

## VECTOR BORNE TRANSMISSIBLE ZOOSES IN MONTENEGRO

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### SUMMARY

Vector borne transmissible zoonoses are becoming more and more important in the group of *emerging* and *re-emerging* infections. We present the characteristics and actuality of this group of infectious diseases in Montenegro for the period 1998 - 2011. In examinations, standard epidemiological, clinical, serological, pathohistological diagnostic methods are employed. Natural conditions in Montenegro make it an important endemic area for more vector borne transmissible zoonoses. The changes of ecological characteristics, the vectors and infective agents, present the accident for expansion and increasing importance of these infections in national pathology. According to the fact that it is an international port of nautical, continental and air traffic, Montenegro has responsibility for control and management of diseases belonging to the group of the travel and tropical diseases.

**Key words:** Transmissible zoonoses, expansion, specter

### INTRODUCTION

*Transmissible Arthropod Zoonoses* (TAZ) represent such diseases, the causes of which, in natural environment, could be transmitted from animals and their vectors to humans (1). In the past many of those diseases represented 'pestilence' for humanity (*plague, typhus, arboviral infections*).

Agents that provoke dangerous zoonoses in recent years (*emerging infectious diseases*) include new ones and some old causative agents of zoonoses also, therefore there are returning of some old and forgotten infectious diseases (*reemerging infectious diseases*). (2, 3, 4)

The studies of zoonotic agents, besides specific heterogeneity, have shown rather frequent presence of intracellular / facultative intracellular microorganisms. The intracellular position and capacity of incorporating into cellular structures of the host is of great importance for sheltering from the mechanisms of immunologic defense

of the host during infection. It represents the potential for development of complex immunopathogenic mechanisms of infection, which determine persistence with consecutive chronic progressive course, relapses, development of immune and autoimmune diseases. (4)

Unspecific immunodeficiency that follows infections with intracellular pathogens puts them into important opportunistic agents and potential reactivations of many other latent agents, which after the reactivation can participate in mutual immunological basis of disease. (4, 5)

In Montenegro, natural environment provide ideal ecological basis for existence of TAZ and for their emerging in coinfectious forms. (5). For Montenegro, as an international port of aerial, terrestrial and nautical traffic, it is an impelling order to resolve the problem of dangerous emerging and reemerging zoonoses for the purpose of establishing mechanisms of more efficient control and protection from diseases that can be imported from abroad. The society is in transitive phase with numerous social and economic problems, which should be aborted for effective protection of the population at risk.

There has not been dedicated enough room for research of these diseases within national pathology, therefore their real significance in national pathology has not been estimated within both, current and future flows. (5)

### MATERIAL AND METHODOLOGY

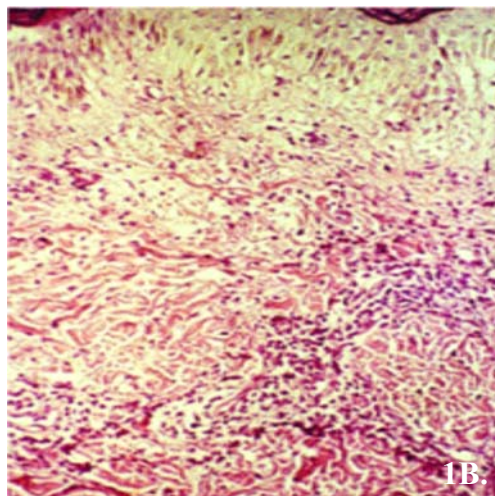
In the analyses are included vector borne transmissible arthropod zoonoses that were registered in Montenegro in the period 1998 – 2011. In this research standard methods of epidemiological, clinical, serological, pathohistological diagnostics were used.

### RESULTS

#### Lyme borreliosis

First cases of *Lyme borreliosis* in Montenegro were registered in 1990 (2 cases). By systematically programmed testing, for the period 1990 – 1996, had confirmed endemic

presence of LB in Montenegro with morbidity level of 8,5%. By the end of 2011, there were registered over 1700 cases. Diagnosis is made on the basis of epidemiological, clinical and serological methods (*Elisa* and *Western Blood*), as well as on *Polymerase Chain Reaction /PCR/* method. Primary affect, *Erythema migrans (EM)*, represents inflammatory skin lesion important for clinical diagnosis (Illustration 1: A and B), but are not criterion for severity of disease.



**Illustration 1.** Erythema migrans /EM/, inflammatory skin lesion in the spot of the tick bite (A).

Pathohistology characteristic of EM: nonspecific lymphocyte-monocyte infiltrate (B)

LB is infectious disease with multiorgan disorders, with rapid dissemination of the causative agent, and later manifestation of progressive forms of the disease (*neuroborreliosis*, *cardioborreliosis*, and *rheumatologic* manifestation). Diagnostic and prognostic problems of disease are presented in practice even in recent time. Consequences are visible in a practical sense on the basis of increased number of infected and frequent registration of advanced stages of disease.

Discoveries of concurrent interrelations between *b. burgdorferi* and other pathogens (*rickettsiae*, *babesiae*, *tick-borne viruses* etc.) defining coinfectious forms or LB syndrome contributed understanding of frequent polymorphism of the clinical display of LB and more frequent appearance of chronic persistent forms of infection, but for the time it is not optimistic about concerning practical solutions of diagnostic problems, therapy, prognostic evaluation and control of the disease.

### **Bartonellosis – Cat Scratch Disease**

Bartonellosis is an example of zoonosis in which the progress in evolution and changes of infectious agents enabled expansion and appearance of severe, disseminated forms of infection and coinfection. Resistance of *Bartonella* species /spp/ to antibiotics additionally complicates disease prognosis. *Cat scratch disease (CSD)* is an entity of bartonellosis which could be registered in our environment (illustration 2: A and B)





**Illustration 2.** Cat scratch disease. **A** - enlargement of regional lymph nodes, **B** - primary affect on the place of the cat scratch

In Montenegro the first few CSD cases were etiologically confirmed, by IIF method date back to 2008. Until the end of 2011, the diagnosis was confirmed in 10 cases. All of the infected had had previous contact with a cat. Primary affect was registered in 5 cases after cat scratch and in 1 case of exanthema after a cat bite. In the cases without primary affect, prolonged fever and generalized adenopathy presented differential-diagnostic problem including glandular form of tuberculosis, infectious mononucleosis, and therapeutical problem.

### Rickettsial Diseases

Montenegro is an endemic area for rickettsial diseases. Historical importance of the epidemic typhus is evident through numerous human victims (Balkan wars, I and II World War). In Yugoslavia were the last reported cases of typhus exanthematicus in Europe (Montenegro in 1965 and Serbia in 1968). It has not been registered since that time, but possibility of reactivating the endemic foci is continuously presenting which is something that recent knowledge indicates as well as the dismantling of inter-epidemic reflection of *Rickettsia prowazekii* in animal nature reservoirs in endemic foci. Since 1997 there has been registered an increased number of cases of the typhus disease over the World.

The first few cases of the *Spotted fever rickettsial diseases* (SFRD) in Montenegro were confirmed in 1996 by identifying *Rickettsia conorii* /*R.conorii*/ and afterwards

coinfections between rickettsial agents (*R. conorii*, *R. typhii*) and *B. burgdorferi* using the IIF method. Until September 2011 the diagnosis was confirmed for 107 cases. In all cases clinical diagnoses of *Spotted Fever Rickettsial Disease*, *R. conorii* was serologically detected in diagnostic titer (in relation 1:160 to 1:1280, by IIF method). In 58 cases coinfections were detected: *R.conorii*, *R.typhii* and *B. Burgdorferi*. Clinical manifestations and the course were the arguments for clinical recognition of the disease (illustration 3: A and B). Distribution of vascular changes was in correlation with the dynamics and the level of systemic disturbances.



**Illustration 3.** Characteristic generalized maculopapular exanthema appears 3 – 4 days from the onset of fever (A). Characteristic primary affect (“tach noire”) on the left leg marks the place of tick bite (B)

Veterinarian testing of the presence of *R. conorii* in the blood of stray dogs showed high prevalence of specific antibodies.

The first etiological confirmations of the *Q fever* within human population in Montenegro date back to 1996. Until the end of 2011 the diagnosis was confirmed in 72 cases. In 17 cases of parallelly tested sera, coinfections of *C. burnetii* with *B. burgdorferi* and agents of *SFRD* group were registered. By clinical testing the most frequent and most consistent registered manifestations were prolonged fever, adenopathy, and respiratory dysfunction. Myopericarditis was registered in 18,2% of examined cases.

Research of the expansion of the *Q fever* among animals has shown the presence of *Coxiellae burnetii* in 0,29%, in relation to the total cattle (sheep) livestock in Montenegro.

Different *Ehrlichia* species are causative agents of severe human infections: *Human Granulocytic Ehrlichiosis (HGE)* and *Human Monocytic Ehrlichiosis (HME)*

The first few etiological confirmations of the ehrlichiosis in Montenegro date back from 2008/2009. *E. canis* was detected in a diagnostic titer in 63 patients (by IIF method). In 57 cases there were coinfections of *E. canis* with other agents of the TAZ complex: *R. conorii*, *C. burnetii*, *B. burgdorferi*.

In clinical manifestations nonspecific, prolonged febrile syndrome and adenopathy represented the starting point for selecting the testing group. The presence of nonspecific antibodies versus *E. canis* and *B. burgdorferi*, are contribution to the results of such testing in Europe which classify *Ehrlichia* species among the most frequent agents of cotransmission and coinfection with *B. burgdorferi*.

### **Hemorrhagic Fever with Renal Syndrome (HFRS)**

*Hantaviruses* belong to old group of viruses but ecological changes are reflected on geographical distribution, dynamics, enlargement of the array of hosts among mouse-like rodents, and expectation of the appearance of new types and subtypes of these viruses.

Four pathogenic hantaviruses are circulating in Europe (*Puumala*, *Beograd / Dobrava*, *Hantan*, *Sarema*). Key manifestation of *Hemorrhagic Fever with Renal Syndrome (HFRS)* is increased capillary permeability. Viral replication takes place into vascular endothelial cells.

HFRS is endemic in Montenegro and has been registered since 1954. It includes severe forms of infection with renal dysfunctions, pulmonary edema, disseminated intravascular coagulopathy and hemorrhagic syndrome. Serological records in many cases show coinfections, in which participate more serotypes of *Hantaviruses*. Within period of 1998 - 2011 years, in majority of cases of the infected patients within almost the same diagnostic titer, 3 serological types of *Hantaviruses* were proved (*Hantan*, *Puumala*, *Beograd/Dobrava*).

Until the 2002 year, there are 116 clinical cases of HFRS, and 33 of them were serologically no confirmed, which likely due to possible appearance of some new serotypes of *Hantaviruses*.

### **Arboviral neuroinfections**

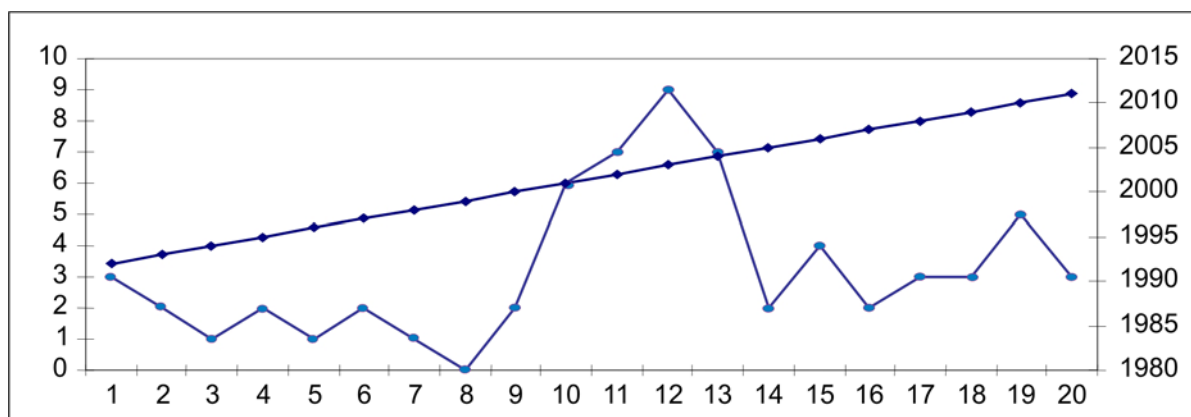
Arboviral neuroinfections in Montenegro had not been the subject of research until now, which is in opposition with data about the number of registered cases, etiologically non differentiated viral infections of *Central Nervous System /CNS/*.

On the base of testing of WNV /*West Nile Virus*/ expansion in the territory of Montenegro, *Vesenjak Hirijan* in 1991 published that specific antibodies were found on WNV in 1% out of 8267 of analyzed human sera in Montenegro.

The expansion of virus of *Mid-European tick encephalitis (TBE)* and the fact that in Europe it corresponds to the distribution of the reservoirs – *Ixodes* ticks, and endemic areas of *L. borreliosis*, classify this severe arboviral disease as important diagnostic and differential-diagnostic problem. The fact that TBE is very easy to prevent by vaccination, facing the problem of TBE in our territory would be significant for an efficient prevention of the disease.

### **Leishmaniasis (Kala-azar)**

*Leishmaniasis (Kala-azar)* is widely expanded in the World. In endemic areas, where there are numerous hosts (*small rodents, small mammals, wild and domestic dogs*), infection is transmitted to humans through the sting of the infected flies (*phlebotomies*). In Montenegro, visceral leishmaniasis (*Kala-azar*) is endemic. The disturbances of the ecosystem, the increase of the vectors density, the reservoirs of infections, provoked an expansion of the endemic foci and increased number of the infected. Since 2005 there are 1-3 registered cases per year. (Fig. 1).



**Fig. 1.** Dynamics of *visceral leishmaniasis* in Montenegro from the 1992 to 2011.

Clinical manifestations of the leishmaniasis depend on the outcome of the complex interactive relations of the parasites and immunological defense of the host. Every infection does not lead to the clinically manifested diseases, however, the persistence of live leishmaniasis long after the infection itself, puts it into the group of important opportunistic agents, what is confirmed by the increase of the number of those immunosuppressed hosts with visceral leishmaniasis.

In clinically manifested infections, nonspecific symptoms, and changes in routine laboratory parameters (anemia, leucopenia, thrombocytopenia) have observed. Diagnosis is confirmed by biopsy of the bone marrow and serological testing.

### Babesiosis

*Babesiosis* is blood hemolytic, malaria like disease. In 100 species of *babesia* identified, only a handful has been documented as pathogenic in humans. The primary vectors for transmission of parasites are different tick species. Prevalence of infection in the World provides a wide range of animal reservoirs of parasites (domestic and wild mammals). The epidemiological examinations in Europe present broad distribution and show that *Babesia* spp. is the most frequent agent in coinfection with *B. burgdorferi*. In Montenegro the first few diagnosed cases of human *babesiosis* /total 10 cases/ are registered in short period of September to November 2011. Diagnosis was based on microbiological and pathohistologic methods, on detection of intraerythrocytic ring forms of parasite in the peripheral blood (colored by *Giemsa-i*), as well as microscopic slides of bone marrow biopsy (colored by *Romanovski*). In 6 examined cases, by serologic methods was detected coinfection of *babesia* parasite with *B. burgdorferi*.

### Malaria and the problem of imported infections

Malaria is the most frequent transmissible parasitic disease in the World. The causative agents are the members

of *Plasmodium* species. The reservoirs of parasites are infected humans and animals. Transmitters are mosquitoes /*Anopheles* species/. In the last decade, two important factors in endemic areas have an impact on the increased number of infected patients: resistance of vectors to the insecticides and resistance of parasite to the antimalarial drugs. In our environment malaria is eradicated after the World War II, but it has been continually registered imported cases of this disease (seamen, travelers to endemic areas). For Montenegro this fact is important, because of possibility for potential reactivation of old malaria foci, under conditions of existence of transmitter, favorable climate and other natural conditions for their survival. The prompt preventive measures are essential and crucial.

### DISCUSSION AND CONCLUSIONS

Analyses of the TAZ in Montenegro indicated their importance about human and veterinary pathology. (5, 6, 7)

Montenegro is classified among ideal natural locations for existence of complex parasite systems of TAZ, connecting their historical and contemporary significance (2).

Supportive are the facts that on this small size area, concerning its surface, important endemic foci for various zoonoses could be found. (8, 9, 10, 11)

It appears that ticks and phlebotomies for the time being are the most important vectors of the transmissible zoonoses in Montenegro. More and more attention is recently dedicated to the bugs, mites, lice, potential vectors of dangerous, old ones and new transmissible zoonoses, especially under conditions of global disturbances of ecosystems. (15, 16, 17)

Numerous infectious agents from the complex of transmissible zoonoses are defined as potential bioterrorist weapons. Their features must be faced from that aspect in our country too, as well as from the aspect of travelers and tropical medicine (15).

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