

HUMAN LEPTOSPIROSIS IN BULGARIA – EPIDEMIOLOGICAL ANALYSIS OF THE INFECTION, 2014-2023

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ABSTRACT

Background

Leptospirosis is a zoonothonosis with natural outbreaks, of great significance for public health worldwide. According to data from the latest ECDC report, there are 765 confirmed cases of leptospirosis in Europe. Human cases of leptospirosis in Bulgaria have been reported since 1952. A trend towards a decrease in the number of registered cases of leptospirosis in our country has been observed in the last two decades. This study was focused on the analysis of circulating serovars of the genus *Leptospira* and their distribution, and epidemiological characteristics of laboratory confirmed cases of human leptospirosis for a ten-year period (2014-2023). It was interesting to reveal the relationship between clinical symptoms, transmission mode and sources of infection, and to assess the status of the problem in our country.

Material and methods

A total of 1152 samples suspected of leptospirosis collected from all regions of the country, were tested by MAT analysis. Of them, paired blood samples (at the beginning of the infection and during the convalescent phase) were taken from 62 patients and were examined by PCR (nested and real time). Suspensions of live serovars from 9 different *Leptospira* serogroups known to circulate in Bulgaria were used as antigens.

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Results

Average annual incidence of the disease was 0.16/100,000 population. The overall case fatality rate was 4.3 % in this period. Analysis of case distribution according to sex showed a high predominance of male patients -75/93 (80.65%), from the age group 50-59 years. Serogroup *Icterohaemorrhagiae* had the leading aetiological role in 2015-2023, but in 2014 *Sejroe* was the most commonly detected serogroup. The highest incidence was observed in the district of Sofia city, and far fewer cases were registered in Shumen, Montana, Bourgas and Pleven. The monthly distribution of leptospirosis cases showed that most of the cases appeared in summer and fall with a peak incidence in late summer and early autumn. Analysis of the possible mode of transmission showed that the infection was acquired mainly through occupational exposure (70.67% of cases), mostly livestock farming. Rats were the main risk factor to contract leptospirosis during this period (38.67% of cases). Data analysis showed that 44.08% of the patients suffered from severe disease (Weil's disease). Acute renal failure, diagnosed in 36.56% , was the main cause for fatality, which was noted also in the previous studies. Anicteric cases (55.91%) had a milder course, in most cases shown to occur as a self-limiting flu-like infection.

Conclusions

The results from this ten-year period show that leptospirosis in Bulgaria is an infection with limited distribution, but the risk of leptospirosis should not be underestimated. The development of real-time PCR method for rapid diagnosis of leptospirosis is very important for the diagnosis of individual cases or during outbreak situations.

Key words: Leptospirosis, serogroups, PCR

INTRODUCTION

Leptospirosis is one of the most important zoonotic bacterial infections worldwide (1, 2). It most commonly affects resource-poor populations, resulting in significant morbidity and many deaths. The infection is estimated to cause one million cases and around 58 900 deaths annually, with a case fatality rate of 6.85% (3). Leptospirosis is a zoonothonosis with natural outbreaks and of great significance for public health worldwide. The disease is more common in temperate and tropical regions. According to the latest ECDC report, there are 765 confirmed cases of leptospirosis in Europe for 2022 (4).

Various wild and domestic animal species serve as maintenance hosts. Humans are accidental hosts of the infection. While between maintenance hosts infection

is spread by direct contact, humans can be infected either by direct contact with urine or tissues of infected animal, or indirectly - with contaminated water (1).

Human cases of leptospirosis in Bulgaria have been reported since 1952 (5). In recent years, leptospirosis remains one of the important diseases for public health, reported as an emerging infection in many countries. In Bulgaria, the average annual morbidity of leptospirosis for the period 2009-2013 was significantly lower - 0.13/100 000, and the average mortality was 0.016%, in comparison to the period 2006-2009 when the cases of leptospirosis were 0.18 / 100 000. A decreasing trend of registered cases of leptospirosis in our country has been observed.

This study was focused on the analysis of circulating serovars of the genus *Leptospira* and their distribution, and epidemiological characteristics of laboratory confirmed cases of human leptospirosis for a ten-year period (2014-2023). It was interesting to reveal the relationship between clinical symptoms, mode of transmission and sources of infection and to assess the status of the problem in our country.

METHODS

Epidemiological Data

This study was based on all reported laboratory confirmed cases of human leptospirosis in Bulgaria for 10 years, from 2014 to 2023. Confirmed case of leptospirosis was defined as a patient with clinical evidence of leptospirosis and laboratory confirmation of infection. All cases were laboratory confirmed in the National reference laboratory of Vector-borne infections, at the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria.

Patients with confirmed leptospirosis were analyzed using questionnaires that included data such as age, sex, location, month of onset. In addition, data from epidemiological investigation concerning the possible source of infection and mode of transmission as well as symptoms of manifestation of the disease evaluated by infectious diseases specialists were included.

Microscopic agglutination test

All patients were diagnosed with the reference meth-

od for serological diagnosis and identification of the infecting *Leptospira* serovar, the microagglutination test (MAT), following established protocols (7). Suspensions of live serovars from 9 different *Leptospira* serogroups known to circulate in Bulgaria were used as antigens: *serogroup Australis (serovar Bratislava Jez Bratislava)*; *Autumnalis (serovar Nikolaevo)*; *Ballum (serovar Ballum)*; *Bataviae (serovar Bataviae van Tienen)*; *Canicola (serovar Canicola)*; *Icterohaemorrhagiae (serovar Copenhageni)*; *Pomona (serovar Pomona)*; *Sejroe (serovar Sejroe)*; *Tarassovi (serovar Tarassovi Perepelicin)* and *Semaranga (serovar Patoc)*. Serum samples were tested first at 1:100 dilution and positive sera were further examined for reactivity to the detected *Leptospira* serovar at 2-fold dilutions starting with 1:100. The end point titer was the highest dilution of serum at which 50% agglutination occurred. In the current Bulgarian case definition, a titer 1:400 is used for laboratory confirmation. All patients had titers of agglutinins above 400, in some cases as higher as above 12 800. Titer of the specific antibodies was processed in the positive sera subsequently (6).

DNA detection by nested PCR

PCR method based on the amplification of the *rrs* genes (16S) of leptospires (8). *Leptospira* DNA extraction using Dneasy Blood & Tissue Kit (QIAGEN GmbH, Germany), were performed. Four oligonucleotide primers (BIONEER, Korea) were used (Table 1). This method was applied to *Leptospira interrogans* in blood samples from patients taken in the initial phase of infection, before antibiotic treatment was started. The size of the first round products was 331 bp, from the second round – 289 bp.

Nested PCR reaction was performed using a commercial kit Illustra Ready-To-GO PCR Beads, GE Healthcare. The PCR reaction conditions are described by Merien et al (8). PCR reaction was fulfilled in DNA thermal cycle 2720 (Applied biosystems).

DNA extracted from *L. interrogans* serovars: *copenhageni*, *pomona* and *canicola* (18 day cultures) was used as a positive control. The amplified products were analyzed electrophoretically on a 1.5% agarose gel. Electrophoretic analyses were performed on an Electrophoresis Power Supply EPS 301 apparatus (Amersham

Table 1. Primers using in nested PCR

Primers	Nucleotide sequence 5' to 3'	Product size in bp
Lepto A	5'-GGCGGCGCGTCTTAAACATG-3'	331 bp
Lepto B	5'-TTCCCCCATTGAGCAAGATT-3'	
Lepto C	5'-CAAGTCAAGCGGAGTAGCAA-3'	
Lepto D	5'-CTTAACCTGCTGCCTCCCGTA-3'	289 bp

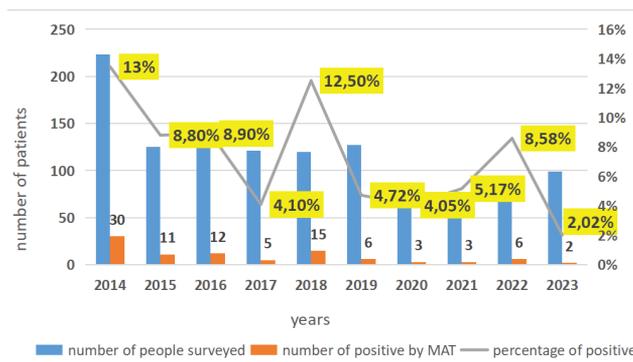


Fig 1. Distribution of the number positive patients for leptospirosis tested by MAT test.

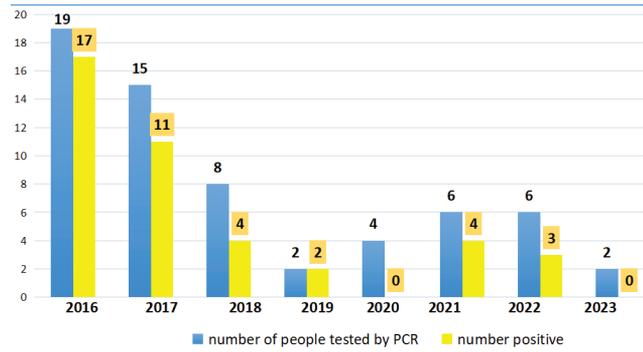


Fig 2. Distribution of number positive patients for leptospirosis by PCR (nested and real time).

Biosciences). Visualization of amplified products and molecular markers were recorded on a UV-transilluminator (MS -Major Science- UVDI).

DNA detection by real time PCR.

Isolated DNAs were stored at -20°C. A real-time PCR assay using a TaqMan probe targeting lipL32, which is present only in pathogenic *Leptospira spp.* The modified primer set of LipL32-45F (5'-AAG CAT TAC CGC TTG TGG TG-3') and LipL32-286R (5'-GAA CTC CCA TTT CAG CGA TT-3') were selected to amplify a fragment of 242 bp, which was detected by the probe, LipL32-189P (FAM-5'-AA AGC CAG GAC AAG CGC CG-3'-BHQ1) (9, 10). Target genes, primer sequences, and FAM fluorescently labeled probes applied for species identification *Leptospira interrogans* were used as described (9). The volume of the real-time PCR reaction was 20 µl, containing a mix of the following: 10 µl MM 2x, primers- For 1 µl, Rev 1 µl, Pr 0,5 µl, H₂O 3,5 µl and 4µl of extracted DNA. A kit Eurx Taqman qPCR for amplification were use. PCR reaction conditions are described by Stoddard et all (9). Real-time PCR was performed with Gentier Real-Time PCR System 96E.

RESULTS

Occurrence of leptospirosis in Bulgaria

A total of 1152 samples suspected of leptospirosis collected from all regions of the country for the period from 2014 to 2023, were tested by MAT analysis. Anti-

bodies against *Leptospira* were detected by MAT in 93 (8.07%) of the investigated samples (Fig. 1).

Paired blood samples (at the beginning of infection and during the convalescent phase) taken from 62 patients with a clinical diagnosis of leptospirosis were examined. In 41/62 samples taken in the acute phase of infection (66.13%), leptospira DNA was detected by PCR (Fig. 2). These samples were previously tested by MAT, and the results were compared. Using PCR analysis, we were able to detect *Leptospira interrogans* DNA in blood samples of patients who died before seroconversion. The average annual incidence of the disease was 0.16/100,000 population (range 0.59-0.01/100,000) (Fig. 3). The average annual mortality from leptospirosis per 100 000 for this period was very low. Not a single deceased patient was registered over the years: 2015, 2016, 2017, 2018, 2020, 2021 (Fig. 4). The overall case fatality rate was 4.3 % (4 patients).

The aetiological agent in fatal cases was serogroup *Icterohaemorrhagiae* and acute renal failure was proposed as the cause of death.

Analysis of case distribution according to sex showed high predominance of male patients -75/93 (80.65%) over female 18/93 (19.35%). The most affected age groups were 50-59 years 32/93 (34.41%), 60-69 years (20.43%) and 40-49 years (18.28 %), and then 30-39 years (16.13%) (Fig. 5).

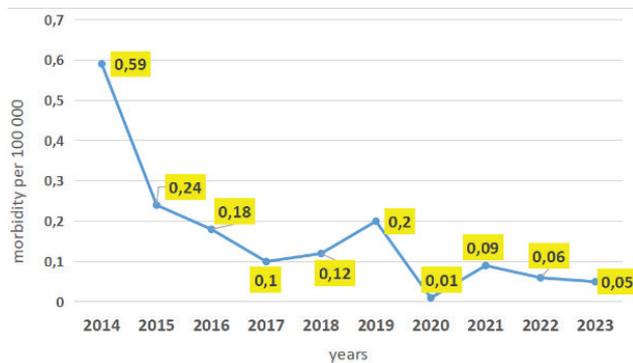


Fig 3. Average annual morbidity of leptospirosis per 100 000 for the period 2014-2023.

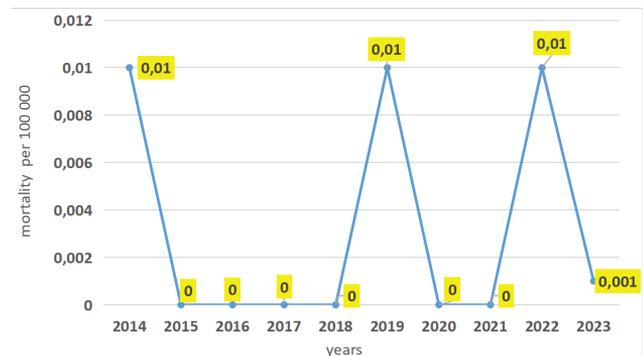


Fig 4. Annual average mortality of leptospirosis per 100 000 for the period 2014-2023

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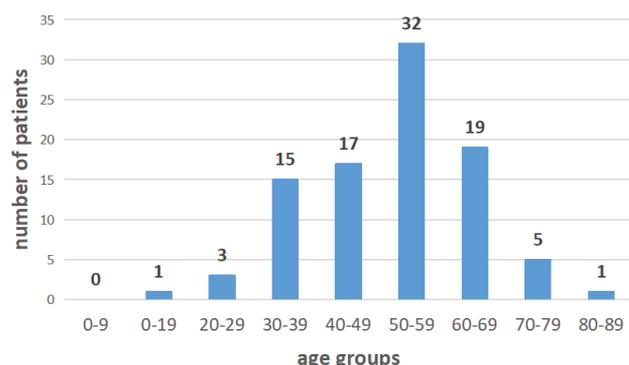


Fig 5. Age distribution of serologically positive patients for leptospirosis for the period 2014-2023.

Leptospira serogroups in aetiology

Leptospira serovars belonging to 7 different serogroups caused infection during the 10-year study period (Table 2). Serogroup *Icterohaemorrhagiae* had the leading role in aetiological structure in 2015-2023. However, in 2014 *Sejroe* was the most commonly detected serogroup. The overall proportion of serogroup *Icterohaemorrhagiae* was 53.76% (50/93 cases), the overall proportion of serogroup *Pomona* was 23.65% (22/93) and of serogroup *Sejroe* was 13/93 (13.98%) for the whole study period (Table 2). Serogroup *Tarassovi* was less common, causing a total 5 cases (5.38%). In fact, all cases caused by serogroup *Tarassovi* were detected in 2014. Serogroups *Autumnalis*, *Australis* and *Bataviae* were extremely rare (1/93), giving proportions of 1.08%, re-

spectively.

Eighteen of 28 Regional Health Inspectorates H(RHI) reported serologically confirmed cases of leptospirosis. Distribution of *Leptospira* serogroups according to districts is shown in Fig. 6.

The highest incidence was observed in the district of Sofia city, where 16 clinical cases from 93 in the country (17.2%) for the entire study period were registered, followed by Gabrovo – 12 cases (12.9%) and Lovech - 9 cases (9.68%). Six cases were registered in each of the districts Shumen, Montana, Bourgas and Pleven, for this period and 5 cases - in the districts Vratsa and Yambol (Fig. 6). One case (1.08%) per year was reported in each of the areas of Blagoevgrad, Sofia region and Sliven. Serogroup *Icterohaemorrhagiae* was registered in almost all districts, with the highest number of cases in Sofia city, Pleven and Bourgas. Serogroup *Pomona* was detected in the areas of Lovech, Shumen, Vratsa and Gabrovo. Serogroup *Australis* was registered only in Lovech and serogroup *Autumnalis*- only in Shumen (Fig. 6).

Monthly distribution of leptospirosis cases showed that most of the cases appeared in summer and fall with a peak incidence in August-September-October (Fig. 7). However, leptospirosis patients were detected throughout the year. Serogroup *Icterohaemorrhagiae* was found from May to December, serogroup *Pomona* – from January to October and serogroup *Sejroe* – throughout the

Table 2. Proportions of *Leptospira* serogroups in the aetiological structure of human leptospirosis in Bulgaria, 2014-2023.

Years	Leptospira serogroups number of cases/total cases per year (%)						
	<i>Icterohaemorrhagiae</i>	<i>Pomona</i>	<i>Sejroe</i>	<i>Bataviae</i>	<i>Tarassovi</i>	<i>Autumnalis</i>	<i>Australis</i>
2014	5/30 (16.6%)	8/30 (16.6%)	10/30 (33.3%)		5/30 (16.6%)	1/30 (3.33)	1/30 (3.33)
2015	4/11 (36.36%)	4/11 (36.36%)	2/11 (18.18%)	1/11 (9.09%)			
2016	8/12 (66.66%)	4/12 (33.33%)					
2017	2/5 (40%)	2/5 (40%)	1/5 (20%)				
2018	14/15 (93.33%)	1/15 (6.66%)					
2019	4/6 (66.66%)	2/6 (33.33%)					
2020	2/3 (66.66%)	1/3 (33.33%)					
2021	3/3 (100%)						
2022	6/6 (100%)						
2023	2/2 (100%)						
Total	50/93 (53.76%)	22/93 (23.65%)	13/93 (13.98%)	1/93 (1.08%)	5/93 (5.38%)	1/93 (1.08%)	1/93 (1.08%)

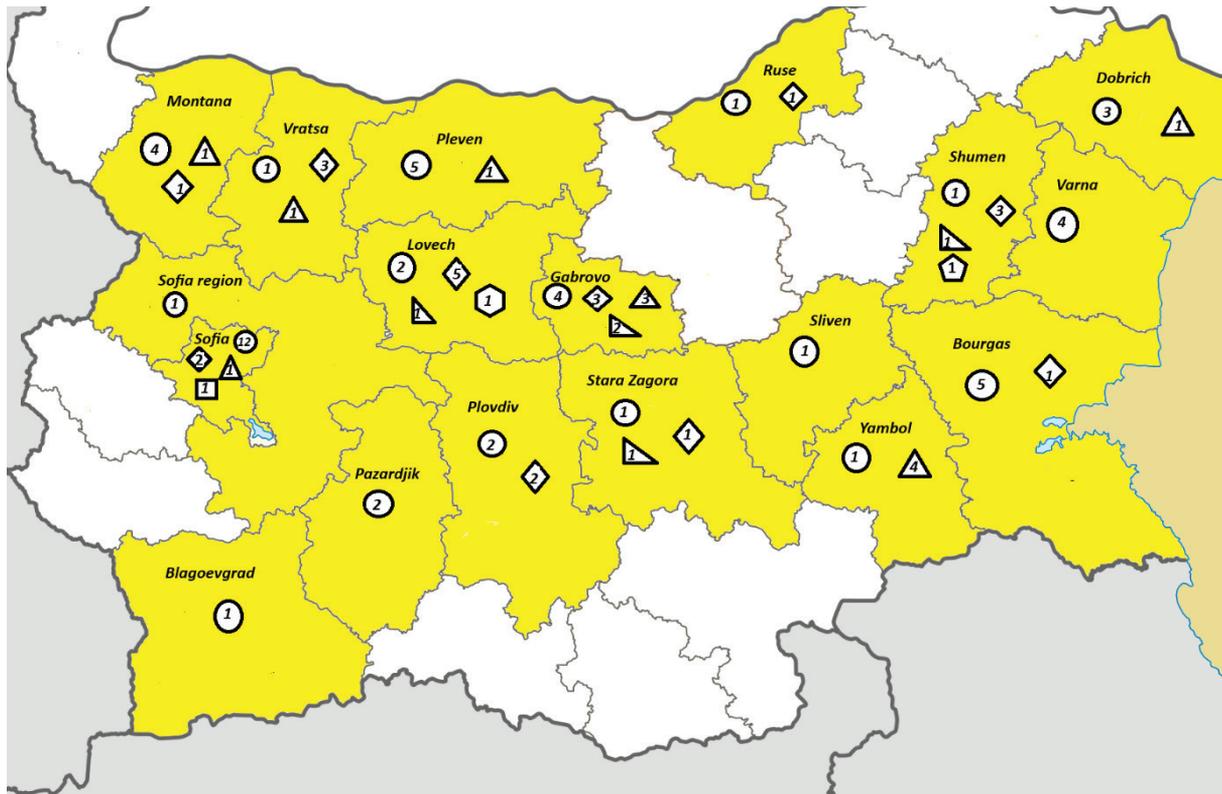


Fig.6. Distribution of leptospirosis cases by serogroups in the different regions of the country for the study period.



year. Serogroup *Tarassovi* was recorded mostly in August (5 cases) and only in 2014 after the heavy floods in Central Bulgaria.

In fact, in 2014, the most diverse spectrum of leptospira serogroups was observed, namely (*Icterohaemorrhagiae*, *Pomona*, *Autumnalis*, *Australis*, *Tarassovi* and *Sejroe*). Only serogroups *Icterohaemorrhagiae* and *Pomona* have been proven in the last 5 years.

Epidemiological data

Data on the possible modes of transmission and sources of infection were available for 75 patients with leptospirosis (80.65%).

Analysis of the possible mode of transmission showed that infection was acquired mainly through occupational exposure: 53 (70.67%) cases were associated with livestock farming; 38 (50.67%) with work in carpentry workshops, and a total of 13 (17.33%) – with work in a slaughterhouse. The second most frequent mode of transmission was by recreational exposure: 13 (17.33%) patients including swimming in lakes and open reservoirs; 7 (9.33%) fishing – 6 (8%); at the third place were activities related to additional factors - 9 (12%) (Table 3). Analysis of possible source of infection revealed that rats was the main risk factor to contract leptospirosis during 2014-2023 – 29/75 (38.67%) cases (Table 3). Twenty six (17+9)/75 (34.67%) patients were infected through direct or indirect contact with domestic animals (swine, goats and sheep). Overall, feed from the warehouses was the third common source of infection – for 10 (13.33%) cases.

Twenty (26.67%) of the patients were exposed to several risk factors – livestock farming, presence of rodents

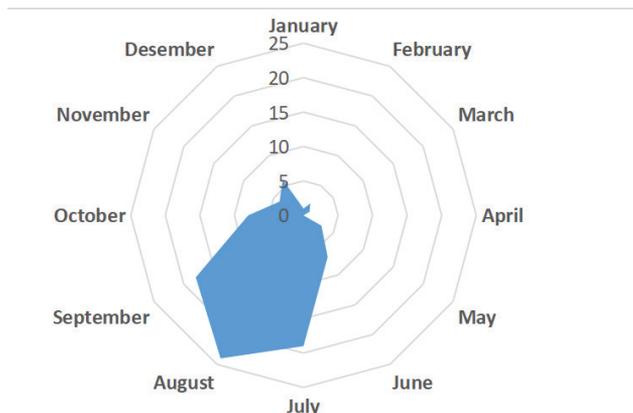


Fig 7. Seasonal distribution of leptospirosis cases, 2014-2023

Table 3. Possible sources of infection and mode of transmission of cases of leptospirosis in Bulgaria for 2014-2023.

Mode of transmission	Patients n (%)	Sources of infection	Patients n (%)
Activity		Contact with:	
I. Related to occupation		1.Rats	29 (38.67%)
1. Livestock production	38 (50.67%)	2.Swine	17 (22.67%)
2. Work in carpentry workshop	8 (10.67%)	3.Sheep and goats	9 (12%)
3. Securing the farm	2 (2.67%)	4. Feed from warehouses	10 (13.33%)
4. Working in a slaughterhouse	5 (6.67%)	5.Sewage	5 (6.67%)
Total	53 (70.67%)	6.Unknown source	5 (6.67%)
II. Recreational Activities		Total	75 (100%)
1. Swimming in lakes and open reservoirs	7 (9.33%)		
2. Fishing	6 (8%)		
Total	13 (17.33%)		
III. Additional factors			
1. Contaminated food	5 (6.67%)		
2. Contaminated water	4 (5.33%)		
Total	9 (12%)		
Summary	75 (100%)		

in houses and/or working places, consumption of food contaminated with rodent excreta, etc.

Clinical data

The clinical spectrum of symptoms was analyzed in all patients (n=93) with leptospirosis (Fig. 8). A lot of combinations of symptoms were observed. Data analysis showed that 44.08% (41/93) of these patients suffered from severe disease (Weil’s disease), probably because the majority of benign forms remained undiagnosed. Fever and myalgia were the most frequently reported symptoms in 56 (60.22%) and in 52 (55.91%) patients,

respectively. Jaundice and hepatomegaly were observed in 41 (44.09%) and 34 patients (36.56%), respectively. The disease was accompanied by nausea in 35 patients (37.63%), hepatomegaly and oliguria in 34 (36.56%), arthralgia – in 31 (33.33%) and vomiting – in 29 (31.18%). Acute renal failure, diagnosed in 34/93 (36.56%) patients, was the main cause for fatality, which was noted also in the previous studies (5,11). Rarely, the disease manifested as rash, anuria and neck stiffness (Fig. 8). Anicteric cases (n=52, 55.91%) had a milder flu-like course, while all icteric cases manifested as severe diseases, meaning that clinical jaundice is an important predictive marker.

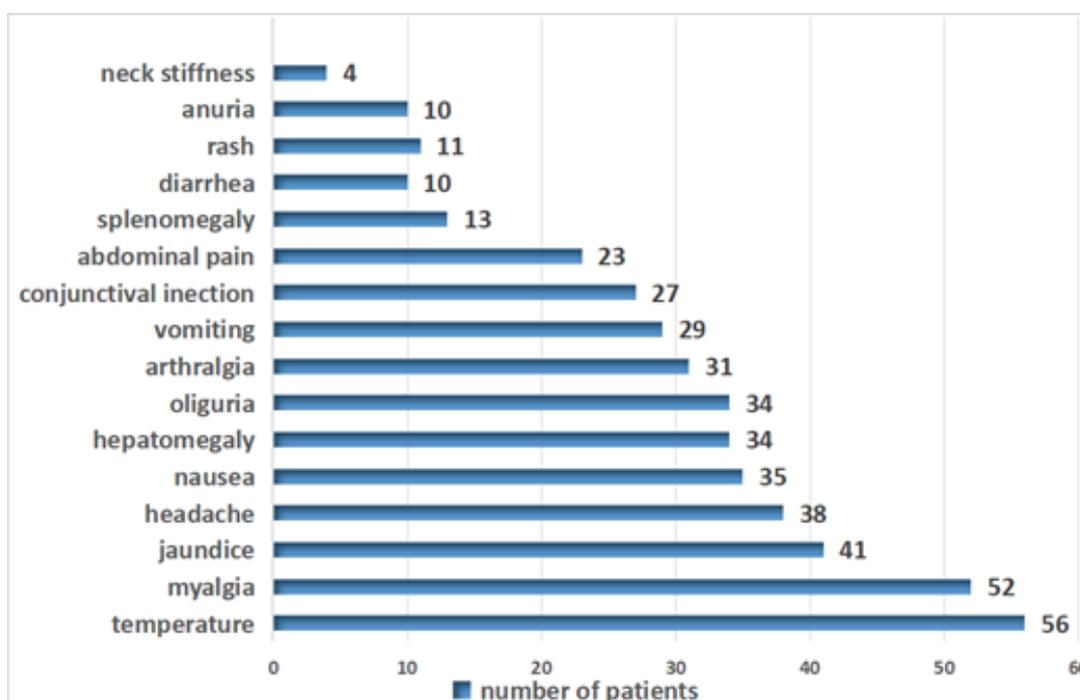


Fig.8 Clinical spectrum of the symptoms in the infected with leptospirosis for the period 2014-2023

DISCUSSION

Human leptospirosis is an officially notified infection in Bulgaria. In this study, all clinically and laboratory confirmed leptospirosis cases in Bulgaria for a 10 year period were analyzed. Compared with previous years (5,11,12), the incidence of leptospirosis showed a marked decline, from 53 reported cases in 1999 (0.64/100 000) to 6 cases in 2013 (0.08/100 000). However, in 2014, a fivefold increase was observed, with 30 reported cases (0.42/100 000) compared to 2013.

The average morbidity decreased to 0.18 / 100000 for the period 2006-2009 (11) and 0.19/100,000 population for the period 2010-2014 (12). In the last 4 years, the incidence has decreased very sharply -0.05/100 000 for 2023. The decline of leptospirosis cases in 2020, followed by an increase in the number of cases in 2021, may be associated with the changes in population behaviors during the COVID-19 pandemic (e.g. less travel and more limited or impaired surveillance activities during the first year of the pandemic) (19).

There has been a decrease in the number of deaths compared to previous studies (12). Many European countries reported a decrease in leptospirosis notifications in the second half of the 20th century in relation to a decline in the number of agricultural workers and improvement of living standards. This trend has been registered in Germany, France, the Slovak Republic and Denmark (13). In Bulgaria, we detected peak in human leptospirosis cases only in 2014, which correlated well with a heavy rainfall and floods in central Bulgaria. Germany also reported increased incidence of leptospirosis cases in 2014 and attributed it to the warm and humid climate (14).

All 93 registered confirmed leptospirosis cases analyzed in the study occurred on the territory of Bulgaria. In accordance with previous investigations in Bulgaria as well as with numerous studies from Europe and Asia (15-18), our study showed that the disease affected mainly men of working age. The average male/female ratio among cases was 4:1. Distribution by sex of patients with leptospirosis in the country was comparable to similar studies from Italy, Germany, France (18, 19). In this study, the age groups of 50-59 years (34.41%) were mainly affected. Such observations were also made in a similar study in Greece (21). This is probably related to the share of increased outdoor activities outside professional activities. In Denmark, however, a similar study was found that the most affected age group was of adult men aged 40-49 years (13).

Most leptospirosis cases in Bulgaria for the last 10 years occurred in late summer and early autumn as has been also reported from Europe, more specifically in Denmark

(19, 20). Obviously, appropriate combination of optimal temperature and rainfall at this time supports efficient survival of leptospirae in the environment. However, in Bulgaria, leptospirosis cases could occur throughout the year. About 66% (61/93) of leptospirosis cases occurred during the months July, August and September. A slight shift of the peak towards the beginning of summer was observed as compared to previous studies (11,12).

Serovars belonging to 7 different serogroups caused infection during the 10-year study period. Serogroup Icterohaemorrhagiae had the leading role in the aetiological structure during 2010-2013 in our country (12). Similar findings about the leading role of this serogroup have been reported in Denmark and Portugal (13, 20). In recent years, domestic cases registered in the Azores have had a significant impact on the total number of cases and the high notification rate reported by Portugal. The Azores is considered an endemic area for leptospirosis. It has subtropical climate and harbors a high density of rats, both favorable for the transmission of leptospirosis (13). However, according to this study, in 2014 Sejroe was the most commonly detected serogroup, while for the period 2015-2023 the leading role in the etiological structure had serogroup Icterohaemorrhagiae. The overall proportion of serogroup Icterohaemorrhagiae was 53.76% (50/93 cases). A significant increase in cases caused by this serogroup was observed - 33.8%, as compared to previous studies (12). The overall proportion of serogroup Pomona was 22/93 (23.65%), a slight decrease compared to previous studies (25%) was observed (12). Serogroup Sejroe was detected only in 2014, 2015, 2017, with the total percentage for the period amounting to 13/93 (13.98%). This serogroup was the second most frequently detected, found in 21.43% of cases in previous investigation in our country (22).

Serogroup Tarassovi was less common, causing a total of 5 cases (incidence 5.38%). In fact, all cases caused by serogroup Tarassovi were detected in 2014. Serogroups Australis, Autumnalis and Bataviae were extremely rare 1/93 (incidence 1.08% resp). In the current study, an increase in the number of leptospirosis cases was observed in the areas of Sofia, Pleven and Gabrovo, as compared to previous studies (11, 12, 22), with serogroup Icterohaemorrhagiae mainly occurring in these areas. In the districts of Lovech and Shumen, a slight increase in the number of cases was also observed, but the leading serogroup was Pomona (22). In Yambol district, the same trend was observed, but serogroup Sejroe had the leading role there. One case of serogroup Australis serovar Bratislava was detected in the Lovech region (i.e. in Central Bulgaria). However, according to

published data, cases of leptospirosis with this serogroup are characteristic of coastal regions, for example in Turkey (23).

All patients were infected on the territory of Bulgaria. Data analysis showed a rising trend of the cases associated with occupational activities in the ten-year period and decrease of those associated with recreational activities. These data differ from the previous studies in our country (22). In Bulgaria, before 1990, the disease was relatively common among rice workers and miners (5). In the following years, along with the radical economic changes in Bulgaria, these professions faded and nowadays most occupationally exposed people are livestock farmers, followed by workers in carpentry workshops and slaughterhouses. Notably, the occupational risk was higher for farmers breeding pig than for farmers breeding cattle. Fishing and bathing in lakes with stagnant water accounted for 17.33% of the infections. The overall distribution of cases by transmission mode did not change substantially over the study period, contact with animals being the most frequent mode in all years. Studies in some European countries have observed this trend of increasing occupationally-related cases of leptospirosis (19). Contact with animals was the most frequent transmission mode in Slovenia (90.5%), Portugal (76.5%), Spain (52.6%) and Austria (52.4%). On the contrary, in Romania, infections were associated with recreational water contact in 76.3% of cases (13). Nowadays, contract leptospirosis in Bulgaria through recreational activities (fishing and bathing) is less likely than through occupational activities. This is in concordance with previous studies for Bulgaria (12). This is because the main age group engaged in animal husbandry is a men 50-59 years old, while more young people under 30 are engaged in swimming and fishing. Although the mode of transmission of leptospirosis for fishermen in Bulgaria and fish farmers in Denmark (24) is different, the source of infection is similar.

Contact with rodents, mainly rats, was another common source of infection as concluded from the results of our study (38.67 %). Patients reported having rodents in their farms and country houses. Generally, leptospirosis was contracted more frequently through indirect contact with animal excretions (via contaminated water, soil, or food) and less commonly through direct contact with animal urine or tissues (1, 2). House mice also play a significant role in the transmission of infection. For example, a significantly high transmission risk from house mice was observed in Portugal emphasizing the need to develop integrated control programs to reduce the risk of human disease (15). Several studies in Europe have reported the role of wild and domestic animals as driv-

ers of leptospirosis, which has led to various animal species, in particular rodents, being described as a source of human infection (18). In this study, another risk factor was revealed - improper food storage and possibility for contamination with excreta of rodents - 6.67%, which is a fairly low percentage compared to some European countries: Lithuania (75.0%) and Slovakia (29.0%) (18,19). During the study period, the infection was detected in only 6.67% of slaughterhouse workers. In contrast, in New Zealand, the incidence of leptospirosis is highest among agricultural and slaughterhouse workers. Meat processing work involving the removal of offal is at high risk, with infected animals spraying urine. Interventions are therefore being developed in the Pacific Islands, which include adjustments to occupational practices and promotion of animal vaccination (25).

It is known that the majority of leptospiral infections are either subclinical or result in very mild illness and recover without any complications. However, a small proportion develop various complications due to involvement of multiple organs and systems. In such patients, the clinical presentation depends on the predominant organs involved and the mortality rate can be about 40% or more (16). Furthermore, analysis of the clinical signs and symptoms of registered leptospirosis cases in Bulgaria 2014-2023 showed prevalence of the severe Weil's disease over flu-like forms of the disease. The most likely explanation is that the majority of milder forms were not clinically recognized. The severe form of leptospirosis was usually accompanied by jaundice and oliguria. In about one third of the patients, acute renal failure was established (36,56%). As in the previous years, acute renal failure was the main cause of death in fatal cases (5). Fever and jaundice were the most frequently reported symptoms. There was a decrease in the percentage of patients with jaundice compared to previous studies - from 73.68% to 44.08% (22). The pathogenesis of severe leptospirosis is poorly understood, however, it is thought to be due to a form of vasculitis. Although jaundice is a prominent feature, death often results from complications of acute kidney injury, myocardial involvement or pulmonary haemorrhage (26). Myalgia is the leading symptom in the current study, which coincides with previous studies in Bulgaria - 55.91% (11, 22). Myalgia can be severe, and usually involves the calves, abdomen (mimicking acute abdomen) and paraspinal muscles (resulting in meningism) (26). The percentage of patients with headache increased from 36.84% to 40.86%, and the percentage of those with conjunctivitis decreased from 42.11% to 29.03% compared to previous studies (22). Rash was observed in 11.83% of the patients, which is higher

than in previous studies - 4.41% (12). Although rare, some manifestations of leptospirosis are important and should not be stated - meningitis, pneumonia, endocarditis. In contrast, a study in Greece reported that mainly pulmonary involvement and hemorrhage were associated with disease severity. This is due to the subtropical Mediterranean climate with mild and wet winters that characterize the country (21). Anicteric cases (n=52, 55.91%) had a milder course, in most cases shown to occur as a self-limiting flu-like infection. An increase in this percentage is observed compared to previous surveys for the country (32.1%) (12).

Symptoms are dependent on certain known and unknown factors, such as immune status, and it is thought that the causative serovar might play a role. This makes it hard to distinguish from other diseases such as influenza, hepatitis, viral haemorrhagic fevers, or enteric disease, resulting in mis- or under-diagnosis (4). This fact, together with the subclinical nature of the disease in animals and people and the need of technically demanding laboratory tests to diagnose the disease, has led to a lack of awareness of leptospirosis and therefore the focus should be on addressing this problem in the future.

CONCLUSION

Leptospirosis in Bulgaria, located in the temperate climate region of southeastern Europe, is endemic with a low incidence but with significant fatality rate. Analyzing the results from this ten-year period, we can conclude that leptospirosis in Bulgaria is an infection with limited distribution, but the risk of leptospirosis should not be underestimated. More efficient measures for rodent control are needed since contact with rodents was shown to be an important risk factor.

The wide spectrum of clinical symptoms of the disease, the development in some cases of severe infection leading to a fatal outcome, requires the search for new, more reliable methods for laboratory diagnostics. Early initiation of adequate and effective treatment is essential, which reduces the risk of developing vasculitis and pulmonary hemorrhage. Development of real-time PCR for rapid diagnosis of leptospirosis is very important both for the individual cases or during outbreak situations.

There are currently no reliable scoring systems or predictive models to determine which patients with leptospirosis are likely to develop severe disease. This is an area for future research. This study provides useful information as a starting point for further research on leptospirosis in Bulgaria and the problems associated with this infection.

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REFERENCES

1. Levett PN. Leptospirosis. *Clin Microbiol Rev*, 2001; 14:296–326. <https://doi.org/10.1128/CMR.14.2.296-326.2001>.
2. Haake DA, Levett PN. Leptospirosis in humans. *Curr Top Microbiol Immunol*, 2015; 387:65–97. https://doi.org/10.1007/978-3-662-45059-8_5.
3. Costa F, Hagan JE, Calcagno J et al. Global morbidity and mortality of leptospirosis: A systematic review. *PLoS Negl Trop Dis*, 2015; 9:e0003898. <https://doi.org/10.1371/journal.pntd.0003898>
4. European Centre for Disease Prevention and Control. Leptospirosis. In: ECDC. Annual Epidemiological Report for 2022. Stockholm: ECDC; 2024 <https://www.ecdc.europa.eu/en/publications-data/leptospirosis-annual-epidemiological-report-2022>
5. Christova I, Tasseva E, Manev H. Human leptospirosis in Bulgaria, 1989-2001: epidemiological, clinical, and serological features. *Scand J Infect Dis*. 2003;35:869-872. PMID: 14723364. <https://doi.org/10.1080/00365540310016709>.
6. Gancheva E., Manev Ch., Halacheva M., Epidemiological and epizootiological analysis of leptospirosis, 1986-1990. *Bulletin of the National Center for Infectious and Parasitic Diseases*, 1990, 6: 57-59. (in Bulgarian)
7. Faine S., Guidelines for control of leptospirosis. WHO Offset Pub., 1982, 67, Geneva:WHO. <https://iris.who.int/handle/10665/37219>.
8. Merien, F., P. Amouriauz, P. Perolat, G. Baranton, and I. Saint Girons, Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples., *J. Clin. Microbiol.*, 1992, 30:2219–2224. <https://doi.org/10.1128/jcm.30.9.2219-2224.1992>.
9. Stoddard R.A., Geera J.E., Wilkinsa P.P., McCaustlandb K., Hoffmastera A. R., Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the *LipL32* gene, *Diagnostic Microbiology and Infectious Disease*, 2009, 64, 247–255. <https://doi.org/10.1016/j.diagmicrobio.2009.03.014>.
10. McBride AJ, Athanzio DA, Reis MG, Ko AI, Leptospirosis. *Curr Opin Infect Dis*, 2005, 18:376–386. <https://doi.org/10.1097/01.qco.0000178824.05715.2c>.
11. Taseva E., I. Christova, T. Gladnishka, I. Trifonova, I. Ivanova, Clinical and serological aspects and prognostic factors of leptospirosis in humans in Bulgaria, a retrospective study for the period 2006-2009, *Infectology*, 2010, 47, 2: 36-43. (in Bulgarian)
12. Christova I., Tasseva E., Human leptospirosis in Bulgaria, 2010-2014, *Problems of Infectious and Parasitic Diseases* 44(2): 2016, 23-29.
13. Beaute J., Innocenti F. et al., Epidemiology of reported cases of leptospirosis in the EU/EEA, 2010 to 2021, *Euro Surveill.*, 2024; 29 (7), :2300266. <https://doi.org/10.2807/1560-7917.ES.2024.29.7.2300266>.
14. Bundesinstitut für Risikobewertung (BfR). Robert Koch-Institut. Leptospirose - eine seltene, aber immer häufiger auftretende Erkrankung [Leptospirosis - a rare but increasingly prevalent disease]. Berlin: BfR; 2014. German. Available from:

- <http://www.bfr.bund.de/cm/343/leptospirose-eine-seltene-aber-immer-haeufiger-auftretende-erkrankung.pdf>.
15. Vieira ML, Gama-Simoes MJ, Collares-Pereira M. Human leptospirosis in Portugal: A retrospective study of eighteen years. *Int J Infect Dis.* 2006;10: 378-386. <https://doi.org/10.1016/j.ijid.2005.07.006>
 16. Vijayachari P., Sugunan A. P., Shriram A. N., Leptospirosis: an emerging global public health problem, *J Biosci.* 2008 ;33 (4): 557-569. <https://doi.org/10.1007/s12038-008-0074-z>.
 17. Lee MJ, Miki S, Kitagawa M, Lee WC. Comparative study on the epidemiology of human leptospirosis in Korea and Japan, 2006-2012. *Jpn J Infect Dis.* 2016; 69:259-261. Available online: <http://doi.org/10.7883/yoken.JJID.2014.589>.
 18. Cataldo C., Bellenghi M., Masella R., Busani L. One Health and sex and gender-related perspective in the ecosystem: Interactions among drivers involved in the risk of leptospirosis in Europe. A scoping review, *One Health* 19, 2024, 100841, <https://doi.org/10.1016/j.onehlt.2024.100841>
 19. European Centre for Disease Prevention and Control. Leptospirosis. In: ECDC. Annual Epidemiological Report for 2021. Stockholm: ECDC; 2023, https://www.ecdc.europa.eu/sites/default/files/documents/LEPT_AER_2021_Report.pdf
 20. Eves C, Kjelsø C, Benedetti G, Jørgensen CS and Krogfelt KA, Trends in human leptospirosis in Denmark, 2012-2021. *Front. Cell. Infect. Microbiol.*, 2023, 13:1079946. <https://doi.org/10.3389/fcimb.2023.1079946>
 21. Papa A., Kotrotsiou T., Leptospirosis in Greece, *Acta Trop.*, 2015 :149:135-137. <https://doi.org/10.1016/j.actatropica.2015.05.025>.
 22. Tasseva E., I. Christova, T. Gladnishka, I. Trifonova, V. Ivanova, Human leptospirosis in Bulgaria - clinical, epidemiological and serological aspects of the infection, 2010-2011, *Probl. Inf. Parasit. Dis.* Vol. 40, 2012, 1, 15-21.
 23. Kuloglu E., Issever K., First report of human infection with *Leptospira interrogans* serovar Bratislava in the Eastern Black Sea region of Turkey, *Rev Inst Med Trop São Paulo.* 2023; 65:e48. <https://doi.org/10.1590/S1678-9946202365048>
 24. Van Alphen LB, Lemcke Kunoe A, Ceper T, Kahler J, Kjelso C, Ethelberg S, et al. Trends in human leptospirosis in Denmark, 1980 to 2012. *Euro Surveill.* 2015; 20(4):21019. <https://doi.org/10.2807/1560-7917.es2015.20.9.21053>.
 25. Kharwadkar S, Weinstein P and Stanhope J, Drivers of human *Leptospira* infection in the Pacific Islands: A systematic review. *Epidemiology and Infection*, 2024, 152, e118, 1–19 <https://doi.org/10.1017/S0950268824001250>
 26. Rajapakse S., Leptospirosis: clinical aspects, *Clinical Medicine* 2022 Vol 22, No 1: 14–17., <https://doi.org/10.7861/clinmed.2021-0784>