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Human Vector-Borne Transmissible Parasitic Diseases in Montenegro

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Abstract

Montenegro is an endemic country for a significant number of vector-borne diseases (VBD). Natural conditions and geographical position (Mediterranean area) are favorable for the existence of the disease, and its expansion (1). Current vector-borne transmissible parasitic infections that have been registered in Montenegro includes: leishmaniasis, babesiosis, malaria, and filariasis (dirofilariasis).

The causes of leishmaniasis are the members of protozoa leishmania species (spp). The phlebotomies are the primary vectors in transmission of parasites. Documented cases of visceral leishmaniasis (VL) from 1992 to 2014 in Montenegro present 84 cases with of Kala-azar, and the 1 case of skin leishmaniasis. In 2014 the coinfection of leishmaniasis and HIV/AIDS for the first time was registered in one case.

Babesiosis is a parasitic infection similar to malaria.

In transmission of parasites, the primary vectors have different tick species, possibly the other blood meal vectors (sand flies, mosquitoes, and bugs).

Dispersion of the infection in the worldwide is enabled by a wide range of reservoirs of parasites. Examinations in Europe proved that babesia is the most frequent agent of co-infection together with *Borrelia burgdorferi*. The first diagnosed cases of human babesiosis in Montenegro were registered in 2011. By the end of 2013, 12 cases were diagnosed. The coinfection of babesia and *B.burgdorferi* were registered in 73% cases.

Malaria is the most known parasitic transmissible disease in the world. The causative agent is *Plasmodium*, a genus of *Apicomplexa*, which is transmitted by mosquitoes of the genus *Anopheles*. In Montenegro, the disease was officially eradicated after World War II, but we continuously register 04 cases of imported malaria per year (sailors, travelers to endemic areas). These facts are significant because of the existence of the

transfers and the favorable conditions for their maintenance, and therefore the fear that the epidemiological focus can be rebuilt.

In 2014 one case of human dirofilariasis was diagnosed for the first time in Montenegro.

Keywords: Leishmaniasis, Babesiosis, Malaria, Dirofilariasis

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1. Introduction

The transmissible way of spreading infection from animals to humans by different vectors is of great importance in contemporary world infectious pathology. This group includes a large number of vectors that cause different emerging diseases, the so called zoonoses. [1, 2]

Vectors play complex role in the epidemiology and pathogenesis of these diseases, with abundance of species that provide the resources for expansion into new geographic areas and direct participation in the pathogenic immunity mechanisms of infections [3]

The natural factors are adjacent to the problems of globalization and increase the importance of these diseases in the world due to: numerical magnification of human population, frequent contact between human and animal population, and behavioral changes in the human population. Also climate change is of special interest [3, 4]

Mediterranean area presents the region with frequent representations of a wide specter of vectorborne diseases (VBD). Montenegro is a Mediterranean country, geographically situated in the Balkan area. Natural conditions in Montenegro represent an ideal ecological basis for the existence of VBD [5]

New characteristics of parasitic VBD present increased frequency, severe clinical courses, and difficulties in diagnosis of nondefined way and prognosis of diseases. On the basis of this fact, they are imputed in the group of emerging zoonoses, occupying an important place [6,7,8]. Frequent multitietiological (coinfective) forms of diseases represent additional problem in this group of parasitic VBD [9], based on the natural factors (coexisting of different causers in common endemic areas, natural hosts, cotransmissions of these agents with common vectors).

Based on the changes of the immunological characteristics of parasitic agents in infected humans, parasitic VBD can be provided by opportunistic action and reactivation of different and numerous intracellular microorganisms and cooperative action, with negative reflection on difficulties and uncertain prognosis of coinfections (eg: coinfection of *Leishmania* parasites and HIV, *babesia* parasites and *B. burgdorferi* [10,11]. In addition, coinfections represent big diagnostic problem.

Increasing multiresistance to drugs and necessary application of combined therapy represent multiple problems [12,13]. In national human pathology in Montenegro, there not enough

research space has been dedicated to parasitic VBD hence they have not received attention in the light of their significance for the present or for the future.

An analysis observed four different parasitic transmissible zoonotic diseases, in different periods of the last decade, by using available diagnostic methods.

Current transmissible parasitic zoonoses that are registered in Montenegro and that should be a field for future research work include: leishmaniasis, babesiosis, and malaria. In 2014, the first registered case of autochthonous dirofilariasis in Montenegro presented a new dimension of the needs and seriousness of the disease, showing that new factors of transmissible parasitic zoonoses should be paid much more attention [14].

2. Methodology

2.1. Leishmaniasis

The number of diseased cases refers to the period 1992 to 2014. In the diagnostic procedure epidemiological, clinical, and laboratory methods were used. Diagnosis was etiologically confirmed through bone marrow biopsy analysis by direct microscoping of serial sections colored with Giemsa (stain), reticulin, and PAS method and by immune-biochemical methods (TdT, CD34, CD117, CD15, glucophorin A, CD31, CD79a, CD20, CD3, CD45RO, CD38, kappa, lambda, IgG, IgM, IgA, CD68). Serologically, with agglutination test, the diagnosis was confirmed in 56% of the cases.

Taking into consideration that in our country wild jackals and domestic dogs are the primary natural carriers of Leishmania parasites, significant for infections in humans, a screening of 1500 serum samples of both asylum dogs and stay dogs was done by using the indirect immunofluorescence (IIF) method.

2.2. Babesiosis

The clinical characteristics of human babesiosis vary from predominantly asymptomatic (silent disease) to fulminate malignant forms, which depends on the degree of parasitemia and the strength of the immune response of the host.

Babesiosis may be suspected in cases as with tick exposure and history of persistent fevers and hemolytic anemia. The definitive diagnosis was confirmed by detection of intraerythrocytic ring forms of parasites in the periphery blood and by microscopic slides of bone marrow biopsy colored with Giemsa-i and Romanowsky stains. Serological testing (ELISA and Western blot (WB) and *polymerase chain reaction* (PCR) were used for detecting coinfections of babesia parasites with *Borrelia burgdorferi*. Microscopic slides of bone marrow biopsy colored with Giemsa-i and Romanowsky stains were also used in differentiating babesia from the malaria parasite. Detectable antibody response takes about a week s time post infection. Serologic testing may be falsely negative in an early stage of the disease progression.

2.3. Malaria

In patients with malaria malady epidemiological data play a very important role particularly with regard to residence in endemic areas and the frequent inadequate use of prevention drugs (in our sailors patients).

The golden standard for diagnosis of malaria is microscopic examination using thick and peripheral blood smears.

About 5 % of people with malaria have infections caused by several kinds of parasites therefore the analyses ought to take this into account.

2.4. Filariasis

The difficulty in making diagnosis of filariasis appears to be because of the long absence of disease symptoms in the initial stage. Therefore most people initially do not expect to have the disease. Later, symptoms are only slight and nonspecific. Eosinophilia was not observed in the blood of the patients. The serum IgE levels were normal, and signs of a specific humoral response to antigens of *Dirofilaria* spp. were absent, although slightly elevated antibody levels of antigens of *Onchocerca volvulus* could be demonstrated in all the patients.

Surgical removal of the worm and biopsy help in both diagnosis and treatment. Morphological examination of the matured adult worm has limitations in the identification of the exact species since a large number of zoonotic *Dirofilaria* spp. have been described. The molecular identification is not widely available. It is also possible that there are different strain variations of *Dirofilaria* parasites. Molecular analysis of the highly conserved mitochondrial 12S rRNA gene of *D. repens* showed a 3% deviation from other filarial parasites.

3. Results

3.1. Leishmaniasis

The first cases of leishmaniasis (kala-azar) in Montenegro were registered in 1924/1925, on the Montenegrin coast (Lustica, Baosici). In period 1930 to 1932, new cases were registered in the southern coastal region, between Bar and Ulcinj. Accurate records of the number of patients with disease manifestations do not exist for the period before 1995.

Our study covers the period from 1992 to 2014, with 86 registered cases of leishmaniasis. The visceral leishmaniasis (VL) has been diagnosed in 84 cases, and 1 (1.20%) case with skin leishmaniasis. Coinfection of HIV/AIDS and leishmaniasis in one case was registered for the first time in 2014. The trend of increase in the number of patients with confirmed diagnosis was present with 0-4 cases per year among 646000 inhabitants.

In the study sample, the child population participates approximately in 37% of the cases and adults in 48%. The diagnosis confirmation was based on microbiological-laboratory and pathohistological methods (Figure 1).

Figure 1. Bone marrow biopsy prepares colored with Romanowsky, visualizing amastigote forms of *Leishmania* parasites extracellular and in leukocytic cells. (Original prepares courtesy of Prof. Mileta Golubovic, Medical faculty – University of Montenegro)

According to the geographic area where VL was diagnosed, the expansion of endemic foci of the disease in Montenegro is evident. Three to four years ago, disease development was registered solely in the endemic area known between Bar and Ulcinj. According to the collected data, these areas now include the entire coastal area of Montenegro from Ulcinj to Herceg Novi, the Skaadar Lake area, including Podgorica and Cetinje, and even some northern parts of Montenegro.

In our country wild jackals and domestic dogs are the primary natural reservoirs of *Leishmania* parasites, significant for infections in humans. The screening of 1500 serum samples of both asylum dogs and stray dogs, from different parts of Montenegro, indicated the high infectiveness of dogs with *leishmanias*, which is 83%.

The investigation done in our country presents two types of leishmanias: *L.donovani* and *L. infantum*. The primary vectors of parasites are phlebotomi. Epidemiological studies conducted during the period 1996-1999 and in 2003 in the endemic area of VL (southern part of the Montenegrin coast) on 4770 samples of phlebotomi showed presence of five kinds: *Ph. perfiliewi* (1%), *Sergentomyia minuta* (12%), *Ph.papatasi* (11%), and *Ph.neglecticus* (60%). Predominantly *Ph.neglecticus* is mostly found in indoor areas. It is assumed that the main vector can be found in the Bar-Ulcinj region and in the northeast Mediterranean. Evolutionary adaptability continuously allows phlebotomies to significantly expand their potential as vectors for causers of vector-borne diseases (VBD). The correlation with global changes of ecologic environment and natural base of VBD is already evident in practice and there is a tendency of further growth.

The results of clinical investigations indicate an increase in the number of clinically manifested disease syndromes of VL in humans. The clinical manifestations of Leishmaniasis are not specific and they do not make diagnosis easier. The results of complex interactions between invasiveness and tropism of parasites in relation to side and the immune response of the host. Hypothesis on long-term persistence of live leishmanias after the infection, classifies them into a group of significant opportunistic agents. Coinfective forms of disease, especially in HIV/AIDS patients, have increased in the worldwide. In Montenegro in 2014, the first coinfection

of leishmaniasis and HIV/AIDS was registered in one patient. Incidental confirmation of coinfections of *L. donovani* and *B. burgdorferi* (three cases) in a common endemic area was registered in 2003.

Pentavalent antimony drug *Glucantime*, was relatively satisfactory in therapy of leishmaniasis over a long period of time in Montenegro. Problems arose because of the increasing resistance, which has been rapidly progressing. In our study, which took place in 2008 / 2009, there were registered recurrences in 12% of patients of the total sample. Repeated treatment with *Glucantime* was not successful, neither was the use of *Miltefosine*. The best results were obtained by using liposomal *Amphotericin B*.

3.2. Babesiosis

Babesiosis (piroplasmosis) is a malaria-like vector-borne parasitic disease, the so called tick-malaria. It was first described in 1883/1884 in the Balkan (Romania) in sheep. As a cause of human infections, babesia species (spp.) were detected in 1957 in Japan.

In Montenegro the disease was first confirmed in September 2011 based on hematological and microbiological examinations. Since 2013, there have been 12 cases detected. Fourteen patients were with positive anamneses of tick bite. Six patients were with skin manifesting erythema migrans (EM) identified in examinations that were associated with Lyme borreliosis (LB). By serological methods ELISA and Western blot and PCR method, the diagnosis of coinfections of babesia parasite and *B. burgdorferi* was confirmed in 72% of the patients, a total of 12 patients with confirmed babesiosis.

Analyzing demographic characteristics showed all 12 cases of the diagnosed babesiosis to be between 35 and 65 years of age, with professional exposure in rural parts of Montenegro. In the clinical presentation of all patients, nonspecific symptoms are dominant. There are several dominant symptoms: prolonged febricity, feebleness, and headache and changes in the laboratory findings (anemia, indirect type of hyperbilirubinemia, and moderately increased activity of serum aminotransferases, hypoproteinemia, and hypoalbuminemia) (Table 1).

Anemia	80 %
Leukopenia	45 %
Thrombocytopenia	11 %
Increased level of serum aminotransferases	10 %
Transitory respiratory disturbances	7 %
Syndrome of infective mononucleosis	15 %
Syndrome of acute leukosis	2 %
Prolonged febricity	29 %

Table 1. Clinical Laboratory Disturbances in Manifested Babesiosis in Our Patients N-12

The confirmation of etiological diagnosis of the disease was based primarily on the fact that intraerythrocytic annular forms of the parasite have been found in the peripheral blood, stained according to with Giemsa, and on the basis of microscopic slides of bone marrow biopsy stained with Romanowsky in 12 cases (Figure 2).

Figure 2. Ring forms of babesia parasite in intraerythrocytic position in our patient. (Courtesy of Prof Mileta Golubovic, Institute for pathology Medical faculty University of Montenegro 2011)

In endemic areas, the asymptomatic forms of babesiosis are the most frequent. Asymptomatic parasitemia can last for months, even years. This latent infection can be reactivated by stress, splenectomy, and immunosuppressive therapy. In human infections, babesia is a significant opportunistic agent.

The largest number of infected cases with babesia does not require a specific therapy treatment (silent disease). After the diagnosis was made in all cases, treatment was administered. There were two types of drugs used: *quinine* and *clindamycin* (within 7 to 10 days), which we also applied in our patients. Recent studies emphasize efficiency of *atovaquone* and *azithromycine*. Supportive and symptomatic treatment is important in severe cases.

3.3. Malaria

Malaria is the most frequent transmissible parasitic disease in the world. The causative agent *Plasmodium* is a genus of Apicomplexa parasites. Of the over 200 known species of *Plasmodium*, at least 11 species are continually competent for infection in humans. The most frequent are *Pl. falciparum*, *Pl. vivax*, *Pl. ovale*, and *Pl. malariae*. The mosquitoes of the genus *Anopheles* are carriers of parasites in humans. There are about 500 different species of *Anopheles*, and 60 of them can transmit the disease. The parasite always has two hosts in its life cycle: a vector – usually a mosquito – and a vertebrate host.

Based on the historical documentation, in the period from 1923 until 1943 in Montenegro there were 28486 registered cases of malaria (data from the *Jovan Kuljaca* 1925). Most severe cases of the disease have been recorded in the vicinity of Ulcinj, Skadar Lake, Rijeka Crnojevic, and Zeta. Mild cases of disease were registered in Podgorica, Danilovgrad, Niksic, Berane, Bijelo

Polje, Andrijevic, and Plav. Extermination of mosquitoes began in 1947, so that malaria was officially eradicated after World War II in our country, but the cases of imported malaria (sailors, travelers to endemic areas), 1–4 cases per year, continued to be registered. Considering this the fear that the endemic foci can be rebuilt is justified. Fortunately some climatic factors do not favoring the anophel species therefore malaria is very rare or even absent in the United States and Europe.

Imported malaria is a diagnostic and therapeutic problem. In the period from 2006 to 2013, we followed nonspecific laboratory analysis in 16 febrile returnees from Africa. It was found that the non-specific laboratory results were useful as an additional parameter for making diagnosis of malaria, with quick and simple diagnostic orientation. This is primarily related to thrombocytopenia, leukopenia, and hyperglycemia and increased level of serum aminotransferases activity and *lactate dehydrogenase* (LDH), urticaria, and hypocholesterinemia. It has been shown that hypocholesterinemia, severe anemia and elevated fibrinogen significantly prolonged the patient's recovery. Thrombocytopenia and increased activity of LDH were significantly associated with enlarged spleen and liver.

The most important groups of antimalarial drugs are: *Quinolone*, *Artemizin*, and *antifolates*.

New antimalarial drugs is *Atovaxon*. The big problem of treatment is resistance, particularly of *Pl. falciparum*. In more severe forms of the disease, in suspected resistance, combined therapy is applied. In severe forms of malaria, parenteral treatment is required.

3.4. Filariasis (Dirofilariasis)

Filariasis has systemic parasitic (worms) zoonosis from the group of VBD. Blood-feeding arthropods are those that can be transmitted. In most of cases the infective larvae (*microfilariae*) are injected through mosquito bites. A large number of mosquito species participate in transmission. Some species of fleas (*black flea*), lice, and ticks are also presumed to act as vectors. Different types of thread-like nematodes are the cause of disease in humans. The most frequent cause of filarial disease in the world is *Wuchereria bancrofti*. Among the many species of *Dirofilaria*, the most prevalent are two main filarial species (*D.immitis* and *D.repens*) that have adapted to canine, feline, and human hosts. At the same time, both the *D. immitis* and *D. repens* are themselves hosts to symbiotic bacteria of the genus *Wolbachia*. For the past few years, the incidence of human filariasis was increasingly reported in many parts of the world, making the disease part of the group of emerging zoonoses. The infection caused by *D.repens* is the most widely reported dirofilariasis with endemic foci in Eastern and Southern Europe, and Asia. In the Mediterranean area, the incidence of human dirofilariasis has increased, especially the subcutaneous and pulmonary forms of the diseases (Italy, Romania, Serbia, Germany, and France). Human dirofilariasis is typically manifested as either subcutaneous nodules or lung parenchyma disease, in many cases, asymptotically [60%]. Patients infected with *D. repens* notice a subcutaneous lump in the affected area which most commonly includes the face and conjunctiva of the eye and sometimes the chest wall, upper arms, thighs, abdominal wall and male genitalia (Figure 3). Ocular involvement is usually periorbital, orbital, subconjunctival, or subcutaneous infection. Human *D.immitis* infection has been associated with the human

pulmonary dirofilariasis and is usually asymptomatic. Symptoms of the disease are fever, chills, malaise, cough, localized retrosternal chest pain, and pleural effusion.

Figure 3. Human infection with *D. repens* are predominantly subcutaneous and most often evolved into a granuloma (Courtesy Hariish S. Permi, Department of pathology KS Haegde Medical Academy, India, 2011, (2): 199 – 201)

Our patient is a resident of Kotor and a civil servant, who has never left Montenegro. The first polymorphic symptoms occurred in January 2014 a feeling of discomfort and wriggle. Addition symptoms including the ocular disturbances that occurred later in the form of pain in short light flash, abdominal pain and dry cough that lasted for long, resulting in pneumonia. After an extensive examination was conducted, he was admitted in the surgical ward of the General Hospital of Kotor with suspected impacted epigastric hernia. On October 10, 2014, the surgeon using intraoperative method succeeded in extirpating entirely the solid fibrous granuloma, site epifascial, the midline supraumbilically. There was an indeterminate thread parasite 9.7 cm long in the excised granuloma. The pathological findings showed a granulomatous tissue with new blood vessels and giant cells of foreign body-type cells and concluded that such images can be found in filarial infections. Multipattern blood test for microfilaria was negative. Histological examination of the worms identified *Diir*ofilaria, based on morphological exclusion of *Wuchereria bancrofti*, *Loa-loa* and *Onchocerca volvulus*. Serological examination of antibodies to *Toxocara* and *Trichinella* proved to be negative. After the surgical procedure the patient was treated with oral *ivermectin* (150 mg per kg) and dovicine 2 x 100 mgr. He feels good so far.

4. Discussion

Parasitic transmissible zoonoses (PTZ) in Montenegro belong to a group of emerging infections, and it is a growing public health problem. Considering the fact that enough research activity has not been devoted to this group of infectious diseases, the consequences will be reflected in the future [5,10].

Based on epidemiological studies, the extension of endemic focus of leishmaniasis in our environment is evident, based on the number of registered cases in nonendemic areas of Montenegro. Veterinary studies of domestic and stray dogs from different parts of Montenegro confirmed their high level of infection with *Leishmania* parasites up to 83% [16].

With filariasis (dirofilariasis), we do not have significant experience. The different types of thread-like nematodes are the cause of human diseases. Mediterranean region as to be endemic for dirofilariasis. In January 2014, the first case of human dirofilariasis in Montenegro was diagnosed. Veterinary service does not have data on the prevalence of infections in dogs in Montenegro. Numerous human cases have been reported for the European Union [16, 17, 18, 19]. In 1999, most reported cases originated from the Mediterranean area, where *Dirofilaria* spp. are traditionally endemic (Italy, France, Greece, Spain, Serbia), with sporadic reports of small outbreaks of subcutaneous/ocular infections caused by *Dirofilaria* in Germany, the Netherlands, the United Kingdom and Norway. Canine dirofilariasis was not reported earlier in Central and Northern Europe.[19, 20]

Drastic changes of ecosystem [21, 22, 23] give the basis for epidemiological changes that are characteristic for this group of infectious diseases. Agent's adaptability coverage and expansion cover the spectrum of natural hosts and vectors [24] thanks to their easy and quick transition from enzootic to zoonotic transmission cycles. This has enabled significant expansion and has given new importance to cotransmissible and coinfective forms of the diseases, with consequent difficulties in diagnosis, therapy, and prognostic assessment. [6, 10, 12, 14]

Parasitic transmissible zoonoses in our study represent a big problem due to diagnosed coinfection. Coinfection of *Leishmania* parasites and HIV was diagnosed for the first time in 2014 [25, 26]. Earlier studies had proven a coinfection of *Leishmania* parasite and the bacterial agent *B. burgdorferi* [27].

The latest studies in Europe have confirmed the significance of babesia parasites as the most frequent tick-borne agent in cotransmission, and participant in coinfection with *B. burgdorferi*. In our study common infection with babesia and *B. burgdorferi* is detected in 76% of cases [27, 28, 29]. Elsewhere in the world coinfections of the causative agent of malaria *Pl. falciparum* and the other types of *Plasmodium* were detected. During our investigations in 1996, *Pl. falciparum* and *Pl. ovale* were detected in the peripheral blood smear of one case, a sailor returning from Africa with a tick stroke.

Coinfective forms of diseases are not uncommon in VBD. Their occurrence highlights two possibilities. Epidemiological path for the formation of coinfection is the consequence of cohabitation of vectors transmitted agents in common endemic areas and in the hosts and

vectors. To confirm there were test results, certifying that the bacterial agent *Bartonella hensellae* can exchange their proteins and genetic material with *B. burgdorferi* and other microorganisms in a shared host or vector, which is part of their remarkable adaptability, and agent identification itself is a big problem for researchers [30].

Another possibility is the complex pathogenesis mechanisms that occurs in the infected organism caused by complex material of the agents provoking the immune response of the host, via cellular and humoral mechanisms that are able to overcome the pathogens, or contribute to the resistance against it, and against chronic infection, recrudescences, and initiation of immune and autoimmune mechanisms of infection.

The bottom line and the failures of the therapy are possible deviations. In the study it is shown that the elimination of *Wolbachia* induces extensive apoptosis of germ cells in adults, and somatic cells in embryos (microfilariae, larvae). The American Heartworm Society nonetheless recommends doxycycline therapy due to its beneficial effects [31].

A major practical problem is resistance or multiresistance to the therapeutic agents by which parasitic diseases have previously successfully been treated. Malaria resistance to hintonol and artemisine derivatives has led to greater practical problems [32]. During our investigations, the first cases of resistance to antileishmania drugs occurred in 2008/2009. Repeated cure of treatment with *Glucantime* has not been successful, as well as the use of *Miltefosine*. We received good results after the introduction of *amphotericin B* in the therapy of our patients.

There is an opinion that therapy treatment for infection of *B. microti* for patients with good function of the spleen is not necessary and that those infections are self-confining. Therapy for infection with *B. divergens* is more problematic because it is more frequently found in asplenic and immunodeficient patients, with high level of parasitemia. The treatment requires combination therapy with *quinine sulphate* (600 mgr per os 3 x a day) and *clindamycin* (600 mgr per 3 x a day) for 710 days. *Pentamidine* can be an alternative drug. Examination of animal models has shown that good effects of cure can be achieved with *azithromycine*. American therapy schemes recommend curing of the heavy forms of babesiosis with combined therapy of *atovaquone* + *azithromycine* or *clindamycine* + *quinine*. In coinfections with *B. burgdorferi*, it is given the advantage to the cure of babesiosis and afterward it is carried on with curing borreliosis. In our study, common infection with babesia and *B. burgdorferi* has been detected in 73% of cases.

The much changes of natural and evolutionary factors put babesiosis in emerging human diseases. Clinical manifestations or asymptomatic infections are not always correlated with the severity of the disease. Asymptomatic parasitemia can be reactivated and take a malignant course in conditions of insufficient therapeutic treatment of coinfection and immunodeficiency. Grave manifest forms of diseases usually occur as an opportunistic infection.

Dirofilariasis has to be considered as a differential diagnosis in patients with subcutaneous or pulmonary disturbances (pneumonia). Effective therapy is possible by surgical removal of the adult worms with oral *diethylcarbamazine* (DEC) (2 mg per kg t.i.d.) over a period of 4 weeks was added to the surgical treatment in patients, only oral *ivermectine* (150 mg per kg).

5. Conclusion

In Montenegro endemic areas of leishmaniasis cover the southern part of the Montenegrin coast. More recent studies testify the extension of endemic areas in the entire coastal region and in accordance with the great expansion of the vector – Phlebotominae. Altered characteristics of leishmaniasis in Montenegro include: increase in the number of clinically manifested cases from the extended endemic area, which now includes the entire coastal areas, and increase of resistance to common therapeutic agents and drugs. The first cases of leishmaniasis resistant to *Glucantime* and *Miltefosine* were registered in 2008/2009, achieving significant results with *Amphoteracin B*.

The first cases of HIV coinfection with leishmaniasis were registered in 2014, as well as coinfection with other agents from complex VBD. Babesiosis is a parasitic disease that shares endemic areas with *B. burgdorferi*, which was proved in our investigations.

Dirofilariasis is a parasitic disease from the group of filariasis, which was first diagnosed in our country in 2014. There are no data in Montenegro on the experiences of veterinarians in the diagnosis of this disease among natural hosts and dogs. But it is surely present, based on data from Europe and countries in the immediate environment.

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