Zoonoses and vector-borne diseases in Croatia – a multidisciplinary approach

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Summary
Emerging and re-emerging infectious diseases create constant and serious concerns for public health. The majority of emerging infectious diseases (EID) are wildlife zoonotic diseases and vector-borne diseases. Croatia has a long tradition in the control, management and research of EID zoonotic diseases and vector-borne diseases. There has also been a long and advantageous tradition in the collaboration of different experts and professionals in EID research in Croatia involving physician clinicians in infectious diseases, microbiologists, pathologists, veterinarians and animal scientists, ecologists, forestry experts, wildlife scientists, public health specialists and epidemiologists and laboratory scientists. The University Hospital for Infectious Diseases in Zagreb established the Centre for Emerging and Re-emerging Infectious Diseases in liaison with national and international partners from Europe and the United States. This Centre is working in line with the ‘One Health initiative’ which recognises the inter-relationships between human, animal and environmental health.

Keywords
Croatia, Emerging disease, Environment, Infection, One Health, Public health, Vector, Zoonosis.

Zoonosi e malattie vettoriali in Croazia. Un approccio multidisciplinare

Riassunto
Le malattie infettive emergenti e riemergenti rappresentano un pericolo serio e costante per la salute umana. Le malattie infettive emergenti (EID) sono malattie vettoriali trasmesse da animali selvatici. La Croazia vanta una lunga tradizione nelle attività di controllo, gestione e ricerca delle zoonosi emergenti e delle malattie vettoriali, così come collaborazioni fruttuose e di lunga data tra esperti e professionisti di vari settori, tra cui clinici specializzati nelle malattie infettive, microbiologi, patologi, veterinari e ricercatori nel campo della salute animale, ecologi, esperti di silvicoltura, ricercatori nel campo della fauna selvatica, specialisti di salute pubblica, epidemiologi e tecnici di laboratori. In collaborazione con partner

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nazionali e internazionali, sia europei che statunitensi, l’Ospedale universitario per le malattie infettive di Zagabria ha creato il Centro per le malattie infettive emergenti e riemergenti che opera secondo i principi dell’iniziativa “Una sola salute”, ovvero nel riconoscimento delle interrelazioni tra la salute umana, animale ed ambientale.

Parole chiave
Ambiente, Croazia, Infezione, Malattia emergente, Salute pubblica, Una sola salute, Vettore, Zoonosi.

Emerging infectious diseases and zoonotic and vector-borne diseases

It was recently reported that between 1940 and 2004, a total of 335 infectious diseases emerged in the global human population. The majority (60.3%) of emerging infectious diseases (EID) were caused by wildlife zoonotic diseases, while vector-borne diseases were responsible for up to 22.8% of these events. The EID origins correlate with socio-economic, ecological and environmental factors and have a significant impact on public health and global economies (24).

Different factors influence EID, such as: changes in human demographics and behaviour, climate and weather, ecosystems, international travel and commerce, microbial adaptation and change, a breakdown of public health measures, poverty and social inequality, wars and the lack of political will to stop some diseases. Additional variables which mostly influence zoonoses and vector-borne diseases include the following: human population density and growth, latitude, rainfall, wildlife and non-wildlife host population number (24, 41, 46). To be able to recognise and to control EIDs different actions and tools are required, in particular: epidemiological field investigations, the application of various disease prevention and control measures, training, development of diagnostics, basic and applied research activities including technology transfer. To be successful in the war against EID microbes, trained, experienced and devoted experts and professionals are required, including clinicians in infectious diseases, microbiologists, pathologists, veterinarians and animal scientists, ecologists, forestry experts, wildlife scientists, public health specialists and epidemiologists, as well as laboratory scientists. Also of importance are diagnostic tools, vaccines and drug manufacturers that invest in the development of research and technology transfer. These are essential components for the prevention and eradication of zoonotic EIDs and vector-borne diseases. Although new technology and communication have vastly improved in the last decade, still there is the need for better exploitation of communication technologies, such as the Internet and other media in the field of EIDs (15, 24, 37, 41, 46).

Zoonoses and vector-borne diseases in Croatia

Croatia has a long tradition in the control and management zoonotic EIDs and vector-borne diseases and has also extensive experience in research. For example, the first human case of eperythrozoonosis was discovered in Croatia (58). There is also a long and advantageous tradition between different experts and professionals working together in the field of research of zoonotic EIDs and vector-borne diseases in Croatia (physician clinicians in infectious diseases, microbiologists, pathologists, veterinarians and animal scientists, ecologists, forestry experts, wildlife scientists, public health specialists and epidemiologists as well as laboratory scientists). The oldest Croatian manufacturer of biologicals, vaccines, diagnostics, and blood products, the Institute of Immunology, established in 1893, has contributed significantly to the research and prevention of EIDs.

Some important zoonotic EIDs and vector-borne diseases that have occurred in Croatia are discussed here.

Anthrax

Anthrax is not a significant public health problem in Croatia. However, in the past century, cutaneous anthrax cases with many
different clinical signs of septicemia were collected in Croatia; mortality rates were low (1.64%) (8).

**Avian influenza**

To date, avian influenza has not created a problem among poultry stocks and veterinary medicine in Croatia. However, the H5N1 virus (strain A/Cygns olor/Croatia/1/05) was isolated from swans in Croatia, but also detected in wild birds in the Baranja region and surrounding areas of Zagreb and Trogir. Fortunately, no human cases have ever been recorded (1, 71). The multidisciplinary board which included physicians, veterinarians, agriculture workers, epidemiologists and forestry workers managed different activities, including euthanasia of domestic poultry in the regions where the virus was detected in wild birds (I. Kuzman and I. Puljiz, personal communication). This prompt and multidisciplinary approach is believed to have prevented avian flu in humans in Croatia.

**Bartonella henselae**

*Bartonella henselae* occasionally causes cat-scratch disease, a self-limiting infectious disease with lymphadenopathy in Croatian patients who are mostly children who have been in contact with cats. Early diagnosis was essential to ensure the success of antimicrobial treatment (28, 53, 70).

**Borreliosis (Lyme disease)**

Borreliosis is endemic in north-western Croatia (45). In a serological and epidemiological study, human sera were analysed using the indirect immunofluorescent assay (IFA), and ten out of 134 serum samples were positive for *Borrelia burgdorferi* antibodies. In a serological/epizootiological study, wildlife and domestic animals were tested by inhibition enzyme-linked immunosorbet assay (ELISA). Antibodies to *B. burgdorferi* were found in 9 out of 42 sera from roe deer (*Capreolus capreolus*) and, in 3 out of 9 sera from hares (*Lepus spp.*). Sera from wild boar (*Sus scrofa*) (n = 10), cattle (n = 103) and dogs (n = 13) gave negative results for *B. burgdorferi* antibodies. The presence of *B. burgdorferi sensu lato* was detected in *Ixodes ricinus*. Ticks (n = 123) were collected at five different locations and were analysed using the polymerase chain reaction (PCR). *B. burgdorferi sensu lato* DNA was detected in 45% of ticks (20). The disease has also been recorded in other parts of the country, with the exception of the area south of Zadar (45). A serological/epidemiological study was conducted on 520 healthy subjects that were divided into three groups, as follows:

- 234 forestry workers (residents of Gorski Kotar)
- 100 residents of various professions in the same region
- 186 subjects of various professions from the neighbouring region.

Sera from 10 hunting dogs from Gorski Kotar were also analysed. The IgG antibodies to *B. burgdorferi sensu lato* were found in 4.7% of forestry workers in 3% participants from the second group and in 2.7% from the third group. Four out of 10 dogs had IgG *B. burgdorferi* antibodies. The results showed that the forest and mountainous area of Gorski Kotar has the characteristics of a low seroprevalence area, in contrast to the endemic neighbouring areas (49). In Croatia, *B. burgdorferi* was first isolated in 1991 at the Department of Dermatology and Venereology of the Zagreb University Hospital Centre, from the skin of a patient, and was named P1 Zagreb (65). Phylogenetic tree analysis placed the borrelial isolates with *B. afzelii* sequences into a single group (64). The clinical picture of Lyme borreliosis in Croatia is dominated by erythema migrants, followed by neurological manifestations. The diversified clinical picture is consistent with reports from other European countries, as is the isolation of *B. afzelii* and *B. garinii*, which are causally related to these forms of the disease (45).

**Boutonneuse fever (Mediterranean spotted fever)**

Boutonneuse fever has been recognised in Croatia. The identification of endemic rickettsiosis in northern Dalmatia, severe clinical forms of the disease and the success of early, adequate antibiotic therapy are a clear
warning that our physicians must be familiar with Boutonneuse fever (17, 56, 57, 59, 61).

**Brucellosis**

Brucellosis does not present a significant health problem among animals or humans in Croatia. However, on account of the increasing problems caused by brucellosis and continuous outbreaks in the neighbouring countries (Bosnia and Herzegovina, Bulgaria, the former Yugoslav Republic of Macedonia and Greece), there is a continuous need for international collaboration to prevent and hopefully eradicate brucellosis from all countries in the region (66). Wild boar were found to be reservoirs of *Brucella suis* biovar 2 in Croatia (11) and *Brucella suis* biovar 3 has been isolated from horses in Croatia (12).

**Chlamyphila psittaci**

*Chlamyphila psittaci* was recently confirmed among pigeons in free-living birds in Croatia. A very high percentage (95.6%) of sera collected from 278 pigeons (*Columbia livia*) and 54 birds of 11 other free-living species captured in various locations in Zagreb were seropositive. Additional laboratory diagnostics confirmed that 15.83% of pigeons were antigen-positive. Although the pigeon serovars of *C. psittaci* are considered to be of moderate pathogenicity for humans, such a high percentage of antigen-positive pigeons presents a potential source of infection to humans, especially for elderly people and immunodeficient patients (50). In humans, *C. psittaci* was found to cause about 19% of all atypical pneumonias in Croatia (69). However, according to our data and clinical experience, the incidence of psittacosis in Croatia is very low and is declining (I. Puljiz and I. Kuzman, personal communication). We consider that the reason for this situation is due to efficient action and to controls conducted by veterinarians in collaboration with epidemiologists.

**Dengue fever**

Dengue fever appears to be one of the most important emerging disease problems among international travellers. Croatia is dengue-free but, the recently discovery of the presence of *Aedes albopictus* in Croatia (25) and two imported cases of dengue in 2007 have attracted the attention of public health and medical professionals in Croatia (16, 35), especially because Croatia seems to have become an attractive tourist destination in the recent years.

**Hantaviruses**

Hantaviruses are endemic in Croatia (32). To date, two viruses, Puumala and Dobrava, have been identified as causative agents of haemorrhagic fever with renal syndrome (HFRS) in Croatia (9, 27, 34, 38, 43). Additionally, the Tula virus, which is considered a non-pathogenic hantavirus, was detected in small mammals (62). However, *Myodes glareolus*, *Apodemus agrarius* and *A. flavicollis* are the principal reservoirs of hantaviruses in the country (27, 34, 38). The incidence of HFRS varies in a cyclic fashion, with peaks registered every couple of years, coinciding with peaks in rodent populations (9). Two extensive HFRS outbreaks were recorded in Croatia in 1995 (27, 32, 34) and 2002 (9, 38) with over 150 and 400 HFRS cases, respectively. Dual infections with hantaviruses and leptospirosis were also observed in humans (33) as well as in rodents (10).

**Leishmaniasis**

Leishmaniasis is mostly a problem for veterinarians in Croatia, although several human cases are detected in southern Croatia every year (4, 7, 44, 55, 72). Zoonotic visceral leishmaniasis is a re-emerging disease in the Mediterranean area. 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 (4, 7). Among the species that may act as *Leishmania infantum* vectors in Croatia, *Phlebotomus tobbi* and *P. neglectus* were the most abundant (7).

**Leptospirosis**

Leptospirosis is endemic in Croatia (10, 22, 30, 33, 40, 48, 67). A detailed analysis of *Leptospira* spp. strains in small rodents captured in 11 different regions of inland Croatia was
performed recently. Sixteen *Leptospira* spp. strains were isolated from small rodents and 10 isolates were attributed to the serovar *istrica*, five isolates to the serovar *tsaratsovo* and one to the serovar *lora*. Phylogenetic analysis revealed that the strains belonged to three different species, namely: *L. borgpetersenii*, *L. kirschneri* and *L. interrogans*. *Mus musculus* showed the highest infection level and confirmed its role as a major reservoir of the serogroup *Sejroe*. The occurrence of serovars *tsaratsovo* and *lora* was recently reported in Croatia (67). *Leptospira* infection was also found in the European brown bears (*Ursus arctos*) from three areas of Croatia. Based on the antibody titres, several serovars were implicated, namely: *australis, sejroe, canicola* and *icterohaemorrhagiae*. There was a strong correlation between serovars in bears and serovars previously isolated from small mammals in Croatia (40). Serological testing of patients revealed 18 serological types of *Leptospira*, among which types *L. sejroe, L. pomona, L. australis* and *L.icterohaemorrhagiae* prevailed (48).

**Listeriosis**

Listeriosis is not a considerable public health problem in Croatia. However, cases have been recorded from time to time. Recently, *Listeria monocytogenes* meningitis, associated with rhabdomyolysis and acute renal failure, was described in a sixty-nine-year-old male hospitalised at our clinic (26). Annually, we register 3–4 patients with systemic listeriosis in our hospital (I. Puljiz, personal communication).

**Malaria**

Malaria is a disease that has been known since Ancient times. The first written documents on malaria in Croatia date back to the 16th century and concern the Istria peninsula. Until the end of the 16th century, malaria spread almost throughout Croatia. During the 19th century, areas affected by malaria in Istria and the Neretva Valley were sanitised with the aim of eradicating the disease. However, the first significant results were not achieved until the beginning of the 20th century after the arrival of Robert Koch and his associates in the Croatian islands of Brijuni. They managed to eradicate malaria by systematic use of quinine for the entire population and a number of other procedures such as land improvement and population education. Robert Koch’s method of eradication of malaria showed outstanding results in 1903. According to a report published in 1932, there have not been significant outbreaks of malaria since those times (19, 23). Malaria had been a major public health problem in Croatia until 1964 when it was declared officially free from the disease. No autochthonous infections have been noted since 1958 (42). Although malaria has been eradicated from Croatia, about twelve cases of malaria are imported into Croatia on average per year (44). Malaria is one of the leading causes of fever resulting from travel to tropical or subtropical countries. The risk is very high, especially for sailors (60). Mosquitoes which are malaria vectors, are present in Croatia even today. There are still several vector species of the *Anopheles maculipennis* complex in this region (36, 42). Sporadic cases of autochthonous malaria in neighbouring countries, from which malaria was officially eradicated, have provided a warning that cases of autochthonous malaria could also appear in Croatia (36).

**Plague**

Plague is another ‘historic’ zoonosis in Croatia. The 14th century was characterised by the threat and frequent outbreaks of plague, a disastrous pandemic disease which spread across all of Europe. It devastated Dalmatian towns and islands, especially Split and Dubrovnik. In many towns, there were no survivors. However, the first successful preventive measures were implemented. Considering that outbreaks mostly occurred after travellers and ships from different plague-stricken parts of the world entered the Dalmatian towns, an obligatory measure of isolation of people and goods was introduced. The first quarantine station was established in Dubrovnik in 1377 (3).

**Q fever**

Q fever is endemic in rural, coastal, and non-coastal areas of southern Croatia and is
associated with stock breeding. In these areas, Q fever occurs sporadically and epidemically (29, 39). The sera of dogs, goats, sheep and cattle (total: 153) from the central areas of the eastern coast of the Adriatic Sea were tested for antibodies against Coxiella burnetii. The overall percentages of positive sera among the tested animals were 16.4% for C. burnetii. The results revealed that animals in this area were clearly exposed to C. burnetii (54). In the past ten years, Q fever has been found to be endemic in certain regions of north-western Croatia (surrounding Dugo Selo) (52).

**Rabies**

In Croatia, rabies has been recorded in wild animals (mostly foxes) and sporadically in domestic animals (dogs, cats). The last human case was reported in 1964 (2). However, we registered two imported cases in 1989 and 1996, both with a fatal outcome, at our hospital (I. Puljiz, personal communication).

**Tick-borne encephalitis**

Tick-borne encephalitis was first discovered in Croatia in 1953. The only thoroughly documented natural focus of tick-borne encephalitis was in the northern part of the country, between the Sava and Drava Rivers. Alleged cases from other parts of the country still need to be confirmed and analysed and additional research and collaboration between different professionals are required (6, 68).

**Trichinellosis**

A high prevalence rate of trichinellosis has been recorded in domestic animals and humans, and also in wildlife in the north-east of the country, but also in some other regions in south-eastern Europe, such as Bulgaria, Montenegro, Romania and Serbia (13, 14, 31, 47, 51, 63). One of the recent outbreaks was detected in Brod-Posaviska County between October and November 2004. A total of 64 trichinellosis cases were reported to the Institute of Public Health (13). Studies in Croatian pigs indicate a higher risk of infection for outdoor farming in areas where Trichinella is endemic (31, 47). Studies on genotypes suggest that T. spiralis is the most prevalent aetiopathogenic agent of domestic trichinellosis in Croatia and the sylvatic species, T. britovi, can be also present in domestic habitats, which suggests a link between the sylvatic and domestic cycles (31, 47, 63). The parasite was also found to be prevalent in rats. However, the finding that no infected rat was found on farms with T. spiralis-negative pigs suggests that, in the area investigated, the brown rat (Rattus norvegicus) is not a reservoir but only a victim of improper pig slaughter procedures (31, 63).

**Tularemia**

Tularemia is endemic and was recognised as a zoonosis in Croatia many years ago. It is mostly observed during the winter among hunters and others in contact with hares in the Moslavina region along the Sava River (5, 18, 21).

Other zoonoses and vector-borne diseases have been reported, with less documented evidence on the epidemiological, clinical and aetiological features (44, 68).

With the new era of increased emerging and re-emerging infectious diseases, multidisciplinary collaboration should receive additional support in the future. To be prepared for new challenges which zoonoses and vector-borne diseases bring in the form of emerging and re-emerging infectious diseases (REIDs), we have established a new Centre for Emerging and Re-emerging Infectious Diseases (CERID). The background and aims of this project are described briefly below.

**Centre for Emerging and Re-emerging Infectious Diseases**

The University Hospital for Infectious Diseases ‘Dr Fran Mihaljević’ in Zagreb established this centre, together with national bodies (Institute of Immunology, Zagreb; Public Health Institute Brodsko-Posavska County, Slavonski Brod; Faculty of Forestry, Zagreb; Faculty of Veterinary Medicine, Zagreb; Croatian Academy of Sciences and Arts, Zagreb and Institute ‘Ruder Bošković’, Zagreb), and also with the collaboration of international partners from Europe and the United States (Fig. 1).
This project (J-29) was approved in May 2007 by the Croatian Ministry of Science, Education and Sports (Science & Technology Projects (HITRA) – ‘CORES’) and initial funds were allocated to this important initiative.

Through this Centre, we plan to apply a multidisciplinary approach to EIDs/REIDs, as follows:

- **Diagnostics:** to prepare, introduce and evaluate new diagnostic methods and tests for detection of EIDs/REIDs in human and animal reservoirs
- **Research:** to detect reservoirs and/or vectors of EIDs/REIDs, conduct serological, epidemiological, ecological, clinical and immunological research in the general population as well as in professionally exposed groups; *in vitro* research on replication mechanisms of pathogens, immunoreactions and cell signalling mechanisms and the efficacy of existing and new drugs and immunological in prevention and treatment of EIDs/REIDs. Special attention will be devoted to the development of new drug candidates and immunologicals.
- **Education:** to organise national and international courses and conferences on EIDs/REIDs, doctoral studies through existing and new programmes and continuous theoretical and practical education of target populations through national and international frameworks.

Several joint projects are already in preparation. Among the most exciting projects is that devoted to telemedicine in EIDs and infectious diseases in general which has been

**Figure 1**

The Centre for Emerging and Re-emerging Infectious Diseases (CERID) – The University Hospital for Infectious Diseases ‘Dr Fran Mihaljević’, Zagreb, established this Centre, together with links to national and international partners from Europe and the United States.

Applications will be made through this Centre for multidisciplinary projects that target diagnostics, research and education fields for emerging and re-emerging infectious diseases.

This Centre is in strong agreement with the ‘One Health Initiative’ which recognises the inter-relationships between human, animal and environmental health and seeks to enhance communication, cooperation and collaboration by integrating these areas in the interests of the health and well-being of all species.
established between The University of Texas Medical Branch in Galveston (‘UTMB’) in Texas and the University Hospital for Infectious Diseases in Zagreb. Other contributors from Africa and South America are considered as partners in this project.

The future activities of the CERID will include reinforced and productive collaboration between all partners. The projects that have been established since 2002 devoted to research into the hantaviruses and leptospirosis and to ecology, epidemiology and diagnostics involved physicians, veterinarians, biologists and forestry workers form several institutions.

Fields surveys of rodents have been organised with the collaboration of forestry workers from the Faculty of Forestry in Zagreb and from the Finnish Forest Research Institute in Vantaa, with the support of epidemiologists from the Institute of Public Health in Brodsko-Posavsk County, veterinarians from the Faculty of Veterinary Medicine in Zagreb and physicians and biologists from our hospital in Zagreb. In addition, human serological surveys on different zoonoses among the forestry workers has been established by the same partners and diagnosis has been performed in collaboration with the experts from the following institutions: University Hospital for Infectious Diseases in Zagreb, Faculty of Veterinary Medicine in Zagreb; Hartmaan Institute, University of Helsinki, Finnish Forest Research Institute in Vantaa, Medical Faculty University of Ljubljana in Slovenia and Centers for Diseases Control and Prevention in Atlanta, Georgia. The same partners plan future collaboration on the detection and the field research into various zoonoses and vector-borne diseases in Croatia, but also in Bosnia and Herzegovina in liaison with The Medical Faculty University of Mostar and The Institute for Microbiology in Sarajevo.

Future plans devoted to the research of immunopathogenesis of zoonoses and rodent-borne diseases will be conducted with the scientists from the Institute of Immunology in Zagreb and the Institute ‘Ruder Bošković’, also in Zagreb, and other international partners such as the School of Medicine in Albuquerque, New Mexico, The United States Army Medical Research Institute for Infectious Diseases (USAMRIID) and other partners mentioned above. The overall activities will be supported by the consultative services provided by the Croatian Academy of Sciences and Arts Zagreb, Croatia, Medora Global Consultants, McLean, Virginia and Medora Inc. in Zagreb. Furthermore, both Medora consultant companies will provide support to the design of the different projects, for example in research projects (FP7, NIH), but also projects with industry (diagnostics and biologics design and production) at the national (e.g. the Institute of Immunology in Zagreb) and international levels.

This Centre strongly supports the ‘One Health Initiative’ which recognises the essential inter-relationships between human, animal, and environmental health and seeks to enhance communication, cooperation, and collaboration by combining these areas of expertise in the interests of the health and well-being of all species.

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