

REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

**CURRENT STATUS OF MOSQUITO FAUNA IN THE
SOUTH-EAST OF AZERBAIJAN AND THEIR ROLE
IN THE TRANSMISSION OF ARBOVIRUSES**

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Applicant: **Yegana Aligulu Sultanova**

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The dissertation work was performed at the Department of Parasitic and Tropical Diseases of the Scientific Research Institute of Medical Prophylaxis Public legal entity named after V.Y.Akhundov of the Ministry of Health of the Republic of Azerbaijan.

Scientific supervisor: Doctor of Medical Sciences
Akif Ayyub Salehov

Official opponents: Doctor of Biological Sciences,
Associate professor

Etibar Nasrulla Mammadov

Doctor of Philosophy in Biological
Sciences

Aysel Nuhbala Aghayeva

Doctor of Philosophy in Biological
Sciences

Nurana Ali Hajiyeva

Dissertation Council FD 1.09 of the Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan

Chairman of the Dissertation council:

Doctor of Biological Sciences,
Associate professor

Elshad Ilyas Ahmadov

Scientific secretary of the Dissertation council:

PhD in Biological Sciences

Elyana Nail Tahirova

Chairman of the Scientific seminar:

Doctor of Biological Sciences,
Associate professor

Ali Mammad Nasirov



INTRODUCTION

The research relevance and development degree: Blood-sucking mosquitoes are distinguished by their species diversity and wide distribution and are found in areas near the north and south poles and most parts of the world. Being blood-sucking insects, they have a harmful effect on the health of humans and domestic animals. Moreover, mosquitoes are considered effective carriers of many transmissible diseases, among which arboviruses are in the first place¹.

In recent years, as a result of global warming, human impact on nature, restructuring of water management, and intensification of transport networks, not only changes in the mosquito fauna have occurred, but also the expansion of their distribution range has been recorded, which leads to the spread of arbovirus infections outside their original range (to humid and warm tropical countries)². Until the 80s of the last century, a total of 28 species of mosquitoes were found in the fauna of our republic³. Similar information has not been published in the last 40 years.

Migratory waterfowl are considered the primary source of mosquito infection with arboviruses. Thus, infected mosquitoes migrate into human and domestic animal habitats, causing them to contract these infections. One of the largest water bodies in the territory of Azerbaijan is located in the Gizil-Agaj State Reserve, where hundreds of thousands of migratory birds live. In his research, A.Sh.Ismayilov confirms the role of infected mosquitoes in spreading Sindbis, Tahyna, Gizil-Agaj, and West Nile arbovirus infections among humans and

¹ Серкова, М. И. Медицинское и Ветеринарное значение кровососущих комаров // – Москва: Теория и практика борьбы с паразитарными болезнями, – 2021. Вып. 20, – с. 469-473.

² Негоденко, А.О. Анализ результатов мониторинга арбовирусных инфекций на территории Волгоградской области в 2019 г. / А.О.Негоденко, Е.В.Молчанова, Д.Р. Прилепская [и др.] // – Москва: Эпидемиология и Вакцинопрофилактика, – 2021. 20 (1), – с. 51-59.

³ Bağırov, Ə.H., Əliyev, M.İ. Azərbaycan Respublikasının ərazisində qansoran ağcaqanadlarının faunasına dair // – Bakı: Azərbaycan təbabətinin müasir nailiyyətləri, – 2012. №1, – s. 94-96.

animals in this area⁴.

The current status of mosquito fauna and their infection with arboviruses remains unknown and has not been widely studied. In addition, migratory birds can become a source of the global spread of arboviruses, as they fly great distances to Gizil-Agaj State Reserve to spend the winter.

The expansion of the range of *Aedes aegypti* and *Aedes albopictus* mosquitoes, capable of transmitting several types of arboviruses at once, has led to the emergence of new forms of the disease, as well as their epidemic outbreaks in the south of Russia, Europe, North, and South America⁵. This case is completely real for our republic too. It should be noted that serological tests have been carried out mainly among people, and there is practically no information about the infection of domestic animals (they, in turn, can be a source).

Object and subject of the research. The object of the research is blood-sucking mosquitoes, and the subject is humans, domestic animals, and migratory birds.

The purpose and tasks of the research: The main purpose of the research was to study the fauna, ecology, reproduction biotopes, daily places of blood-sucking mosquitoes in the Lankaran-Astara economic region and the Gizil-Agaj State Reserve, current status of their infection with arboviruses, and the epizootological-epidemiological regularities of the circulation of these infections among humans, domestic animals, and migratory birds.

To achieve this purpose, the following tasks were considered important in the research:

1. To determine environmental factors for the fauna, dynamics of change, and reproduction of blood-sucking mosquitoes;
2. To determine the role of migratory birds in the infection of

⁴ Исмаилов, А.Ш., Касымов, М.С. Арбовирусы в Азербайджане // – Баку: Биомедицина, – 2009. – с. 14-16.

⁵ Малецкая, О.В. Природно-очаговые вирусные лихорадки на юге Европейской части России. Лихорадка Западного Нила / О.В.Малецкая, Д.А.Прислегина, Т.В.Таран [и др.] // – Саратов: Проблемы особо опасных инфекций, – 2020. (1), 109-14.

mosquitoes, and identify suitable biotopes for different stages of their development;

3. To research the regularities of circulation of arboviruses among mosquitoes, domestic animals, and birds, and the tension of the epizootological situation;

4. To determine the epidemiological characteristics of arboviruses transmitted by mosquitoes to humans;

5. To determine sanitary education methods for the population in the implementation of prevention and control measures against arbovirus;

6. To determine the possibilities of anti-mosquito measures designed to reduce the tension of the epizootological-epidemiological situation for arboviruses;

Research methods. Entomological, parasitological, epizootological, epidemiological and statistical calculation methods were used during the research⁶. Thus, adult mosquitoes were caught using traps, larvae were collected in photo cuvettes, and their species composition was determined using determinants. The regularities of the circulation of arboviruses among humans, domestic animals and birds were epidemiological and epizootological examination methods and substantiated by statistical methods.

The main provisions of the defense:

1. The role of blood-sucking mosquitoes as vectors of arboviruses, their fauna and ecology;

2. Exposure to arboviruses in domestic animals, birds, and humans which are the main source of food for mosquitoes;

3. Epizootological and epidemiological characteristics of arboviruses;

4. Current status of control and prevention of arboviruses spread among humans, domestic animals, and birds.

Scientific novelty of the research:

1. It was determined for the first time that the changes in the ecosystem as a result of human activity, the intensification of transport

⁶ Балашов, Ю.С. Паразито-хозяйственные отношения членистоногих с наземными позвоночными // Ю.С.Балашов. – Ленинград: Наука, – т. 97. – 1982. – 318 с.

and migration processes affected the modern state of the fauna of blood-sucking mosquitoes, and 26 species of mosquitoes were found in the region.

2. 5 species— *An. plumbeus*, *Ae. versicolor*, *Ae. pulchritarsis*, *Cs. morsitans* and *Cs. fumipennis* previously existed in the mosquito fauna specific to the region were not detected in our research.

3. 3 species— *Ae. cataphylla*, *Ae. dorsalis* and *O. pulchripalpis* found in other regions of the republic for the first time were also observed in this region.

4. For the first time, we found new mosquito species (*Aedes albopictus*, *Aedes aegypti*, *Culex apicalis*) in the Caucasus region.

5. It was found that the primary source of mosquito infection with arboviruses is considered to be numerous species of flocks of migratory waterfowl that fly to Gizil-Agaj State Reserve in April-June to reproduce. Mosquitoes that feed on their blood migrate into residential areas, spreading arboviruses among domestic animals, birds, and humans.

6. It was determined that favourable climatic conditions for the preimaginal and imago stages of mosquitoes, the presence of various biotopes and food sources created a tense epizootological-epidemiological situation, and ensured intensive circulation of 8 arboviruses: West Nile virus (WNV), Sindbis, Tahyna (pre-existing species), Chikungunya, Uukuniemi, Batai, Bhanja, Dhori (new species).

7. For the first time, development biotopes of mosquitoes were defined and classified in Lankaran and Masally districts which are part of the Lankaran-Astara economic region, and Gizil-agaj State Reserve. 14 types of biotopes where the larvae actively feed were identified, and paddy fields predominated.

8. Filling unusable water sources, old paddy fields, ponds and septic tanks in backyards with soil or draining, and covering wells, water tanks, and pools with polyethylene cover was considered effective in preventing the development of mosquito larvae. Also, covering windows, doors, and gaps in farms and all types of buildings with small-hole nets prevented mosquitoes sucking blood.

9. For the first time, a combination of tested and developed anti-

mosquito measures in dwellings was found to be effective in controlling arboviruses in domestic animals, birds and humans.

10. Questionnaires and cluster systems used for the first time in the implementation of arbovirus prevention and control measures were determined to have a high role in increasing people's motivation.

Theoretical and practical significance of the research. The data obtained as a result of the research can help medical and veterinary establishments to monitor arboviruses. Our questionnaires can be used to raise awareness about arboviruses. A cluster system designed to increase residents' motivation can be important in the prevention of arboviruses. Preventing the development of larvae in paddy fields, the set of anti-mosquito measures has effectively reduced the number of their preimaginal and imago stages, these recommendations can be used in the control and prevention of arboviruses, in the regulation of agriculture, medicine, veterinary, and sanitary services.

The dissertation approbation. The main provisions and results of the dissertation work were interpreted, discussed and approved at international and republican-level scientific-practical conferences and scientific seminars. Including:

- Materials of the Republican Scientific Conference "Modern Problems of Biology" (Sumgait, 2018);

- 1st international conference on "One Health: problems and their solutions" (Baku, 2018)

- 16th Medical Biodefense Conference (Germany, Munich, 2018)

- Second International Conference of European Academy of Science (Bonn, Germany, 2018)

- American Society for Microbiology (ASM) Microbe conference (Atlanta, USA, 2018)

- 2nd international conference on "One Health: problems and their solutions" (Baku, 2019)

- 4th Annual BTRP Ukraine Regional One Health Research Symposium (ROHRS) (Kyiv, 2019)

18 scientific works reflecting the content of the research, 7 of them were published in periodical scientific publications where the main results of dissertations were recommended to be published in the Republic of Azerbaijan, and 3 articles, 7 theses were published abroad,

as well as 1 methodical instruction was published.

The name of the organization where the dissertation work was performed. The dissertation work was performed at the Department of Parasitic and Tropical Diseases of the Scientific Research Institute of Medical Prophylaxis Public legal entity named after V.Y.Akhundov of the Ministry of Health of the Republic of Azerbaijan.

The structure and volume of the dissertation. The dissertation includes an introduction (9938 characters), five chapters (I chapter – 43004 characters, II chapter – 10200 characters, III chapter – 36436 characters, IV chapter – 32854 characters, V – 42567 characters), a discussion of the obtained results (21247 characters), results (3489 characters), practical suggestions (1476 characters), a list of 231 references, appendices, and abbreviations. The dissertation contains 19 tables, 3 figures, and 13 graphs. The total volume of the dissertation is 166 pages and 201211 characters.

CHAPTER I.

FAUNA OF BLOOD-SUCKING MOSQUITOES IN MODERN CONDITIONS, TRANSMISSION OF ARBOVIRUSES, THEIR ROLE IN THE SPREAD OF OTHER INFECTIONS AMONG HUMANS AND DOMESTIC ANIMALS

Data from numerous studies in this chapter show that blood-sucking mosquitoes pose a serious nuisance to humans and domestic animals during sucking blood and secreting salivary. Mosquitoes are considered effective carriers of many transmissible diseases. According to the WHO, about 750,000 people die from diseases spread by mosquitoes every year, and the real number of patients is in the tens of millions⁷. The share of arbovirus infections in these statistics is not small, and it has also been met with great interest by medical specialists around the world. Therefore, the Zika fever outbreaks in Brazil and other countries in the Pan-American region may be a clear example of this. They were classified as a public health emergency by the

⁷ Dikid, T.S. Emerging and re-emerging arboviral diseases in India: an overview / T.S. Dikid, K. Jain, A.Sharma [et al.] // Indian J.Med.Res., – 2013, 138, – p. 19-31.

WHO⁸. The chapter also provides information on the study of species composition, ecology, parasitological, epizootological, epidemiological, and virological characteristics of mosquitoes in Azerbaijan and other countries.

CHAPTER II. MATERIAL AND METHODS OF THE RESEARCH

During 2016-2022, research works were carried out in the Lankaran natural province located in the humid subtropical zone in the south-eastern part of Azerbaijan, especially in five selected settlements of Lankaran (Shirinsu, Urga, Goyshaban, Ashagi Nuvadi and Kanarmesha) and Masally districts (Tazakand, Mahmudavar, Arkivan, Miyanku and Eminli) and in the Gizil-Agaj State Reserve.

In the first group of studies, work devoted to the modern ecological-faunistic assessment of the population of blood-sucking mosquitoes in the humid subtropical zone, the species composition of the mosquito fauna, and its dynamics was studied using entomological methods.

The preimaginal stages of mosquitoes were recorded in different types of control water sources with the help of 20x25 cm photo cuvettes and by counting samples in 1 m² of water surface every 4 days during the entire seasonal activity period. A stereoscopic microscope (Ken-A-Vision T-26011C Vision Scope 2 model, USA) and determinants were used to determine the species affiliation of the captured insects (imago and preimaginal).

During the research, 5422 adult mosquitoes were caught using BG-sentinel (Biogents AG, Regensburg, Germany) and CDC light traps (Centres for Control Disease miniature light trap, USA) and species affiliation was determined⁹. 896 mosquitoes were caught from Gizil-Agaj State Reserve, 2312 mosquitoes from the Lankaran district,

⁸ Costa-da-Silva, A.L. Laboratory strains of *Aedes aegypti* are competent to Brazilian Zika virus / A.L.Costa-da-Silva, R.S.Ioshino, H.R. Correa de Araujo // PLoS One, – 2017. 12 (3), e 0174081

⁹ Штакельберг, А.А. Семейство Culicidae Фауна СССР. Насекомые двукрылые / А.А.Штакельберг. – Ленинград: Изд. АН СССР, –1937. Т.3 (4), – с. 257

and 2214 mosquitoes belonging to 7 genera (Anopheles - 1421, Aedes - 1477, Culex - 2421, and other genera - 103) from selected villages of Masally district.

In order to detect the dominant species of mosquitoes according to the temperature regime, monthly, average daily temperature was calculated based on the data of the Lankaran zonal meteorological station.

14 types of biotopes where the development of mosquito larvae took place were found, of which type 1-characterized by reserve, abundant aquatic plants, and 227 larvae were collected. Type 2 – same, but 213 larvae were collected from biotope without plants, type 3- 384 larvae were collected from the reserve, seawater, and abundant aquatic plants, type 4 - same as 3, but 233 larvae were collected from biotope without plants, type 5 – 867 larvae from wells (water sources for irrigation of paddy fields), type 6 – 1327 larvae from biotope with paddy fields, type 7- 844 larvae from open water tanks, type 8- 256 larvae from ditches in dwellings, type 9- 442 larvae from trenches, type 10- 263 larvae from puddles form after rain, type 11- 281 larvae from ponds near little rivers and springs, type 12- 260 larvae from ponds, pools in the yard, type 13- 324 larvae from open septic tanks, and type 14- 888 larvae from swampy areas in the roadside, a total of 6807 larvae were collected. The number of larvae was calculated per 1 m² of the studied area.

In the second group of studies, the assessment of the tension of the epizootological situation, and the regularities of the circulation of arboviruses among blood-sucking mosquitoes, domestic animals, and birds was carried out by epizootological methods¹⁰. To study mosquito infection, they were divided into four groups depending on their number: >1000 pools (30%), 400–900 pools (8–16%), 50–300 pools (1–6%), <150 pools (<1%). Such division is important for saving serological diagnostic test systems - for this purpose, a suspension of mosquitoes was prepared. Selected first group suspension included >12 mosquito pools, second group suspension included 8-11 mosquito pools, third group

¹⁰ Балашов, Ю.С. Паразито-хозяйинные отношения членистоногих с наземными позвоночными / Ю.С.Балашов. –Ленинград: Наука, – т. 97. – 1982. – 318 с.

suspension included 4-seven mosquito pools, 4th group suspension included ≤ 3 mosquito pools. The suspension was crushed and made into a homogeneous solution using the Mini-BeedBeater-16 homogenizer device.

All suspensions included mosquito species that were the most dominant and effective vectors of arboviruses. Serological diagnostics were obtained commercially by "Bioservice" BTK (made in Russia).

The pools were tested by the IFA method using a Microplate washer REF 5165000 and a Spectrophotometer (Thermo) according to the generally accepted scheme, and manufacturer's instructions. A total of 357 pools were serologically tested to identify 11 types of arboviruses. 48 pools for West Nile virus, 47 pools for California serogroup (CSG), 28 pools for Sindbis, 32 pools for Zika, three pools for Chikungunya, 59 pools for Uukuniemi, 29 pools for Tahyna, 31 pools for Batai, 51 pools for Bhanja, 3 pools for Dengue, and 26 pools for Dhori were examined. That is, local mosquito species were infected with 7 types of arboviruses. 31 pools of mosquitoes in Gizil-Agaj State Reserve, 182 pools in villages of the Lankaran district, and 144 pools in villages of the Masally district were prepared. In order to determine the prevalence of mosquito seropositivity, the amount of examinations were calculated depending on the distance from the reserve. As mentioned, 31 pools were studied in the reserve, 23 pools at a distance of < 250 m, 22 pools at a distance of 250-499 m, 87 pools at a distance of 500-749 m, 83 pools at a distance of 750-999 m, 85 pools at a distance of 1000-1249 m, 26 pools at a distance of ≥ 1250 m were tested.

In total, 326 mosquito pools were studied through serological test systems in ten biotopes (used by mosquitoes as daily places). Thus, 44 pools from dwellings, 31 pools from agricultural facilities, 30 pools from outdoor sanitary facilities, 34 pools from basements, 34 from places where cattle are kept, 30 pools from chicken coops, 27 pools from tea houses, 27 pools from cafes, 29 pools from medical facilities, 40 pools from meat and dairy farms were examined.

In order to assess the role of domestic animals (cattle), its character was first determined together with local veterinary staff in individual farms. In twelve villages, 28-42 individual farms (376 farms in total) were studied spontaneously. The samples for serological tests were taken during the slaughter of the cattle after prior agreement with

their owners, and for this purpose, the test systems used during the examination of the mosquito pool were used. The level of awareness of cattle care and treatment was assessed.

The role of poultry in the circulation of arboviruses was observed among three groups of chickens, and samples of collected blood serum were studied.

The third group of studies is devoted to the study of epidemiological regularities of the circulation of arboviruses among humans¹¹. The only source of human infection with arboviruses is considered to be bloodsucking by infected mosquitoes. Taking into account the great epidemiological importance of the data on their fauna, ecology and infection, we carried out a seroepidemiological study of arboviruses. It should be noted that the studied region is considered highly endemic for malaria, as earlier epidemics of these parasitic infections were observed here. After the survey, doctors, nurses, and paramedics collected blood samples for serological tests. Residents were presented with a health assessment scheme. It consisted of seven items and the importance of each item was rated from one to seven points. The average index (norm) of the points listed above was calculated.

In the fourth group of studies, a cluster system was developed to assess the level of awareness of residents on the prevention of arboviruses. This system consisted of five clusters, each of which was rated from one to five points according to the level of importance. The approbation of the system was carried out in a private farm where cattle were kept, awareness was evaluated at three levels: general-medical, infection and arbovirus. Public awareness was considered adequate when the average score was above four points.

A questionnaire with questions on the most important elements of arbovirus control and prevention was developed, and after 9-12 months, awareness was assessed among 685 residents out of 833 initially registered residents. According to the percentile table, the answer to any four out of

¹¹ Домацкий, В.Н. Эпизоотологическое и эпидемиологическое значение кровососущих двукрылых насекомых в условиях Крайнего Севера (обзор) / В.Н.Домацкий, О.А.Фёдорова, А.Н.Сибен // Российский паразитологический журнал, – 2018. Т.12. №4. – с.73-76.

twenty questions received one point, the answer to eight questions received two points, twelve questions received three points, sixteen questions received four points, and seventeen questions received more than five points.

Carrying out such complete information-explanatory work is important for increasing the residents' activity and the implementation of recommended preventive measures. A cluster system was developed to assess the motivation level of residents.

A comprehensive action plan for the prevention and control of arboviruses spread among humans and domestic animals was developed. Windows, doors and gaps were covered with netting. Preimaginal and imago stages of mosquitoes were recorded at the beginning and end of the study.

Detailed statistical processing of the obtained data was carried out¹², and the methods of calculating the average mathematical grade, Student's criterion, correlation coefficient, χ^2 criterion, and Van-der-Waarden criterion were used. The mentioned calculations were performed using SPSS version 10.0 for Windows (SPSS, Chicago, Illinois, USA). A P value less than 0.05 was considered statistically reliable.

CHAPTER III.

ECOLOGICAL-FAUNISTIC ASSESSMENT OF THE MODERN POPULATION OF BLOOD-SUCKING MOSQUITOES IN THE HUMID SUBTROPICAL ZONE

According to the research conducted, 26 species belonging to 7 genera were found in the fauna of mosquitoes in the south-east of Azerbaijan. (Table 1).

The dominant mosquito species in terms of number and distribution intensity were *An. sacharovi* (14.9±0.5%), *Ae. vexans* (15.7±0.5%) and *Cx. pipiens* (29.8±0.6%). The frequency of detection of other species ranged between 0.2±0.06 and 9.2±0.4%. In the Gizil-Agaj State Reserve, the detection of mosquitoes was also high and reached 63.6±15.2% in some species (*Cs. annulata*).

¹² Герасимов, А.Н. Медицинская статистика / А.Н.Герасимов. – Москва Медицинское информационное агентство, – 2007. –480 с.

Table 1

Comparison of the general indicators of mosquito fauna in the southern region with the indicators of previous studies (n=5422 ind.)

Detected adult mosquitoes			Our results (%)	
Anopheles	1	<i>An. maculipennis</i> Meis	302	5.6±0.3
	2	<i>An. sacharovi</i> Favr	806	14.9±0.5
	3	<i>An. subalpinus</i> Grass	16	0.3±0.07
	4	<i>An. superpictus</i> Grass	29	0.5±0.1
	5	<i>An. hyrcanus</i> Pall	81	1.5±0.2
	6	<i>An. plumbeus</i> Stef	-	-
	7	<i>An. claviger</i> Linn	187	3.5±0.2
Aedes	8	<i>Ae. caspius</i> Pall	497	9.2±0.4
	9	<i>Ae. vexans</i> Mg.	852	15.7±0.5
	10	<i>Ae. geniculatus</i> Ol.	11	0.2±0.06
	11	<i>Ae. pulchritarsis</i> Ron.	-	-
	12	<i>Ae. cataphylla</i> Duc.	35	0.6±0.1
	13	<i>Ae. dorsalis</i> Mg.	46	0.8±0.1
	14	<i>Ae. versicolor</i> Dob.	-	-
	15	<i>Ae. albopictus</i> S.	16	0.3±0.07
	16	<i>Ae. aegypti</i> L.	20	0.4±0.09
Culex	17	<i>Cx. modestus</i> F.	35	0.6±0.1
	18	<i>Cx. mimeticus</i> N.	480	8.9±0.4
	19	<i>Cx. pipiens</i> Hin.	1618	29.8±0.6
	20	<i>Cx. theileri</i> Th.	150	2.8±0.2
	21	<i>Cx. tritaeniorhynchus</i>	25	0.5±0.1
	22	G.	57	1.1±0.1
	23	<i>Cx. hortensis</i> F.	46	0.9±0.1
	24	<i>Cx. territans</i> W. <i>Cx. apicalis</i> A.	10	0.2±0.06
Culiseta	25	<i>Cs. annulata</i> S.	11	0.2±0.06
	26	<i>Cs. longiareolata</i> Macq.	23	0.4±0.09
	27	<i>Cs. morsitans</i> T.	-	-
	28	<i>Cs. fumipennis</i> S.	-	-
29	<i>Uranotaenia. unguiculata</i> Ed.	24	0.4±0.09	
30	<i>Mansonia richiardii</i> F.	26	0.5±0.1	
31	<i>Orthopodomyia pulchripalpis</i> R.	19	0.4±0.1	

The altitude of the area also significantly affects the ecology of mosquitoes. For the first time, the ecological-faunistic characteristics

of the preimaginal stages of mosquitoes were studied. Fourteen biotopes (Table 2) were identified mainly for the development of larvae, four of them were located in the reserve.

Table 2
Biotopes where mosquito larvae develop and their characteristics

Types of biotopes and their characteristics	Number of mosquitoes (%)		Number of larvae (%)	
1st type: Reserve. Seawater. Rich vegetation.	44	7.9±1.1	227	3.3±0.2
2nd type: The same area, but without plants.	39	7.0±1.1	213	3.1±0.2
3rd type: Reserve. Rich vegetation.	38	6.8±1.1	384	4.8±0.3
4th type: The same area, but without plants.	43	7.7±1.1	233	3.4±0.2
5th type: Water resources for rice irrigation (ditch)	28	5.0±0.9	867	12.7±0.4
6th type: Paddy fields	47	8.4±1.2	1325	19.5±0.5
7th type: Open water tanks	33	5.9±1.0	844	12.4±0.4
8th type: Ditches in residential areas	48	8.6±1.2	256	3.8±0.2
9th type: Trenches	49	8.8±1.2	442	6.5±0.3
10th type: Puddles form after rain	36	6.5±1.1	263	3.9±0.2
11th type: Ponds near rivers and springs	35	6.3±1.0	281	4.1±0.3
12th type: Ponds, pools in the yard	29	5.2±0.9	260	3.8±0.2
13th type: Open septic tanks	42	7.5±1.1	324	4.8±0.3
14th type: Roadside swamps (permanent)	47	8.4±1.4	888	13.0±0.4
Total	558	100.0±0.0	6807	100.0±0.0

The population of mosquito larvae in biotopes ranged between 5.2±0.9 and 8.8±1.2% ($t=2.40$; $p<0.01$). According to the intensity indicators, the following biotopes: wells, paddy fields, open tanks in the yard for drinking water, and non-draining roadside swamps were more populated by larvae (from 12.4±0.4 to 19.5±0.5%). Mosquitoes larvae

and imagos (adults) of the same type were found in biotopes. The population density of larvae in biotopes accounted on average 2.11 ± 0.25 per 1 m^2 of water surface. The density of *An. sacharovi*, *Ae. vexans* and *Cx. pipiens* larvae ranged from 2.28 ± 0.81 to 3.25 ± 0.83 larvae/ m^2 ($t=0.84$; $p>0.05$). Determining the species affiliation of $0.2 \pm 0.05\%$ of captured larvae was impossible.

Cx. pipiens was abundant in all biotopes and sometimes reached $76.7 \pm 2.9\%$, the intensity of mosquito detection did not adapt to this area in any way and ranged between 5.4 ± 1.0 and 34.7 ± 3.5 larvae/ m^2 ($t=8.05$; $p<0.001$).

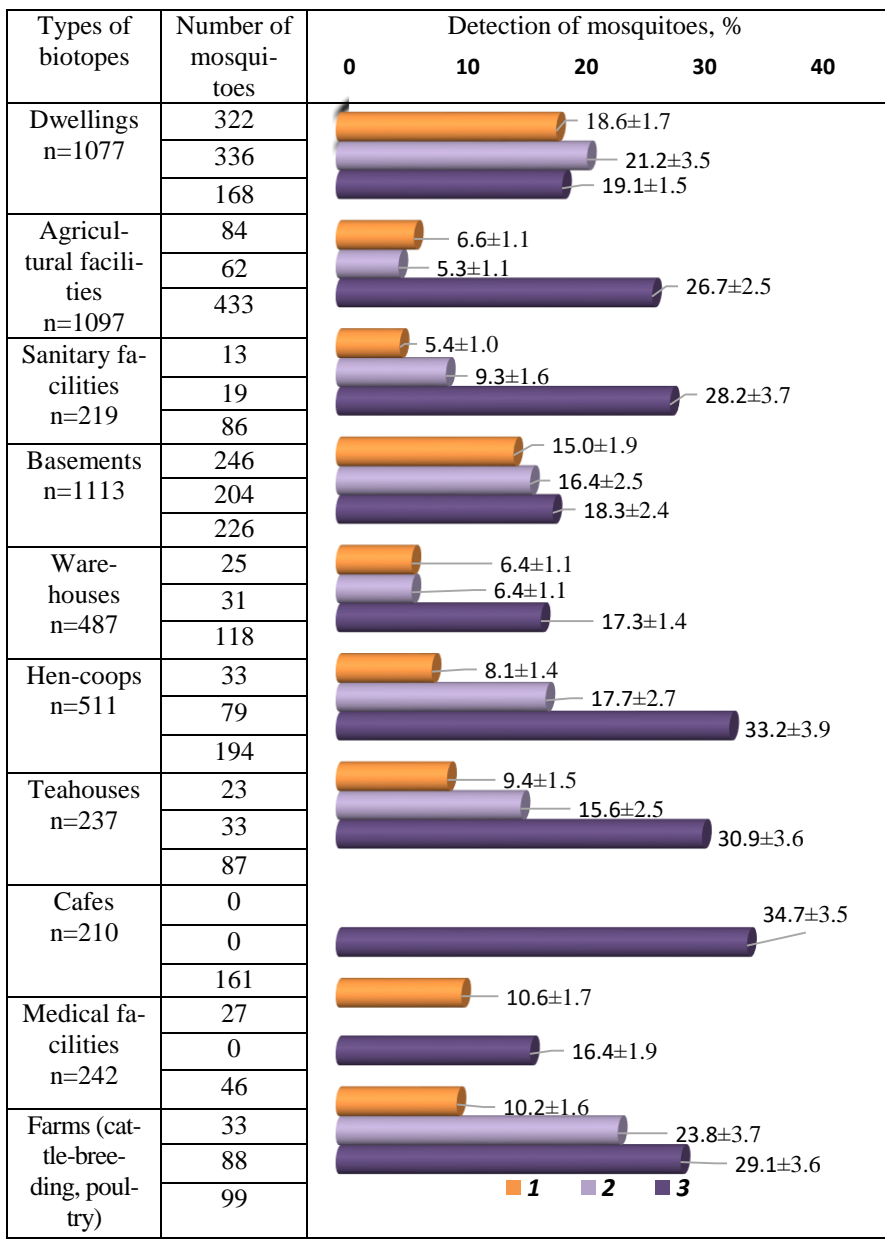
The species affiliation of 6,807 mosquito larvae captured from 14 biotopes was studied. A total of 558 mosquitoes were recorded, including ditches for rice irrigation (28), paddy fields (47), and open water tanks in yards (33). It was determined that roadside swamps (47) were more populated by mosquitoes.

To study the adaptation of blood-sucking mosquitoes in the imago stage to different types of biotopes, ten biotopes were identified (Graph 1).

The most common biotopes of captured mosquitoes were: dwellings (1077 mosquitoes), agricultural facilities (1097) and basements (1113). Based on the three dominant mosquito species, it was determined that the blood-sucking *An. saccharovi* mosquito species were found in biotopes consisting of human habitats - dwellings and basements, where their population level was 29.9 ± 1.4 and $22.1 \pm 1.2\%$, respectively ($t=4.24$; $P<0.001$), daily- intensity was also high and accounted for 18.6 ± 1.7 and 15.0 ± 1.9 ind./ m^2 ($t=1.02$; $P>0.05$).

Ae. vexans mosquitoes also prefer these biotopes, accounting for 31.2 ± 1.5 and $18.3 \pm 1.2\%$ ($t=6.72$; $P<0.001$), but their frequency of detection was high in farms and reached $38.4 \pm 3.2\%$ ($t=2.11$; $P<0.05$), mosquito intensity ranged between 16.4 ± 2.5 and 23.8 ± 3.7 ind./ m^2 ($t=2, 10$; $P<0.05$).

Cx. pipiens mosquitoes use all biotopes daily with high intensity- it ranges from 23.3 ± 1.3 to 69.8 ± 3.0 ind./ m^2 ($t=14.22$; $P<0.001$), intensity ranges from 16.4 ± 1.9 to 33.2 ± 3.9 ind./ m^2 ($t=3.87$; $P<0.001$). Based on the obtained results, it is possible to organize and implement mosquito control measures in a more rational way.



Graph 1. Population intensity of dominant mosquito species in different types of biotopes (daily) 1. *An. sacharovi* (n=801); 2. *Ae. vexans* (n=852); 3. *Cx. pipiens* (n=1618)

CHAPTER IV. ASSESSMENT OF THE TENSE OF THE EPIZOOTOLOGICAL SITUATION AND CIRCULATION OF ARBOVIRUSES AMONG BLOOD-SUCKING MOSQUITOES, DOMESTIC ANIMALS, AND BIRDS

The chapter presents the results of serological tests conducted among mosquitoes, domestic animals, and birds. Commercial test systems (IFA) were used, and 357 mosquito pools, and blood samples of 324 calves, 321 sheep, 300 chickens, and 61 chicks were analyzed by a serological test. According to a prior agreement with the animal owners, veterinary staff took blood samples during the slaughter of domestic animals and birds.

The infection of the local population of mosquitoes, primarily *Cx. pipiens* and *Ae. vexans*, with different types of arboviruses, was quite high and averaged $10.6 \pm 1.6\%$. Considering that *Cx. pipiens* and *Ae. vexans* are widespread in most other regions of the republic, then we should expect arboviruses to spread beyond the limits of humid and subtropical zones.

A positive response was obtained confirming the presence of seven types of arboviruses in the results of the serological test of mosquito pools. Three of them- West Nile virus, Sindbis, and Tahyna viruses were previously identified, indicating that they circulated in mosquitoes for a long time. The previously detected Gizil-Agaj virus was not detected due to the lack of appropriate test systems. Four more new arboviruses were observed for local conditions. We have already mentioned the Tahyna virus which belongs to CSG, while Batai and Bhanja viruses are considered new. The Uukuniemi virus and the entire group of CSG were also detected.

Out of 5422 mosquitoes, 896 ($16.5 \pm 0.5\%$) were captured from the reserve, while their number in the dwellings was 2312 ($42.6 \pm 0.7\%$). In the dwellings of the Masally district, there were 2214 mosquitoes ($40.8 \pm 0.7\%$) or 2.47-2.58 times more mosquitoes compared to the reserve. Despite the small number of captured mosquitoes, their infection with arboviruses in the reserve was high, accounting for $19.4 \pm 7.2\%$. However, in Lankaran and Masally districts, mosquito infection was low and it was $10.4 \pm 2.7\%$ and $9.0 \pm 2.3\%$, respectively. In this case, the noticeable difference was not reliable due to the small number of infected

mosquitoes ($\chi^2=2.03$; $P>0.05$).

This situation suggests that the main sources of arboviruses in the reserve are waterfowl, birds in the water areas (both permanent residents of the reserve and those that fly here for nesting), as well as large flocks of migratory birds. Ten biotopes (dwellings and agricultural facilities, basements, places where animals and birds are kept, farms, public facilities, etc.) where mosquitoes live daily in villages were identified, and infected mosquitoes from 7.4 ± 5.1 to $17.6\pm 6.6\%$ were detected in all of them.

Based on the survey of 376 owners of cows (calves) and sheep, different disease levels were observed in animals, 38.8 ± 2.5 and $23.7\pm 2.2\%$, respectively ($t=4.53$; $P<0.001$). Their owners noted that the animals had lost their appetite, and lethargy, diarrhea, temperature, bellow, etc. were observed. Antibiotics, antidiarrheal and antiparasitic drugs were prescribed to the animals. Animals were treated, or the disease progressed slowly. Animal mortality among cows, especially baby animals, was observed in 24 cows ($16.4\pm 3.1\%$) and 6 sheep ($6.7\pm 2.3\%$; $\chi^2=4.67$; $p<0.05$). It is quite clear that the cause of the disease in cattle was an arbovirus. When the disease occurs, the owners rarely use the help of veterinarians and treat the animals by themselves.

Therefore, the level of awareness of people about the sanitary-veterinary norms for keeping cattle, the treatment, and the prevention of disease was studied. Thus, the satisfactory level was $20.6\pm 2.2\%$ in hosts and $15.0\pm 2.2\%$ in housewives ($t=1.80$; $p>0.05$), and the sufficient level was 7.8 ± 1.5 and $6.6\pm 1.5\%$, respectively ($t=0.57$; $p>0.05$).

The conducted examinations showed that poultry (chickens, chicks) also play an important role as food sources for mosquitoes, which are reservoirs for arboviruses, and in the recirculation of infection. Their seropositivity averaged $5.0\pm 1.1\%$, and the same eight types of arboviruses were detected, the frequency of which varied from 2.6 to 12.1%. Seropositivity was higher in selected chicks accounting for $11.5\pm 4.1\%$, and it was $7.4\pm 3.6\%$ ($\chi^2=0.55$; $p>0.05$) in households chickens, and it was less than all of them in chickens in poultry factories (farms) accounting for $2.8\pm 1.1\%$ ($\chi^2=2.84$; $p>0.05$). It should be noted that the sanitary-veterinary norms were followed in most of

these farms, facilities were disinfected, windows were covered with nets, etc.

CHAPTER V. EPIZOOTOLOGICAL-EPIDEMIOLOGICAL BASIS OF PREVENTION AND CONTROL OF ARBOVIRUSES SPREAD AMONG HUMANS AND DOMESTIC ANIMALS

In this chapter, based on the obtained data, a complex of measures against arbovirus was developed and its trial approval was carried out at the population level. In addition to epizootological examinations, their epidemiological characteristics were also studied. Arboviruses were detected in 97 out of 633 residents ($15.3\pm 1.4\%$) using a serological test. $20.4\pm 2.3\%$ of cases were recorded in residents with fever but did not seek medical care, and $8.9\pm 1.7\%$ of cases were recorded in residents without fever ($\chi^2=15.83$; $p<0.01$). Among the residents, mostly Sindbis virus was found at $30.5\pm 4.5\%$, followed by the Tahyna at $26.2\pm 4.4\%$ ($\chi^2=0.27$; $p>0.05$), Uukuniemi, West Nile virus, Batai, and Bhanja were not higher than $10.4\pm 2.9\%$ ($\chi^2=9.21$; $p<0.01$). According to the rules of extrapolation, the most common symptoms in seropositive animals were a temperature of $38-40^\circ\text{C}$, fever for 5-7 days, chills, muscle pains, headache, sleep disturbance, and conjunctivitis, their frequency ranged between $68.6\pm 1.8\%$ and $80.4\pm 1.6\%$ ($t=4.90$; $p<0.001$). 13 more symptoms were found, the frequency of which was not higher than $55.6\pm 2.0\%$ ($t=4.81$; $p<0.001$). Fixation of symptoms and antiviral treatment of infected persons were carried out by local doctors. The self-assessment of residents infected with arboviruses was 3.04 ± 1.7 points on average when the norm was seven points.

To increase the awareness of the residents, we prepared a questionnaire consisting of twenty questions, which allowed us to increase their awareness to the required level. Moreover, a cluster system was developed for a reliable assessment of their level of awareness. This system was made up of five clusters and each of them was given 1-5 points depending on its importance (the norm is >4 points on average). At the beginning of the work, general medical and veterinary awareness was 3.16 ± 0.5 points on average, awareness about infectious dis-

eases was 2.56 ± 0.4 points ($t=0.93$; $p>0.05$), awareness about arboviruses was 1.62 ± 0.3 points ($t=1.88$; $p>0.05$). As a result of the training-explanatory work conducted among the residents for 9-12 months, the level of awareness among them increased to 4.87 ± 0.9 points ($t=1.66$; $p>0.05$), 4.71 ± 0.7 points ($t=2.65$; $p<0.01$), 4.66 ± 0.8 points ($t=3.58$; $p<0.001$), respectively.

A cluster system was also developed to assess the level of informativeness of residents. It was composed of six clusters, each of which was evaluated with 1-6 points depending on its importance (norm is >4 points on average). As a result of the work carried out during 9-12 months, the residents' motivation increased from 2.55 ± 0.7 to 4.82 ± 0.6 points ($t=2.47$; $p<0.05$).

To protect people and animals from mosquito attacks, the doors, windows, and gaps of various buildings were covered with nets. After 9-12 months, the number of mosquitoes in dwellings decreased from 33.6 ± 2.0 to 4.9 ± 0.9 mosquitoes/m² ($t=9.17$; $p<0.001$), in places where cattle are kept decreased from 42.7 ± 3.6 to 4.4 ± 0.8 mosquitoes/m² ($t=10.38$; $p<0.001$), in outbuildings decreased from 45.6 ± 4.1 to 7.9 ± 1.2 mosquitoes/m² ($t=8.83$; $p<0.001$).

Thus, the complex of epizootological-epidemiological measures for controlling and preventing arboviruses spread among humans and animals was effective and realistic in practical experience. They are accessible and do not require additional logistical and personnel resources.

Additionally, three groups of research data are presented in this chapter. After the explanatory work, blood samples were taken for serological tests by field doctors, nurses, and paramedics in the first group. 633 blood samples (serum) taken from residents were examined. Clinical signs of infections were recorded, and 21 specific symptoms of arbovirus infection were revealed. Residents were presented with a scheme consisting of seven sections for assessing their health, each of which was evaluated from one point to seven points.

In the second group, a five-point cluster system was developed to assess the level of awareness of residents on the issues of prevention of arboviruses, and a six-point cluster system was developed to assess their motivation in the implementation of preventive measures. 833

residents observed in the study were distributed leaflets reflecting the main preventive measures for the disease. The evaluation of the effectiveness of cluster systems was conducted among 685 residents after 4-6 months.

In the third group, the level of application of the integrated complex developed for the control and prevention of arboviruses spread among humans and domestic animals was evaluated. The following measures were taken against mosquitoes in the research areas: 41 out of 76 unusable backyard water sources were filled with soil and drained, and this process was partially carried out in another 29 water sources; 66 out of 108 old rice fields were drained (28 of which partially); 127 puddles in 176 yards were drained (49 partially); 47 out of 85 wells (12 partially) and 283 out of 376 water tanks and pools (193 partially) were covered with polyethylene cover; 113 out of 211 septic tanks (67 partially) were neutralized with chlorine. Windows, doors, and gaps were covered with nets in 223 out of 376 dwellings (117 partially); in 161 out of 376 places where cattle are kept (152 partially); and in 109 out of 324 outbuildings (138 partially). Preimaginal and imago stages of mosquitoes were recorded at the beginning and end of the work in the above-mentioned facilities.

DISCUSSION OF THE OBTAINED RESULTS

The discussion of the obtained results provides information on the fauna, ecology, reproduction biotopes of blood-sucking mosquitoes in Lankaran, Masally districts, and Gizil-Agaj State Reserve located in the south-east of Azerbaijan, as well as their daily places, their infection with arboviruses, comparative analysis based on the results aimed at studying the parasitological, ecological, as well as epizootological-epidemiological regularities of the circulation of these infections among humans, domestic animals and birds.

RESULTS

1. Anthropogenic factors and the intensification of transport have led to changes in the mosquito fauna, a total of 26 species of mosquitoes were found in the studied areas, of which three species (*Ae. albopictus*, *Ae. aegypti*, *Cx. apicalis*) were found for the first time.

An. sacharovi, *Ae. vexans* and *Cx. pipiens* were dominant species, and their final specific weight in the fauna was $59.5 \pm 0.7\%$. The detection of mosquitoes was $16.5 \pm 0.5\%$ in the Gizil-Agaj State Reserve, $42.6 \pm 0.7\%$ and $40.8 \pm 0.7\%$, respectively in the settlements of Lankaran and Masally districts [1, 3, 4, 7, 15, 17, 18].

2. Seasonal activity of mosquitoes, depending on temperature, is observed from April to the first half of November, and the highest level ($20.6 \pm 1.4\%$) occurs in June - the first half of July and October. As the increase of the height of the area above the sea level by more than 1250 m, the number of *Cx.pipiens* mosquitoes decreased by $9.1 \pm 1.0\%$, *Ae. vexans* decreased by $5.8 \pm 1.2\%$, *An. sacharovi* was no longer detected [16].
3. 14 biotopes suitable for the development of the preimaginal stages of mosquitoes were found, four of which were in the Gizil-Agaj State Reserve. In biotopes at an altitude of 1250 m above sea level, the number of larvae decreased by 1.63-5.20 times, and it was determined that the number of larvae was not significantly affected by the seasons [5,16].
4. Ten biotopes were found in the settlements used by mosquitoes as their daily habitats. Dwellings, agricultural facilities, basements, and farms were more densely populated by mosquitoes, where the number of mosquitoes was 13.6 ± 1.8 mosquitoes/m² [18].
5. Using serological tests, pools of *Cx. pipiens*, *Ae. vexans*, *Cx. mimeticus*, *Ae. albopictus*, *Ae. aegypti*, *An. maculipennis*, *An.sacharovi*, *Ae. caspius*, *Cx.modestus* species were examined. $10.6 \pm 1.6\%$ of pools were seropositive, of which $19.4 \pm 7.2\%$ was in Gizil-Agaj State Reserve, $10.4 \pm 2.7\%$ was in Lankaran, and $9.0 \pm 2.3\%$ was in Masally districts. Infected mosquitoes were found in all biotopes from 7.4 ± 5.1 to $17.6 \pm 6.6\%$. Mosquitoes were found to be infected with arbovirus species- Sindbis, West Nile virus, Tahyna (pre-existing), CSG, Uukuniemi, Batai, and Bhanja (new species) [15,16].
6. The main source of food for mosquitoes in the reserve was nestlings without feathers (April-June). After the nestlings leave their nests, mosquitoes migrate to villages and attack cattle as a source of food. The seropositivity of cows and calves was the same and averaged $17.9 \pm 2.0\%$, and that of sheep was $3.7 \pm 1.6\%$. Among them, a new

8th type of arbovirus - Dhori was detected. Infection of chickens averaged $5.0 \pm 1.1\%$. [6,8].

7. Six types of arboviruses were found among residents: Sindbis, West Nile virus, Tahyna, Batai, Uukuniemi, and Bhanja. Seropositivity averaged $15.3 \pm 1.4\%$, reaching $20.4 \pm 2.1\%$ among those with fever. Most infections occurred in May-June and September-October when mosquitoes were most active [9, 10].
8. Evaluation of their health by the infected people was 3.04 ± 1.7 points (norm - seven points) [11].
9. The information-explanation work carried out for 3-12 months on the implementation of anti-arbovirus recommendations allowed the residents' motivation to increase from 2.55 ± 0.7 points to 4.82 ± 0.6 points (norm >4 points) [12].
10. As a result of the recommendations, from $53.6 \pm 3.4\%$ to $75.3 \pm 2.2\%$ of suitable sources for the development of mosquito larvae were drained or filled with soil [13, 18].
11. It was found that, once a week, dewatering water sources where mosquito larvae developed led to a reduction of larvae from 37.3 ± 3.1 to 7.3 ± 1.1 larvae/m² [14, 18].

PRACTICAL SUGGESTIONS

1. Establishment of regional small medical and veterinary laboratories (doctor, laboratory assistant) to monitor arboviruses, timely diagnosis and treatment of infected persons.

2. Carrying out information work on increasing the motivation of the people in the implementation of preventive measures with the help of prepared questionnaires and cluster systems.

3. The following mosquito control measures are recommended to reduce the conditions for mosquito reproduction and their attacks on humans, animals, and birds:

3.1. Filling water sources unsuitable for agriculture, ditches, backyard ponds, and old paddy fields with soil, or draining, covering wells, water tanks, and pools with polythene cover, disinfecting septic tanks with chlorine;

3.2. Covering doors, windows, and gaps in dwellings, places where cattle and birds are kept with nets;

3.3. Destructing all unnecessary buildings used as daily places by mosquitoes. Adhering to veterinary and sanitary rules in the territories of individual farms, dewatering paddy fields once a week to prevent the development of mosquito larvae;

3.4. Strengthening the regular control of yards by medical and veterinary workers for timely detection of infected people and domestic animals;

3.5. Organizing and carrying out measures to combat and prevent arboviruses spread among people and domestic animals with the coordinated participation of municipal authorities, medical, veterinary, and sanitary-epidemiological services.

The list of the publications on the topic of the dissertation

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Address: Str.A.Abbaszadeh, 1128th side street, 504th block, AZ 1004, Baku.

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