VECTOR-BORNE DISEASES IN THE REPUBLIC OF MOLDOVA. UPDATES AND PERSPECTIVES

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Abstract. The National Center for Public Health has been monitoring Ixodidae tick species more than 15 years. In this paper, data concerning Ixodidae tick species diversity, their density during the maximum biological activity in spring-autumn period, and the presence of pathogens in these vectors were analyzed. Collections were made during 2005-2009 in accordance with standard methods, targeting selected areas for study. Out of the 22 species of ixodide ticks registered in the country, in the reference period five species were identified: *Ixodes ricinus, Dermacentor marginatus, D. reticulatus, Haemaphysalis punctata*, and *H. inermis.* The species *Ixodes ricinus* is one of the most widespread species of vectors of Lyme borreliosis in the Republic of Moldova. The study of mosquito species diversity was initiated in 2009. Mosquitoes were collected from two areas: the northern (district Glodeni – nature reserve Padurea Domnesca (Royal Forest) (May), Ocnita - the middle part of Nistru River (September) and southern (Cahul district Prut river floodplain (August). Mosquitoes of 5 genera of fam. Culicidae: *Anopheles, Aedes, Culex, Coquillettidia* and *Uranotaenia* with 9 of 28 species recorded in the country also may be involved in the development and transmission of new infectious diseases in our country. Data on the presence of *Uranotaenia* in the Republic of Moldova were not found in other publications.

Keywords: vector-borne, biotope, natural outbreaks, epizootic study.

Rezumat. Vectorii bolilor transmisibile în Republica Moldova. Actualizări şi perspective. Centrul Naţional de Sănătate Publică exercită o activitate de monitorizare a speciilor de căpuşe ixodide mai mult de 15 ani. În prezenta lucrare au fost analizate datele despre diversitatea speciilor de căpuşe ixodide, densitatea lor în perioada activităţii biologice maximale de primăvară-toamnă şi prezenţa agenţilor patogeni la aceşti vectori. Colectările s-au efectuat în perioada 2005-2009 în conformitate cu metodele standard, vizând teritorii selectate pentru studii. Din 22 specii de căpuşe ixodide înregistrate pe teritoriul Republicii Moldova, în perioada de referinţă, au fost identificate 5 specii: *Ixodes ricinus, Dermacentor marginatus, D. reticulatus, Haemaphysalis punctata* şi *H. inermis.* Specia *Ixodes ricinus* este una din cele mai răspândite specii de vectori ai borreliozei Lyme în Republica Moldova. Studiul diversităţii speciilor de ţânţari a fost iniţiat în anul 2009. Colectarea ţânţarilor s-a efectuat în două zone: de nord (raionul Glodeni - rezervaţia „Pădurea Domnească" (luna mai)), Ocniţa - în parte mijlocie a râului Nistru (septembrie) şi sud (raionul Cahul, lunca râului Prut (luna august). Din totalul de 28 specii de tânţari înregistrate în Republica Moldova, 9 specii aparţinând genurilor *Anopheles, Aedes, Culex, Coquillettidia* şi *Uranotaenia* (Fam. Culicidae) pot fi de asemenea implicate în apariţia şi transmiterea maladiilor infecţioase nou introduse în ţară. Date privind prezenţa genului *Uranotaenia* nu au fost găsite în sursele bibliografice din Republica Moldova.

Cuvinte cheie: vector, biotop, focar natural, cercetare epizootică.

INTRODUCTION

Climate change, globalization, intensive migration of population and development of international trade in the last decades can favor the extension of the specific spreading area for the species of mosquitoes and *Ixodes* ticks, involved in the transmission of infectious diseases. This phenomenon represents a potential risk of appearance and spread of emerging diseases in Europe and other regions of the world.

Surveillance of infectious diseases transmitted by vectors in an area includes both monitoring of environmental elements to determine the variety and density of existing species (mosquitoes, *Ixodes* ticks) and of new species and detection of diversity of the causative agents of the infectious diseases to estimate the hazard and assess the risk for Public Health. In recent years, materials have been published on the dissemination in Europe of Chikungunya, Dengue, West Nile fevers - diseases transmitted by mosquito species, which demonstrates the need to maintain vigilance by public health systems. The WHO report in 2007 on Public Health Security in the world shows that the consequences of anthropogenic activity on the environment are one of the hazards to public health (World Health Organization, 2007).

Dengue, Yellow, Chikungunya fevers viruses are transmitted by *Aedes albopictus* and *A. aegypti* (HOCHEDEZ et al., 2006). The European Center for Infectious Disease Control (ECDC, Stockholm) in its report on *A. albopictus* species spreading area and risk mapping concluded that the population of *A. albopictus* has the tendency to spread and can be determined in other regions of Europe (ECDC Technical report, 2009). The species *A. Albopictus* was registered in Albania in 1986 for the first time in Europe, then in Italy (1990-1996), France (2000), Yugoslavia, and Germany (2001). In Italy, in 2009, this species was recorded in 8 of 102 municipalities located primarily on the mainland coast in the north and central regions of the country (ECDC Technical report, 2009; GALUSHKINA & DREMOVA, 2008). France in 1999 approved a national program of active surveillance of wild mosquitoes *A. albopictus*.

West Nile virus is transmitted through mosquitoes, being isolated from 43 species, mostly of the genus *Culex*. In Europe, the main species involved in the transmission of West Nile virus infection are *C. pipiens, C. modestus* and *Coquillettidia richiardii* (HUBÁLEK & HALOUZKA, 1998; FYODOROVA et al., 2006; REITER, 2010).

According to the data of TIHON (1981) in the Republic of Moldova there were determined 28 species of mosquitoes belonging to the Culicidae family (TIHON, 1981), including species those determined as vectors for West Nile virus.

ECDC experts in West Nile virus infection in Europe have found that the risk of this disease spreading in 2009 increased essentially in the last decade for Italy, Hungary and Romania. These conclusions were made based on the data of recording more than 500 clinical cases of West Nile virus infection with mortality rate of 10% in 1996 - 1997 registered near Bucharest (Romania). This outbreak was the largest recorded in Europe and reaffirmed that the vector-borne viral diseases can be spread in human population, even in temperate climate zones (HUBÁLEK & HALOUZKA, 1999).

Ixodidae tick species have also been recorded as vectors for more than 20 infectious diseases in humans and animals (HOCHEDEZ et al., 2006). Ixodidae ticks are involved in the transmission of viral (encephalitis, Crimeea - Congo haemorrhagic fever etc.), bacterial (Lyme Borreliosis, tularemia etc.) and rickettsiosis (Q fever etc.) infectious diseases (GRATZ, 2005).

In order to monitor the evolution of the situation in diseases transmitted by ticks, reducing the risk of diseases and preventing their spread it is necessary to maintain the preparation activities and implementation of response measures by public health systems by planning specific actions on national and local levels.

Ecological and faunistic situation in the Republic of Moldova at present is influenced both by global climate change, as well as by processes of territories change through anthropogenic activities by destroying forests and aquatic territories, increased urbanization, changes in agro-industrial complex etc. Social changes, intense process of migration of population, trade also maintain the risk of above named diseases, in our country included. In the Republic of Moldova, 22 species of ixodide ticks were registered (USPENSKAYA, 1987). As a result of preliminary studies in our the country, there have been isolated from the species *I. ricinus, H. punctata, D. marginatus* the causative agents of diseases: Crimea - Kongo and West Nile fevers encephalitis (HUBÁLEK & HALOUZKA, 1999; GRATZ, 2005; USPENSKAYA, 1987).

Thus, the task of the surveillance system is to monitor these species of vectors in some areas and to increase preparedness for response in order to minimize the risk and consequences to public health.

Research carried out until now provide a strong basis of knowledge about zooanthroponosis diseases but some features of these diseases remain unidentified: circulation and persistence of the causative agents in the vector and reservoir body, determining the risk of spread in different areas, implementation of laboratory diagnosis algorithm for confirmation, reporting cases of illness with elaboration and implementation of measures to prevent illness in humans.

MATERIALS AND METHODS

The National Center for Public Health has been monitoring Ixodidae tick species for more than 15 years. In this paper, the data concerning Ixodidae tick species diversity, their density during the maximum biological activity in spring-autumn period, and the presence of pathogens in these vectors were analyzed. Collections were made during 2005-2009 in accordance with standard methods, targeting selected areas for study (HUBALEK & HALOUZKA, 1987). Tick density index is the ratio of the tick number collected from the route with a length of 200 m. Using the "flag" in the reference period from plants 4332 specimens of Ixodidae ticks were collected. The territory of study (forest biotopes, forest edge, agrocoenosis, recreation areas, near water basins) was selected in 3 zones of the country: northern (Glodeni, Floresti districts), central (mun. Chişinău, Straseni, Orhei, Ungheni, Hincesti, Ialoveni, Anenii Noi districts) and southern (Cahul and Stefan Voda districts).

The study of mosquito species diversity was initiated in 2009. Mosquitoes were collected from two areas: the northern (Glodeni district – Pădurea Domnească nature reserve (May), Ocnita - the middle part of the Nistru river (September) and southern (Cahul district, the Prut river floodplain (August)). Mosquitoes were collected in the river floodplain biotopes near the Prut and the Nistru Rivers using the method: light lamps and entomological net. At the same reference period, there were made collections of mosquitoes in resting places - the sheepfold. On the whole, 410 specimens of mosquitoes were collected.

All results were subjected to generally accepted statistical calculation using Excel software.

RESULTS AND DISCUSSIONS

The genus *Ixodes* tick in the Republic of Moldova includes a total of 22 species (USPENSKAYA, 1987) that have spread quite widely in different biotopes. The life cycle of ticks of the genus Ixodes includes 4 phases of development stages: egg, larvae, nymphs, and imago. From the beginning of the larval stage, ticks feed with blood and transmit the causative agents to the hosts, which are represented by different vertebrates (reptiles, birds, mammals). During the blood feeding, the infected tick by its saliva can transmit the casual agents of tularaemia, Lyme borreliosis, Q fever, encephalitis etc.

In the Republic of Moldova there were identified three genera of ixodide ticks: *Ixodes, Dermacentor* and *Haemaphysalis*, with 22 species recorded. During the study, five species were identified: *I. ricinus, D. marginatus, D.*

reticulatus, H. punctata and *H. inermis*. In this north, two genera have been identified: *Ixodes* and *Dermacentor*; in the south and center (Chisinau) three genera: *Ixodes, Dermacentor* and *Haemaphysalis*.

Average density index of ixodide ticks in the northern area was 61.5 individuals on 200 m route, being at the same time the highest index in comparison with the other two areas of the country. In the southern area, the average index was 44.8 specimens, in the center - 21.6 specimens, and in Chişinău - 32.2 specimens on 200 m standard route.

During the period 2005-2009 the overall average density index of those three genera of ticks in the country was 36.8 specimens for 200 m. The average density of the genus *Ixodes* was the highest - 25.1 specimens, of the genus *Dermacentor* - 11.4 sp., and respectively *Haemaphysalis* - 0.3 specimens on standard route of 200 m.

Generally, the rate of the genus *Ixodes* ticks in the country is 68% and it was ~ 2 times higher comparatively with compared to the genus *Dermacentor* (31%). The genus *Haemaphysalis* had the lowest percentage - 0.9% of the total number of ticks, being not registered in the north area. High rate of Ixodes genus ticks is conditioned by their tropism and feeding on a large diversity of vertebrates, fact which is not particular for other tick genera.

The distribution of the tick genera varies from one area to another. The highest rate (> 90%) of the genus *Ixodes* was recorded in the north and center. In these zones, the territories covered with woods dominate, the rodent number is high and the grazing of domestic animals is intense. The rodents and domestic animals ensure tick survival and prosperity in all the stages of their development. The ticks of the genus *Dermacentor* in 83% were identified in the Prut river floodplain in the south of the country. In Chişinău, the rates of the genera *Ixodes* and *Dermacentor* were 41% and 58% respectively.

Indexes of tick species density in different biotopes of the 3 areas of the Republic are presented in Table 1.

In the Northern area there was registered the highest average index of Ixodidae ticks density - 61.5 specimens. The distribution of the species of Ixodidae ticks in biotopes showed that the highest average density index was in the rest and recreation area, being – 86.2 specimens. In other biotypes this index was lower: in agrocoenoses - 49.0 sp., forest and forest edge – 34.3 sp. and near water basins – 32.0 specimens.

The distribution of the genus *Ixodes* tick species in the studied biotopes reflects the highest average density index – 59.8 specimens (sp.). In recreation areas, *Ix. ricinus* density is 89.8 specimens, being the highest compared to other species index. The lowest density index was determined in wet biotopes around water basins – 7.5 specimens. The average density of the genus *Dermacentor* was 2.6 specimens (Table 1), and the highest density index of this genus of ticks was recorded in wet biotope - 24 specimens. In forest and forest edge biotopes this index of ticks density was the lowest – 0.3 specimens on 200 m.

In the **Central area,** the average index of ixodide ticks density was more frequently detected in agrocoenosis (30.9 specimens) and in Chişinău in wet biotopes (60.8 specimens). In leisure places and forests of the central zone this index was lower and registered almost the same level ~ 20.0 specimens, ~ 9.0 in wet biotopes. In Chişinău, in forest biotopes the density index of ticks was ~ 32.0 specimens, in agrocoenosis and resting places ~ 11-13 specimens.

Average density index of the genus *Ixodes* in the center was 19.7 sp. (Table 1) and in Chişinău – 13.3 sp. The highest density index of the genus *Ixodes* in the central zone was registered in agrocoenoses – 29.9 sp. and the lowest – near water basins (9.3 sp.). In Chişinău, in forest biotope, forest edge, parks, the density index was the highest – 28.0 sp., in wet biotopes the registered low index (1.2 sp.) is specific for the area of spreading of this genus. The average density of the genus *Dermacentor* in the center was 1.5 sp, in Chişinău – 18.8 sp. (Table 1). In the wet biotopes (ponds) of Chişinău, the genus *Dermacentor* density index was the highest – 59.5 sp., where the habitat conditions are optimal.

The genus *Haemaphysalis* is rarely encountered in the central area (0.4 sp.) and in Chişinău (e.g. 0.1 sp.).

In the **biotopes of the South area** of those three genera of identified ixodidae, the genus *Dermacentor* is the most common one, being 69.5 specimens in wet biotopes near the Prut river floodplain. The genus *Dermacentor* was not found in the forest and forest edge biotopes. The average density of the genus *Dermacentor* specimens was 3.4 on standard route. The genus *Ixodes* was identified in all studied biotopes, the average density index - 5.7 specimens. The lowest density index of the genus *Ixodes* was determined in wet biotopes – 0.3 sp., in the forest and the forest edge – 17.6 sp., the highest in the area. The genus *Haemaphysalis* was detected only in forest ecosystems, forest edge, and agrocoenosis, the average index being – 1.7 sp. (Table 1).

In order to determine the presence of Borrelias in ticks, there has been investigated only *I. ricinus* species of the genus *Ixodes*. The results obtained in the years 2005-2009 revealed the presence of borrelia on average 35.9% out of investigated ticks. It is necessary to note that out of 10 known borrelia genospecies, only four are responsible for developing of the disease in humans. Previous studies have demonstrated the presence of pathogenic genospecies of borrelia (*Borrelia burgdorferi sensu stricto, B. afzelii, B. garinii, B. lusitaniae*) in *I. ricinus* ticks collected from all areas of the Republic of Moldova (GHEORGHIŢĂ, 2007). The percentage of ticks with borrelia varied from one area to another; in the north it was 22.6%, in the center – 34.0%, and in Chişinău - 39.8%. The results obtained in other European countries also reflect a variation from 0% to 58% of borrelia infected ticks rate (HUBÁLEK & HALOUZKA, 1998).

Previous studies achieved in 2003-2005 showed the presence of the causative agent of tularaemia in ticks species *Ixodes apropnophorus* in the south of the country, the Prut river floodplain. But in the reference period this species of ticks was not identified.

In 1997-1999, the causal agent of Q fever was determined in ticks of the species *D. reticulatus, D. marginatus, Ixodes ricinus* in Chişinău and in the central zone.

Table 1. Diversity and density of Ixodidae ticks species collected from different areas of the Republic of Moldova in 2005-2009.
Tabel 1. Diversitatea şi densitatea speciilor de căpuşe Ixodide colectate din diferite zone ale Republicii Moldova în 2005-2009.

Areas	Biotope type	Density index	Density index			Percentages (%)		
			Derma-centor	*Ixodes*	*Haema-physalis*	*Derma-centor*	*Ixodes*	*Haema-physalis*
North Zone	Recreation area	86.2	1.2	89.8	-	-	-	-
	forest	34.3	0.3	34.0	-	-	-	-
	agrocoenoses	49.0	3.0	46.0	-	-	-	-
	wet	32.0	24.5	7.5	-	-	-	-
Total		61.5	2.6	58.9	-	4.2	95.8	0.0
South Zone	forest	12.0	0.0	17.6	2.4	-	-	-
	agrocoenoses	45.5	9.7	9.0	3.4	-	-	-
	wet	76.2	69.5	0.3	0.0	-	-	-
Total		44.8	37.4	5.7	1.7	83.6	12.7	3.8
Chişinău	Recreation area	11.1	0.2	10.9	0.0	-	-	-
	forest	32.8	4.8	28.0	0.0	-	-	-
	agrocoenoses	13.4	8.3	4.3	0.9	-	-	-
	wet	60.8	59.5	1.2	0.1	-	-	-
Total		32.2	18.8	13.3	0.1	58.3	41.4	0.3
Central Zone	Recreation area	20.8	2.2	18.1	0.4	-	-	-
	forest	20.8	1.4	19.1	0.4	-	-	-
	agrocoenoses	30.9	0.6	29.9	0.4	-	-	-
	wet	9.3	0.0	9.3	0.0	-	-	-
total		2.6	1.5	19.7	0.4	7.2	91.1	1.8
National average		36.8	11.4	25.1	0.3	31.0	68.1	0.9

Primary studies of species diversity of mosquitoes from Culicidae family, initiated in 2009 in the Republic of Moldova revealed the presence of five genera: *Anopheles* (339 specimens), *Aedes* (30 specimens), *Culex* (14 specimens), *Coquillettidia* (25 specimens) and *Uranotaenia* (2 specimens). Data on the presence of *Uranotaenia* in Moldova were not found in other publications.

In the northern area, the genera *Anopheles*, *Culex,* and *Aedes* were identified. In different territories, the number of the identified genera was different. In Glodeni district two genera were identified genera: *Anopheles* and *Aedes*. The genus *Anopheles* included the species: *A. maculipennis complex* collected at sheepfolds, *A. cantans* was found in collections from the "Padurea Domneasca" Nature Reserve near the pond surrounded by woods. The work carried out in the Ocnita district in the middle part of the Nistru River allowed the identification of three genera: *Anopheles, Culex,* and *Aedes*. The *A. maculipennis complex* species has also been reported in sheepfolds. Near the water basin, in the recreation places of population in Ocnita, the species *C. modestus, C. pipiens,* and *C. territans* have been identified. The species *C. modestus* was found in the apartment blocks in Ocnita. *A. vexans* and *C. modestus* species were identified in the wet biotope in the middle part of the Nistru River (Naslavcea).

In the South, the species diversity is the largest identified and it includes the genera: *Anopheles, Culex, Aedes*, *Uranotaenia* and *Coquillettidia*. In Cahul district, in wet meadow biotopes near the Prut river (Valeni, Manta) *Coquillettidia richiardii, Uranotaenia unguiculata, A. vexans, C. Modestus,* and *A. hyrcanus* species were identified. As in the northern area, the species *A. maculipennis* complex was identified in places of sheep breeding.

Mosquitoes are the vectors that transmit the causative agents of such diseases as West Nile virus infection, Congo Crimea etc. which are not currently studied in the Republic of Moldova.

CONCLUSIONS

Out of the 22 species of ixodide ticks registered in the Republic of Moldova, in the reference period, five species were identified: *I. ricinus, D. marginatus, D. reticulatus, H. punctata* and *H. inermis*.

The species *Ixodes ricinus* is one of the most widespread species of vectors of Lyme borreliosis in the Republic of Moldova.

The species *I. ricinus, D. marginatus* and *D. reticulatus* maintain the risk of transmission of zooanthroponosis diseases on the territory of our country.

Those five genera from the family Culicidae: *Anopheles, Aedes, Culex, Uranotaenia* and *Coquillettidia* with 9 of 28 species recorded in the Republic may also be involved in the development and transmission of new infectious diseases in our country.

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