Surveillance of wildlife zoonotic diseases in the Balkans Region

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ABSTRACT

The countries of the Balkan Peninsula have become the region with frequent outbreaks of the emerging and re-emerging diseases during the last decade of the 20th and the first decade of the 21st century. The majority of outbreaks were wildlife zoonotic, and vector-borne diseases, such as brucellosis, leptospirosis, listeriosis, tularemia, Q-fever, Lyme disease, anthrax, rabies, viral hemorrhagic fevers, sandfly fever, tick-borne encephalitis and leishmaniasis. Epidemiological factors determined by ecology of causative agents are often the most useful diagnostic clues. The recognition of evolving problems of emerging and re-emerging diseases emphasizes the need for the development of better laboratory diagnostic methods for the surveillance and tracking of the diseases, and for continued research of factors contributing to the transmission of the organisms. The continuous occurrence of previously unidentified infections requires prospective national strategies for timely recognition of the syndromes, causative agent identification, establishment of criteria and methods for the diagnosis, optimization of the treatment regime, and determination of successful approaches to prevention and control. Wildlife diseases surveillance in the most of the Balkan countries has been coordinated by the WHO since 1992. Although new technology and communication have extremely improved in the last decade, there is a need for optimal communication lines among the Balkan countries, better exploitation of communication technologies like the Internet and other media in the field of emerging diseases.

Key words: surveillance, wildlife zoonotic diseases, Balkan Peninsula
INTRODUCTION

A zoonosis is an animal disease that may be transmitted to humans in natural conditions. Almost a half out of 1700 known pathogens affecting humans are estimated to be zoonotic (1). It has been reported that there are up to 335 pathogens which were associated with emerging infectious diseases (EID) in the global human population between 1940 and 2004, and majority of them were caused by the wildlife zoonotic diseases, while vector-borne diseases were responsible for up to 22.8% (1).

Many wildlife zoonotic disease (WZD) outbreaks were reported in the area of the Balkan Peninsula during the last twenty years (2). The wartime period (1992-1995) was associated with socio-economic, human demographics, behavioral, ecological and environmental (climate, ecosystems, microbial adaptation) changes that had significant impact on public health and global economy, as well as to wildlife zoonotic diseases (3). The additional variables which mostly influence zoonoses and vector borne diseases are human population density, human population growth, and wildlife and non-wildlife host species richness (1, 3-5). Breaking of communications channels between the former Yugoslav countries during the wartime and some years in post-war periods, and lack of political will to control the diseases have also greatly contributed to the appearance of WZD outbreaks in the Balkans area.

There is a need for different actions to be undertaken in order to recognize and control EIDs and WZDs. First of all, it is important to improve the reporting system of infectious diseases at the state, regional and World Health Organization level. It is also important to improve several tools for controlling EIDs and WZDs such as epidemiologic field investigation, control, elimination and eradication, training, diagnostic development, and basic and applied research activities including technology transfer. Surveillance of infectious diseases and continuous systematic collection, collation, analysis and interpretation of data and the dissemination of information to the authorities have to be established in order to take any actions (4-7). The purpose of the disease surveillance is to identify the changes of infection and/or health status of animal and human populations, and it is essential to provide rigorous evidence of the disease absence or to determine pathogen prevalence.

To be successful in the understanding WZDs a basic lack of integration between disciplines needs to be eliminated, the most noticeably between human and veterinary medicine and also among different branches within these fields. Although new technology and communications have extremely advanced in the last decade, there is still a need for better exploitation of communication technologies such as the Internet and other media in the field of EIDs (5-7).

WILDLIFE ZOONOSIS IN THE BALKANS REGION

The epidemiological situation related to zoonoses in the Balkan countries is not well characterized due to the difficult circumstances prevailing during the previous decade. There is a lack of reporting and publishing system of the infection and/or health status of animal and human populations. Although there were no readily accessible data concerning epidemiology and epizootology of WZDs, limited official and published data were analyzed.

Anthrax

Anthrax is endemic in some regions of the Balkan Peninsula, but it is not a significant public health problem (2). However, in the past century cutaneous anthrax cases with several clinical signs of septicemia were recorded in Bulgaria, Bosnia and Herzegovina (B&H) and Croatia, with a low mortality rate (1.64%) (7-9).

Brucellosis

Brucellosis is a significant health problem among animals and humans in the Balkan Peninsula. Brucellosis is spread in Greece, the former Yugoslav Republic of Macedonia, Kosovo, Serbia and Croatia (10, 11). It is an endemic disease among animals and humans in the southern part of the Balkan Peninsula, Greece, the Former Yugoslav Republic of Macedonia and Kosovo at the end of 20th century, which was a consequence of the changes in political situation in this area followed by the wartime. Appearance of brucellosis represents a classical example of spreading zoonosis as a result of the population and animal migration (9, 12, 13).

Brucellosis is an EID in B&H and it is still an increasing public health problem. Only one case
of human brucellosis was reported in 1999, while in 2008 there were 988 cases reported (14). The current animal health situation in B&H shows an increase in the number of reported outbreaks in ruminants. The number of seropositive cases as compared with the number of processed serums in last four years has ranged from 0.047% to 1.09% (11).

In Greece, cattle are also affected either by *B. melitensis* or *B. abortus*. Wild boars (*Sus scrofa*) were found to be carriers and reservoirs of *Brucella suis* biovar 2 in Croatia (15), whereas *Brucella suis* biovar 3 was isolated from horses in Croatia (16).

Due to the dimension of the disease-related problems, there is a need to establish cooperation in the elimination and prevention of brucellosis among all countries in the region, supported by World Health Organization.

**Leptospirosis**

Leptospirosis is an endemic zoonosis existing in Croatia and B&H (17-20). This is a widely spread disease with frequent epidemic occurrence, especially among miners in B&H and foresters in Croatia (18, 21, 22). Serological testing conducted in 1983 revealed antibodies to *Leptospira* serovars *pomona*, *grippotyphosa*, *sejroe*, *australis* and *bataviae* (21) in 10.08% of the B&H miners population. Leptospirosis outbreak among miners in B&H occurred 25 years later (2005) but caused by new serovars: *L. ballum*, *L. icterohaemorrhagiae*, *L. sejroe* and *L. tarassovi* (22). Serological tests in patients from Croatia have shown 18 different serovars of *Leptospira*, with the highest prevalence of *L. sejroe*, *L. pomona*, *L. australis* and *L. icterohaemorrhagiae* (23).

Leptospiroseroprevalence among small rodents captured in shafts of the lignite mines in B&H of 37.5% was noted in 1983 (21). An analysis of *Leptospira* sp. among small rodents in Croatia revealed three different species: *L. borgpetersenii*, *L. kirschneri* and *L. interrogans*. *Mus musculus* exhibited the highest infection level which was confirmed as a major reservoir of the serogroup *Sejroe* (23). Leptospirosero infection was also found among European brown bears (*Ursus arctos*) in Croatia (24), and based on the antibody titers, several serovars found were implicated: *australis*, *sejroe*, *canicola* and *icterohaemorrhagiae*. A strong correlation between serovars in bears and serovars previously isolated from small mammals in Croatia was noted (25).

**Listeriosis**

Listeriosis is present in the area of the Balkan Peninsula, but it is not a considerable public health problem. However, occasional cases of different clinical forms of leptospirosis have been recorded recently (26, 27). The analysis of the contamination level of different kinds of raw meat (raw beef, pork and chicken) in B&H showed overall presence of *Listeria* spp. in 49.4% samples; *L. monocytogenes* detected in 23.3%, *L. innocua* in 22.2% and *L. welshimeri* in 3.9% analyzed row meat samples (28). Both beef and pork were mostly contaminated by *L. monocytogenes*, in 34.3% and 25.7%, respectively. Chicken had the lowest level of contamination amounting to 10.0% (28). In a study conducted in Serbia, 45% of all pigs examined harboured *L. monocytogenes* in their tonsils, and 3% were intestinal carriers. *L. monocytogenes* was detected in 29% of swabs from retropharyngeal nodes and in 19% of fecal samples of cattle. *L. monocytogenes* was found in 69% of minced meat (mixed pork and beef) samples, in 19% of raw dry sausages, and in 21% of vacuum-packaged hot smoked sausages. However, *L. monocytogenes* was not detected in the hot smoked sausages heated to internal temperature of 70-75 °C after the fumigation process (29).

**Tularemia**

*Francisella tularensis* has been recognized as a human pathogen for almost 100 years and it is the etiological agent of the zoonotic disease, tularemia. The organism has been isolated from over 250 different species, including fish, birds, amphibians, rabbits, squirrels, hares, voles, ticks and flies. An important aspect of *F. tularensis* pathogenesis is the transmission via arthropod vectors into mammalian host. *Chrysops* spp. and *Tabanus* spp. also designated as deer fly and horsefly, respectively, are common arthropod vectors of *Francisella* transmission to humans, resulting in initial clinical presentation with ulceroglandular form of the disease (30, 31).

Due to its easy dissemination, multiple routes and low dose infection, morbidity and mortali-
ty rates, *F. tularensis* subsp. *tularensis* has been classified as a category A bioterrorism agent by the CDC (32).

Tularemia is an emergent infectious disease in several countries at the Balkan Peninsula. The first outbreak of tularemia occurred in B&H in 1995, during the wartime (33). A large tularemia outbreak occurred in Kosovo in the early postwar period, 1999-2000 (34). Only sporadic cases of tularemia have been recognized and reported since the first epidemic in Kosovo (32, 34). There are no data about tularemia seroprevalence in general population or in the high risk groups, e.g. foresters, hunters, veterinarians, or soldiers.

Epidemiological and environmental investigations were conducted to identify sources of infection, modes of transmission, and household risk factors in Kosovo. The results suggested that the infection was transmitted through contaminated food or water and that the source of the infection were rodents (34). Environmental circumstances in the war-torn Kosovo led to epizootic rodent tularemia and its spread to displaced rural populations living under circumstances of substandard housing, hygiene, and sanitation (34).

Tularemia outbreak areas in 1962 and 1997-2006 periods in Bulgaria were different. The first case of tularemia was reported in 1997. Starting from 1998 to 2008, 296 cases were registered. Amplified fragment length polymorphism (AFLP) and multiple-locus variable number of tandem-repeats analysis (MLVA) typing confirmed epidemic spread and evolution, and comparison of strains isolated from different regions in Bulgaria and Turkey resulted in the finding of the common “Balkan” genovar of *Francisella*. In addition, several novel genotypes of endosymbionts of *Francisella*-like organisms have been found in *Hyalomma* and *Dermacentor* ticks (9).

**Lyme disease**

Lyme borreliosis is an emergent disease in many Balkan countries: Bosnia and Herzegovina, Croatia, Slovenia, Serbia and Bulgaria (35-39). It is a zoonosis transmitted from animals to humans by ticks of *Ixodes ricinus complex*. Lyme borreliosis is caused by *Borrelia burgdorferi sensu lato*, which has four different species (36). In order to evaluate prevalence rate of tick-borne bacterial pathogens, unfed adult *Ixodes ricinus* ticks were collected from vegetation at 18 localities throughout Serbia in 2001, 2003, and 2004, a total of 287 ticks were examined by PCR technique for the presence of *Borrelia burgdorferi sensu lato*; prevalence rate for *B. burgdorferi sensu lato* was 42.5% (36, 39). The presence of five *B. burgdorferi sensu lato* genospecies, namely *B. burgdorferi sensu stricto*, *B. afzelii*, *B. garinii*, *B. lusitaniae*, and *B. valaisiana* was identified by restriction fragment length polymorphism (RFLP) analysis (39). The most frequent *B. burgdorferi sensu lato* genospecies was *B. lusitaniae*, followed by *B. burgdorferi sensu stricto*. These findings indicate a public health threat in Serbia related to tick-borne diseases caused by *B. burgdorferi sensu lato* (39).

**Q-fever**


Analyzing the available epizootiological and epidemiological data on the incidence of Q fever in the region, it could be concluded that beside classical locally focused character of the disease, incidence of new epidemics was also influenced by uncontrolled dislocation of animals, mostly sheep and goats (41- 44). Although some experts deny the significance of Q fever from the viewpoint of veterinary medicine, the latent forms of this disease in domestic animals and abortions in sheep and goats result in huge economic losses, thus confirming the importance of Q fever as a problem of the veterinary practice (39. 41). Moreover, having also in mind the fact that veterinarians together with other professionals are
very exposed to this infection, it is obvious that Q fever must not remain marginalized in the veterinary science. The frequent incidence of this disease in humans should be followed by systematic investigations of the infections in animals and natural reservoirs (42, 43).

Hantavirus infections

The Balkan Peninsula has been known as a highly endemic region for hantavirus infections. So far our research has shown that at least two different Hantaviruses (HTV) (the murine Dobrava (DOBV) and the avricoline Puumala (PUUV) viruses), each carried by a different rodent species, have been circulating in the area (44,45).

So far two viruses, Puumala and Dobrava have been identified as causative agents of hemorrhagic fever with renal syndrome in Albania, B&H, Greece, Croatia, Kosovo, Montenegro, Slovenia and Serbia (45-50). Additionally, Tula virus, which is considered a non-pathogenic hantavirus was detected in small mammals (51). However, Clethrionomys glareolus, Apodemus agrarius and Apodemus flavicollis are the main reservoirs of hantaviruses in the region (46). The incidence of haemorrhagic fever with renal syndrome (HFRS) varies in a cyclic fashion, with peaks occurring every three to four years, coinciding with peaks in rodents population (45). Several HFRS outbreaks were registered in the area of the Balkan Peninsula in: 1967, 1986, 1987, 1989, 1995 (45, 51), and 2002 with more than 1000 HFRS cases. Dual infections with hantaviruses and leptospires were also detected in humans as well as in rodents (52).

In the Balkan states where PUU and DOB viruses co-circulate, seroprevalence in general population is between 1.6% (Slovenia) up to 5.18% in B&H, which has been recognized as a highly endemic region for hantavirus infections for over 56 years (45,51,53). As early as in 1952 the first "probable" HFRS case was described in the region - the victim was a soldier and infection occurred near Fojinica city, B&H. The first documented HFRS outbreak was reported in 1967, followed by five outbreaks in the region and the majority of the affected population was located in B&H. The total number of HFRS reported cases was 1242, but the real number of HFRS cases is probably higher because the surveillance of infectious disease had not been regular until 1992 (53).

Rabies

Rabies remains endemic within a number of countries in Southeast Europe including Romania, Bulgaria, B&H and Turkey (7, 44, 55, 56). With the exception of Turkey, the red fox (Vulpes vulpes) is the principal disease reservoir in Southeast Europe. However, cases of rabies in dog (Canis familiaris) are regularly reported. In contrast to Northern Europe, the raccoon dog (Nyctereutes procyonoides) does not appear to be a vector in the south. In Bulgaria, dogs are the main vectors bringing rabies into contact with humans and livestock. Foxes are the principal reservoir species for rabies in Romania although cases in dogs are regularly reported. Despite a gradual decline in dog rabies, urban pockets of the disease remain in many regions of Turkey. Furthermore, there is some evidence that the foxes have been a significant vector for rabies and might be responsible for increased rabies in cattle. Rabies in Croatia has been registered in wild animals (mostly foxes) and sporadically in domestic animals (dogs, cats). The last human case was described in 1964. The fox and dogs are the principal reservoir species for rabies in B&H. Throughout the region there is evidence of cross-border movement of rabies by both wildlife and canine vectors (55,56).

The lyssaviruses currently consist of 7 established genotypes or lineages of rabies(-like) viruses (56) of which classical rabies virus is found throughout the world and associated with terrestrial mammalian hosts and American bats forms genotype 1. Phylogenetic relationships among sequences of five viruses isolated in B&H have shown the presence of two phylogenetic lines, one which is present in the Northwestern part and the other, which is present in the Northeastern part of the country. Viruses are closely related to Western European isolates of rabies virus (56).

Tick-borne encephalitis

Tick-borne encephalitis virus is the causative agent of the most prevalent arboviral human infections in Europe. Three subtypes have been identified: European (TBEV-Eu), Far Eastern (TBEV-FE) and Siberian (TBEV-Sib). The vector of TBEV-Eu transmission is the tick Ixodes ricinus, whereas I. persulcatus is the vector of other two subtypes. The virus is maintained in nature through cycles involving tick and wild vertebra-
te hosts and also by transovarial and transstadial transmission in its vector. Many different vertebrates have been implicated in the maintenances and circulation of the TBE virus. The major causative role in Central Europe seems to belong to *Apodemus flavicollis* and *Myodes glareolus*, not only because they are abundant in the regions where TBE incidence is high and they are excellent hosts for both nymphal and larval stages of the tick, but also because they promote transmission by co-feeding (44,57,58).

The distribution of TBEV is well coordinated with its vector distribution. There is a lack of data related to TBEV seroprevalence among the wild animals in the Balkan region. Data from Slovenia have shown that the infection prevalence in rodents sampled in Slovenia in the period between 1990 and 2008 entirely varied based on the rodent species, from 14% in *Myodes glareolus* to 2-4% in *Apodemus* species (57-59).

Tick-borne encephalitis (TBE) in Slovenia represents a serious public health problem with hundreds of official reported cases (58). In Croatia TBE was first discovered in 1953. The only really documented natural focus of tick-borne encephalitis was the northern part of the country, between the Sava and Drava rivers. Alleged cases from other parts of Croatia still have to be confirmed and analyzed, and additional research and collaboration between different professionals is required (44, 57, 59). Official data show no occurrence of TBE in Albania, Bosnia and Herzegovina, Bulgaria, the Former Yugoslav Republic of Macedonia and Turkey (57).

**Sandfly fever**

Sandfly fever viruses (SFV) are moderately endemic along the Adriatic coast of Albania, Croatia, B&H, Slovenia and landlocked Serbia, and locally distributed throughout Greece and the former Yugoslav Republic Macedonia (44, 60-65). Historical data of the sandfly fever (Pappataci fever) indicate its origin in B&H at the end of the 19th century (62, 63). Before the World War II, the pappataci fever in the former Yugoslavia was mainly detected in Herzegovina, Dalmatia, Montenegro and especially in Macedonia, where its prevalence coincided with that of *Phlebotomus papatasii* (64, 66). Most of the clinical and epidemiologic studies had been conducted in B&H between 1886 and 1962 (66-68), but there was no published data about the SFV infection between 1971 and 2006. The recent infection was found in 9.38% to 12.50% of patients during the period of three years (2006-2008) (68).

The first extensive serological investigations of the prevalence of the arbovirus infection were reported in 1975-1982 (64, 68-70). The results indicated a high prevalence of the antibodies to Naples and Sicilian sandfly fever viruses in Dalmatian population, North Croatia and Kosovo. On the island of Brač, 57.6% of the population had antibodies for the Naples virus and 15.6% for the Sicilian virus, in Kosovo- 27.9% and 9.6%, respectively. The prevalence of the *Naples virus* infection reached 62.1% in the inhabitants of B&H in 1962 (69). These results indicated the continuous circulation of Naples virus in nature.

Finding the TOSV positive results in clinical samples after more than forty years (66), means that the virus has been circulating in this region, but has not been the point of interest of local researchers. It is very important to direct the attention of clinicians to sandfly fever (papataci fever) because the disease was unrecognized in the region until recently.

**Leishmaniasis**

Leishmaniasis is widespread on the Balkan Peninsula. It is a protozoan parasitic infection caused by *Leishmania infantum* that is transmitted to human beings through the bite of an infected female sandfly (71). The disease occurs in urban centers and rural highland villages in Albania, B&H, Croatia, Greece, Montenegro, Serbia, Romania and Slovenia (71-76). Zoonotic visceral leishmaniasis (VL) is a re-emerging disease in the Balkan area (1, 3-5, 73, 74). The re-emerging is probably due to a combination of factors including increased monitoring, intensified research, demographic change, land-use/land cover changes that create new habitats and/or changes in microclimate, and changes in seasonal climate (2). Some human cases are usually detected in the area every year (74-76). Visceral leishmaniasis in 50 children was reported from Albania in 2009 (72).

Leishmaniasis is mostly a problem in veterinary medicine. The investigation of 272 dogs sera originating from different regions in Albania...
and Kosovo have shown specific antibodies for *Leishmania* in 3.3% of animals. All leishmania-positive animals were stray dogs (74, 75). Those animals contribute to the maintenance of *Leishmania* transmission in endemic areas, and a control of the canine stray population should be considered (74-78). A historical review on human and canine leishmaniasis in Croatia documents the presence of stable disease foci in coastal and insular territories of central and southern Dalmatia since the beginning of the 20th century (71,74). Among the species which may act as *Leishmania infantum* vectors in Croatia, *Phlebotomus tobbi* and *P. neglectus* were the most abundant (74).

**Echinococcosis**

Echinococcosis remains a very serious public health problem in Southeast Europe, although a decrease in incidence has been observed in some endemic areas during the last decades (77-80). However, in some non-endemic areas an increase in infected cases and new foci of animal echinococcosis were registered during the same time (77). The disease is very common in the Balkan Peninsula, with the former republics of Yugoslavia having one of the highest prevalence rates (77-80). Echinococcosis is zoonosis transmitted by dogs in livestock-raising areas and accidentally affects men. The most frequent site of hydatid cysts is in the liver (78%), followed by lungs (17%), and less frequently, spleen, kidneys, heart, bones, central nervous system, and elsewhere (80).

**Trichinellosis**

In the majority of Southeast European countries cases of trichinellosis among the human and animal populations were described in the late 19th or early 20th century (80-81). *Trichinella* infections among wildlife were also described in the aforementioned countries (5, 80). Today, the prevalence of trichinellosis between the Balkans and bordering countries is different (81-83). A high prevalence of trichinellosis in domestic animals and humans has been reported in Bulgaria, Serbia and Montenegro, Romania and Croatia (80-84), and a moderate prevalence was found in B&H. Trichinellosis has not been found among domestic animals and humans in Greece and Macedonia in recent years, while in Turkey and Slovenia human trichinellosis is sporadic (83). A re-emergence of trichinellosis is connected with the changes in the social and political systems in Bulgaria and Romania (81, 84). In B&H, Kosovo, Serbia as well in Croatia, however, the re-emergence of trichinellosis did not only happen due to political and social changes, but also due to the war that took place in these countries during the last years of the 20th century (84).

There are some other zoonoses and vector-borne diseases with more or less documented evidence on epidemiological, clinical or etiological features (1, 8, 44, 85, 86). The special attention deserves tuberculosis and rickettiosis, because the outbreaks affected the history of the Balkan Peninsula.

**THE SURVEILLANCE OF WILDLIFE ZOONOSIS DISEASES**

The last outbreaks of avian A (H5N1) and swine influenza A (H1N1) showed the importance of greater collaboration between physicians and veterinarians as well as for the disease surveillance in humans, domestic animals and wild animals (5). The surveillance of diseases in wild animals is a relatively new activity compared to the surveillance of diseases in humans or domestic animals (5, 7). At the moment, there is no Balkan-wide network of health surveillance. Since 1992, the disease surveillance in most Balkan countries is irregular and coordinated by the WHO. A number of new zoonotic infections emerged or re-emerged during the past 17 years, caused by social, political, climate and environmental changes in the area. The communication channels among Balkan countries were broken, so preventative action has not been done. There is an urgent need for building of a European health surveillance network. It should include the following elements: national surveillance programs that cover the whole country; human diseases, domestic animal diseases, all wildlife species, and all diseases; comparable methodology among surveillance programs, so that it is valid to compare results; strong interaction among surveillance programs, so that new information and knowledge is rapidly and efficiently shared.

Recent initiative called One Health, which supports the unique approach to zoonoses, is a concept of the worldwide strategy for expanding
interdisciplinary collaborations and communications in all aspects of health care for humans and animals (86).

ACKNOWLEDGEMENTS/DISCLOSURES

Competing interests: none declared.

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