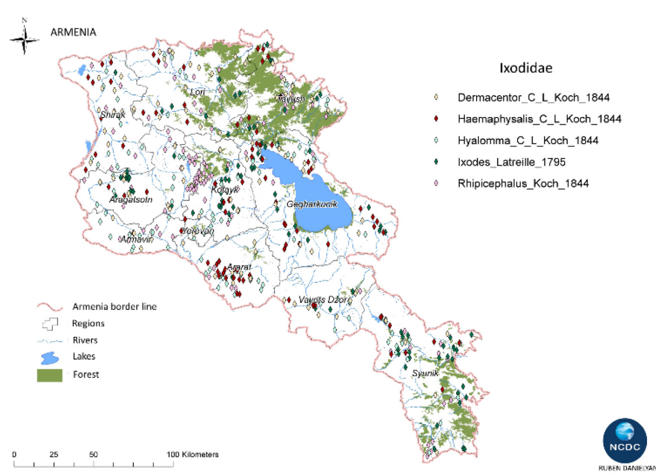


Figure 6. Distribution of individual species of *Ixodes* ticks in Armenia in 2021.



Questionnaire

A total of 499 healthcare workers participated in the assessment. Among them 85% (n = 424) were female, 67% (n = 334) were in the ≥ 50 years age group, 60% (n = 303) were from distant (> 100 km) regions, 12.4% (n = 62) were epidemiologists and infectious diseases specialists, 31.1% (n = 155) were clinicians of other specialties, and 56.5% (n = 282) were family doctors/physicians (Table 2).

Evaluation of the questionnaire scores revealed no association between the age/gender and distance from the capital, and knowledge on arboviral diseases. Epidemiologists, infectious diseases specialists, and family doctors/physicians were more aware of arboviral diseases with an average score of 33.4 (index intervals: 29.7–37.1). Other specialists had poorer knowledge; average score: 11.4 (index intervals: 0.3–22.5).

PCR Results

Based on the diagnostics of ticks and mosquitoes for CCHFV and WNV, we identified 13/525 *Ixodes* tick pools that were positive for CCHFV. The CCHFV positive *Ixodes* ticks were identified as *Hyalomma marginatum* species from the territory of Syunik in the Sisian region. Of the 11 pools containing 473 *Cx. pipiens* mosquitos, none tested positive for WNV.

Discussion

Climate change may lead to increase and spread of existing vector-borne diseases, and there are a number of infections whose importation may result in the transmission of epidemics in Armenia due to the presence of potential vectors. Fast-growing international travel and transport play an important role in the rapid spread of reservoirs, vectors, and pathogens of vector-borne diseases all over the world. Armenia is the homeland for all people of Armenian descent, including those who are residents of other countries, and many of them visit Armenia frequently. This can lead to the import of vectors carrying infectious diseases, and individuals with infections that are capable of being transmitted by vectors. This can lead to their transmission in Armenia and potential export of diseases and vectors to other countries.

Cx. pipiens was widespread in all the regions we studied. Mosquito activity starts in May and has great epidemiological significance as a known vector of WNV, Rift Valley fever virus, Japanese encephalitis virus, dirofilarial parasites and others [14]. WNV has efficiently spread and is present in Africa, Europe, Middle East, North America, and West Asia. Dirofilariasis, another vector-borne disease caused by nematodes of the genus *Dirofilaria*, is found in humans throughout the world. *Dirofilaria* are most commonly found in dogs; and humans are incidental hosts. Dirofilariasis has not yet been registered in humans in Armenia but has been detected in dogs [15]. Several types of mosquitoes in Armenia are capable of transmitting *Dirofilaria* spp. including those from the genus *Ae.*, *Anopheles*, and *Cx*. Additionally, a large entomological investigation performed from 2003–2006 identified multiple arboviruses including tick-borne encephalitis and many mosquito-borne viruses including WNV, Batai, Sindbis, Tahyna, and Gheta virus [1]. This highlights the presence of previously discovered diseases in Armenia and the concerns of spread to humans and animals.

Field observations demonstrate the recent introduction and establishment of *Ae. albopictus* in the north of the country which has vast implications for public health. The presence of *Ae. albopictus* along an important transport road between Yerevan and Tbilisi, Georgia provides additional concerns in terms of risk of arbovirus transmission as this mosquito is capable of

Table 2. Characteristics of participants of questionnaire on knowledge of arboviral diseases in Armenia.

Region	Number of participants		Gender				Age group (years)				Profession						Distance from Yerevan			
			Male		Female		< 50 years		≥ 50 years		Epidemiologists and infectious diseases specialists		Family doctors / physicians		Clinicians of other specialties		< 100 km		≥ 100 km	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Aragatsotn	49	9.8	3	4.0	46	10.8	7	4.2	42	12.6	2	3.2	26	9.2	21	13.5	47	15.5	2	1.0
Ararat	58	11.6	6	8.0	52	12.3	15	9.1	43	12.9	4	6.5	31	11.0	23	14.8	58	19.1	0	0.0
Armavir	44	8.8	6	8.0	38	9.0	18	10.9	26	7.8	3	4.8	29	10.3	12	7.7	44	14.5	0	0.0
Gegharkunik	26	5.2	7	9.3	19	4.5	13	7.9	13	3.9	3	4.8	13	4.6	10	6.5	13	4.3	13	6.6
Lori	32	6.4	11	14.7	21	5.0	17	10.3	15	4.5	5	8.1	20	7.1	7	4.5	0	0.0	32	16.3
Kotayk	21	4.2	5	6.7	16	3.8	12	7.3	9	2.7	4	6.5	10	3.5	7	4.5	21	6.9	0	0.0
Shirak	44	8.8	9	12.0	35	8.3	19	11.5	25	7.5	6	9.7	25	8.9	13	8.4	0	0.0	44	22.4
Syunik	46	9.2	9	12.0	37	8.7	20	12.1	26	7.8	6	9.7	28	9.9	12	7.7	0	0.0	46	23.5
Vayots Dzor	21	4.2	4	5.3	17	4.0	8	4.8	13	3.9	2	3.2	10	3.5	9	5.8	0	0.0	21	10.7
Tavush	38	7.6	7	9.3	31	7.3	11	6.7	27	8.1	5	8.1	19	6.7	14	9.0	0	0.0	38	19.4
Yerevan	120	24.0	8	10.7	112	26.4	25	15.2	95	28.4	22	35.5	71	25.2	27	17.4	120	39.6	0	0.0
Total	**499**		**75**	**15.0**	**424**	**85.0**	**165**	**33.0**	**334**	**67.0**	**62**	**12.4**	**282**	**56.5**	**155**	**31.1**	**303**	**60.7**	**196**	**39.3**

transmitting several infections including Zika, dengue, yellow fever, and chikungunya [4]. Continued studies are needed to understand the complete distribution of *Ae. Albopictus* in Armenia, to estimate and predict the future distribution, and to target surveillance and control efforts that aim to mitigate the spread of arboviral diseases.

The type of pathogens found in Ixodidae ticks varies depending on their location, climatic conditions, density, and predominance of the distribution of certain species over others in different landscape zones. The ticks that are commonly found in Armenia can spread Q fever, Lyme disease, tick-borne encephalitis, and CCHF. High incidence of CCHF, which can cause serious health problems and death, has been recorded in all neighbouring countries of Armenia; thus, highlighting this concern [16]. Although ticks of the genus *Hyalomma* play a significant role in CCHFV transmission, this virus has also been found in 31 other tick species [13]. Ongoing studies to identify the presence and concentration of certain vector species is very important as socio-economic conditions, and climate and environmental conditions continue to change, allowing arboviral infections to spread worldwide [17]. Since Armenia is represented by a distinct variety of landscapes with different climate zones in a relatively small area, the Armenian countryside supports the potential for continued distribution and expansion of the vector species [18].

Entomologists, ectoparasitologists, and zoologists of the laboratory of epizootology investigating the natural foci of infections are engaged in the study of arthropods that transmit vector-borne diseases in particularly dangerous infections. They implement measures to control the natural foci, collect biological material, and test for various pathogens. This has improved in recent years with the introduction of new laboratory diagnostic tools that have made it possible to differentially diagnose arboviral diseases, and the field workers were trained on prevention of disease spread when performing fieldwork.

In Armenia, overall knowledge on arboviral diseases was very low among doctors in all regions. Awareness on the various symptoms of arboviral diseases was low among doctors in all regions, and they were not aware of which diseases could be present in Armenia. The lack of dissemination of information among doctors, veterinarians, and epizootic laboratory specialists led to potential arboviral cases in Armenia to be registered as fever of unknown etiology with no clear diagnoses. An initial step to fill this knowledge gap has been to add information on arboviral diseases to the Health Workforce Continuing Education Program at the National Institutes of Health to improve awareness among healthcare professionals.

We acknowledge that there were limitations in our study. Insufficient resources limit our ability to conduct ongoing and comprehensive research on the complete distribution of all vectors and vector-borne pathogens in Armenia. This limits our understanding and development of comprehensive public health plans for tackling vectors and their diseases throughout Armenia.

Conclusions

The “One Health’’ approach needs to be emphasized and highlighted in future research plans to ensure additional stakeholders such as local governments, veterinary services, and environmental services are collectively involved in vector-borne diseases surveillance and control. It is important to continue to develop and organize updated awareness campaigns among doctors and the general population to improve knowledge on how vector-borne diseases can be contracted and how to inform the physician about risk factors involved with vectors exposure. Following analysis of disease surveillance, healthcare workers should be regularly updated on the potential presence of arboviral diseases in Armenia and the areas of surveillance should be comprehensive to accurately estimate the burden of diseases which will improve effective public health policies and ensure safety of the population.

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Annex – Supplementary Items

Supplementary document 1. Questionnaire: Assessment of the regional medical workers' knowledge on arboviral diseases.

Question 1. Which of the following are arbovirus infections?

○ Leishmaniasis	○ Sandfly (pappataci) fever
○ Tularemia	○ Crimean-Congo hemorrhagic fever
○ West Nile fever	○ Rabies
○ Creutzfeldt-Jakob disease	○ Leptospirosis
○ Q fever	○ Tick-borne encephalitis
○ Relapsing fever	○ Zika
○ Dengue	○ Chikungunya
○ Yellow fever	○ Lyme disease
○ Kyasanur forest disease	○ Malaria
○ Foot and mouth disease	○ Listeriosis
○ Escherichiosis	○ Omsk hemorrhagic fever
○ Japanese encephalitis	○ Ebola
○ Lassa fever	○ Marburg fever
○ Wuchereriasis	○ Oncocercosis
○ Babesiosis	○ Ehrlichiosis
○ Theileriosis	○ Alveococcosis

Question 2. Do you know if there have ever been local cases of arbovirus infections reported in Armenia?
o Yes o No o I do not know

Question 3. Do you know of any local cases of arboviral infections in Armenia in the last 5 years?
o Yes o No o I do not know

Question 4. Which arboviral outbreaks in the world have you heard about in the last decade?

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Question 5. What are the typical symptoms of arboviral infections?
o There are no symptoms
o High fever
o Rash
o Muscle aches
o Bleeding
o Congenital malformations of the fetus
o Influenza-like symptoms
o Arthritis
o Hemorrhages
o Encephalitis
o Jaundice
o Stiffness of the neck muscles
o Disorders of consciousness
o Headache