



## New trends in human and animal leptospirosis in Croatia, 2009–2014



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### ABSTRACT

Leptospirosis is a geographically widespread and globally underestimated zoonosis that affects humans and variety of animals. To identify trends and possible risk factors, joined medical and veterinary teams investigated epidemiology and epizootiology of leptospirosis in Croatia. Retrospective analysis of data obtained from referent diagnostic laboratories included a total of 1917 human and 123964 animal sera tested in the period from 2009 to 2014. We found high human leptospirosis average incidence rate of 1.53/100000 with clear predominance of male patients older than 40 years (sex ratio M/F:3.2; median age  $51 \pm 15.1$  years). Statistical analysis revealed seasonal and annual variations of incidence in humans that were primarily associated with favourable weather conditions (temperature 10–19, 9 °C and precipitation above 100 mm/m<sup>2</sup>). Majority of infections in humans were caused by serogroups Sejroe, Australis and Icterohaemorrhagiae. Notable variations in seroprevalence and changing trends in prevailing serogroups were recorded in most of the domestic animals and during the entire period of investigation. All of the observed findings underline leptospirosis as a significant human and veterinary public health threat and emphasize the importance of continuous multidisciplinary surveillance. We also argue that only input from both professions improves our overall knowledge on leptospirosis and leads to better and more efficient prevention and control strategies.

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### 1. Introduction

Leptospirosis is a re-emerging veterinary and public health problem caused by different pathogenic serovars of the genus *Leptospira* (Hartskeerl et al., 2011). Disease is usually endemic and sustained in a certain environment by variety of animal species with rodents as primary reservoirs. Different animal species are maintenance hosts of distinct serovars. Domestic animals can serve as maintenance hosts (e.g. cattle for serovar Hardjo, dogs for serovar Canicola) or incidental hosts when infected with serovars carried by other domestic or free-living animals. In maintenance hosts disease is usually subclinical or mild, while in incidental hosts infection is often accompanied by the emergence of certain clinical signs that vary depending on the animal species and infecting serovar (Faine et al., 1999; Ellis, 2015). On the other hand, humans are always incidental hosts and disease is caused by the infection with serovars

maintained by the animals in that particular region. Consequently, in spite of the large number of known pathogenic serovars (almost 300), only a limited number occur in certain geographical area and cause disease in humans and domestic animals (Ellis, 2015; Levett, 2015). Therefore, knowledge on prevalent serovars/serogroups in particular area is essential for understanding epidemiology of leptospirosis and allows us to develop effective control and eradication schemes.

Leptospirosis in Croatia has been studied for almost a century. First confirmed case was described in a dog in 1926 (Babic, 1927) and was followed by clinical description of leptospirosis in human patient eight years later (Antunovic-Mikacic, 1935). Since then, a multidisciplinary team consisting of infectious disease specialists, epidemiologists, veterinarians and foresters is engaged in on-going research and monitoring of leptospirosis on Croatian territory. Endemicity of leptospirosis in big river valleys in continental parts of Croatia as well as relatively high incidence of disease in humans is already well established (Pappas et al., 2008; Balen Topic et al., 2010). In addition, certain specificity concerning prevalence of presumptive infective serovars in humans as well as connection

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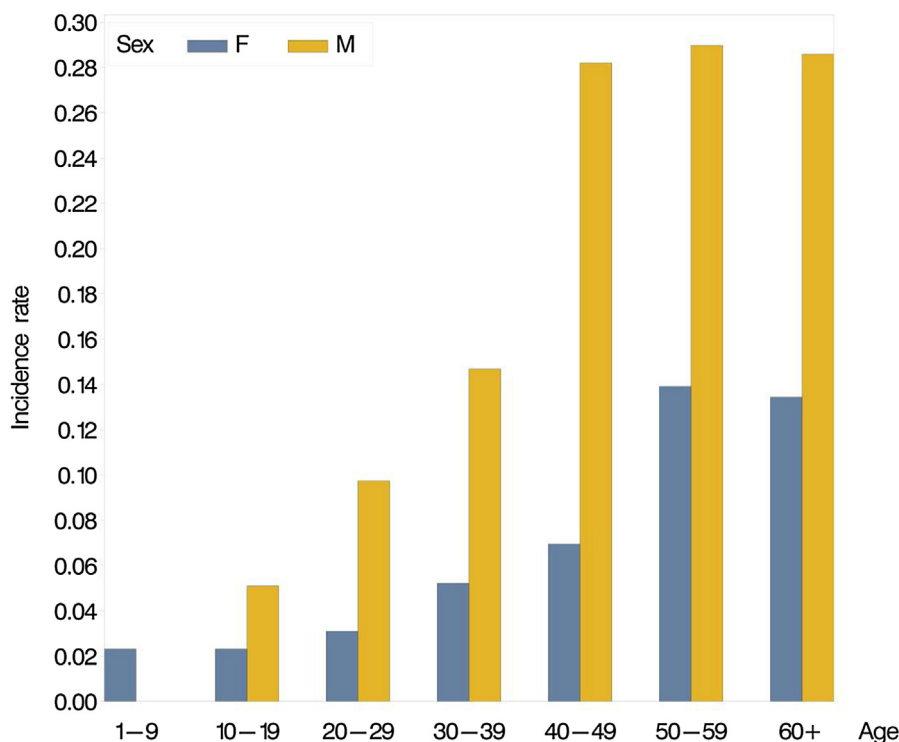


Fig. 1. Correlation of leptospirosis incidence with gender and age of patient, Croatia, 2009–2014.

of increased incidence of human leptospirosis with so-called “mice years” was also noted (Turk et al., 2003; Turk et al., 2009; Balen Topic et al., 2010; Stritof Majetic et al., 2014).

The aim of this study was to analyse recent trends of human and animal leptospirosis and to define certain specificities in epidemiology and epizootiology of leptospirosis in Croatia. In addition, we investigated influence of changing climate conditions on incidence of the disease.

## 2. Materials and methods

### 2.1. Study area

Croatia’s territory covers 5,594 km<sup>2</sup> and has a population of 4.3 million with density rate of 75.9 inhabitants per square kilometre. Croatia lies between latitudes 42° and 47° N and longitudes 13° and 20° E. Forest and forest land cover 48% of the country. Big part of the Croatian territory (53.4%) belongs to Panonian basin with lowlands and elevations of less than 200 m (660 ft) above sea level. This area includes the largest rivers flowing in the country. Because of its climate (moderately warm and rainy continental climate) and geomorphology Croatia has a number of ecoregions and is among the countries with the highest biodiversity in Europe. Current average age of population is 39.9 and 43.4 for men and women respectively. Life expectancy at birth is also age dependant with current estimation of 73.8 for men and 89.9 for women (Anon., 2015).

### 2.2. Study population

During study period (2009–2014) a total of 1917 human and 123964 animal sera were tested for presence of specific leptospiral antibodies. Animal samples included 364 canine, 41016 equine, 22669 bovine, 41752 small ruminant and 18163 pig sera. Human and canine sera were tested when disease was suspected by the clinician’s based on the manifestation of specific clinical signs. Live-stock sera were tested in accordance with the requirements of

annual Orders on measures for protection of domestic animals from infectious and parasitic diseases issued by the Veterinary Directory. Surveillance systems included in this document require serological testing of (1) bulls, rams, goats, boars and stallions used for the production of semen for artificial insemination and natural mating; (2) horses used for sports and recreation and (3) cows, sheep, goats, sows and mares that aborted.

### 2.3. Laboratory diagnosis

Data analysed in this study were obtained from the archives of National Referent Laboratories (Croatian Institute of Public Health and Faculty of Veterinary Medicine University of Zagreb) and Laboratory for bacterial zoonosis and molecular diagnostics of bacterial diseases laboratory at Veterinary Institute. Diagnosis was based on serological results inferred from microscopic agglutination test (MAT). MAT was performed following standard procedure (Dikken and Kmetz, 1978; Hartskeerl et al., 2006) using a panel of 12 *Leptospira* serovars: Grippothyphosa, Sejroe, Australis/Bratislava, Pomona, Canicola, Icterohaemorrhagiae, Tarassovi, Saxkoebing, Ballum, Bataviae, Poi and Hardjo. This panel was supplemented with serovars Autumnalis and Patoc when performing diagnostics in humans.

Cut-off values varied between species. In humans a single titre of  $\geq 500$  in symptomatic patient was considered positive. In accordance with legal regulations pigs, small ruminants and cattle was considered positive at titre  $\geq 100$ . Cut-off value for horses was 1:200 for serovars Australis and Bratislava and 1:400 for other serovars (Anon., 2011). In order to eliminate animals with positive reactions due to vaccination, dogs were considered positive when titre was  $\geq 800$ .

Presumptive infective serogroups were determined by identifying the highest titres to one or more serovars belonging to a certain serogroup. In humans, when identification of presumptive infective serogroup was impossible due to the cross-reactions, e.g. same titre to two or more serovars belonging to different serogroups,

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