ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 2, 285-288
www.aaem.pl

Seroprevalence of leptospirosis in rural populations inhabiting areas exposed and not exposed to floods in eastern Poland

Bernard Wasiński[1], Jacek Sroka[2,3], Angelina Wójcik-Fatla[3], Violetta Zając[3], Ewa Cisak[3], Józef P. Knap[4], Anna Sawczyn[3], Jacek Dutkiewicz[3]

[1] *Department of Swine Diseases, National Veterinary Research Institute, Puławy, Poland*
[2] *Department of Parasitology, National Veterinary Research Institute, Puławy, Poland*
[3] *Department of Zoonoses, Institute of Rural Health, Lublin, Poland*
[4] *Department of Epidemiology, Medical University, Warsaw, Poland*

Wasiński B, Sroka J, Wójcik-Fatla A, Zając V, Cisak E, Knap JP, Sawczyn A, Dutkiewicz J. Seroprevalence of leptospirosis in rural populations inhabiting areas exposed and not exposed to floods in eastern Poland. Ann Agric Environ Med. 2012; 19(2): 285-288.

Abstract

Blood serum samples collected from randomly selected groups of 100 persons inhabiting rural community 'A' located in eastern Poland and exposed to floods by the Vistula river, and 98 persons inhabiting rural community 'B', also located in eastern Poland, but in the area not exposed to floods were examined by the microscopic agglutination test (MAT) for the presence of antibodies against 18 *Leptospira* serovars. Positive results showed 3% of serum samples collected from community 'A', while the seroprevalence in community 'B' was 9.2%, being insignificantly higher than in community 'A'. For both examined communities (n=198), a significant positive correlation was found between the prevalence and titer of seropositive response and age of examined people (r=0.145, p=0.042). No significant differences were found between the prevalence of positive reactions in males and females (p>0.05). The reactions with 10 serovars of *Leptospira* (Australis, Autumnalis, Hebdomadis, Hardjo, Sejroe, Zanoni, Bataviae, Bratislava, Canicola and Grippotyphosa), belonging to 3 species (*L. interrogans, L. borgpetersenii, L. kirschneri*), were found in the examined communities. From both communities, of 12 persons demonstrating positive results in MAT, 9 showed reaction with one serovar, 2 with two serovars and 1 with three serovars. The highest titers found during the examination did not exceeded 800. In conclusion, our results suggest that there is only a slight, if any, hazard of an leptospirosis epidemic after the flood that afflicted eastern Poland in the year 2010 and the general epidemiological situation of leptospirosis in eastern Poland. Although there does not seem to be any cause for concern, further research is needed.

Key words

leptospirosis, sero-prevalence, farmers, eastern Poland

INTRODUCTION

Leptospires are thin, helical bacteria classified currently into at least 13 pathogenic species, comprising in turn more than 260 serovars, and 6 saprophytic species, with more than 60 serovars [1, 2]. Although this classification is dynamic, it is expected that additional new species also exist [1]. Leptospires are capable of surviving in a wide range of moist environmental conditions, including soil, mud, ground water, streams, rivers and lakes [3]; however, the main niche for their maintenance are warm-blooded organisms, mainly mammalians. Leptospires survive usually in the renal tubules of rodents and many other wild and domestic animals [1, 2, 4]. Humans become infected most commonly through occupational, recreational, or domestic contact of skin with the urine of infected animals, either directly or via contaminated water or soil [1, 5].

Leptospirosis is regarded as the most widespread zoonosis in the world that represents a re-emerging health problem because of increasing the incidence among humans and domestic animals [1, 6, 7, 8, 9]. Depending on the serovar, the immune status of the host and many other known and unknown factors, the disease can run as a mild, flu-like illness or a severe infection capable causing serious multiorgan or systemic disorders leading to death. Despite this, the disease is severely neglected and the estimated incidence of about half a million severe human cases annually is probably an underestimation [10]. *Leptospira* spp. present an occupational risk to agricultural workers, slaughterhouse workers, sewage workers, veterinarians and other professions [5, 11]. The mechanisms of pathogenesis of *Leptospira* are largely unknown [8]. With global climate change, extreme weather events such as cyclones and floods are expected to occur with increasing frequency and greater intensity and may potentially result in an upsurge in the disease incidence, as well as the magnitude of leptospirosis outbreaks [12]. The natural foci of leptospirosis may occur in swampy areas and could be described as 'marsh fever' [13, 14].

Leptospiral infection in humans may be symptomless and the only proof of such infection are specific anti-*Leptospira* antibodies. Thus, the serological response rate of the examined population to *Leptospira* antigen may be regarded as an indicator of prior contact with these bacteria and the degree of exposure. Reported response rates in agricultural workers living in the countries located in the tropical climate

Address for correspondence: Jacek Dutkiewicz, Department of Zoonoses, Institute of Rural Health, Jaczewskiego 2, 20-090 Lublin, Poland.
E-mail: dutkiewi@galen.imw.lublin.pl

Received: 10 April 2012; accepted: 20 May 2012

zone (Tanzania, Nigeria, Somalia, India) are within the range of 19.4-62.5% [15, 16, 17, 18], whereas the analogous rates in similar workers living in the temperate climate zone (The Netherlands, USA, Northern Ireland, Italy) are usually lower, ranging between 0.5-23.5% [19, 20, 21, 22, 23, 24].

The aim of this study was to investigate seroprevalence of *Leptospira* spp. among adult farmers living in two rural communities of the Lublin Province in eastern Poland. Community 'A' is situated on the Vistula river, in the western part of the province, an area often exposed to the consequences of raised levels of the river (wet soil, inundations), and in the summer of 2010 was afflicted by two huge floods. By contrast, community 'B' is situated in the central part of the province and does not experience floods.

MATERIALS AND METHODS

Examined population. Blood serum samples collected from 100 randomly selected persons (27 males and 73 females) inhabiting the rural community 'A', exposed to floods, and serum samples from 98 persons (27 males and 71 females) inhabiting the rural community 'B' not exposed to floods, were examined during the study. The mean age of the investigated group for community 'A' (x ± S.D.) was 51.1 ± 13.4 yrs (range 18-79 yrs), while for community 'B' it was 54.1 ± 14.7 yrs (range 18-85 yrs). Collected serum samples were examined by the microscopic agglutination test (MAT) with live antigens.

Antigens. Cultures of reference strains of – *Leptospira interrogans* serovars: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Zanoni, – *L. borgpetersenii* serovars: Ballum, Poi, Serjoe, Tarassovi, – *L. kirschneri* serovars: Cynopteri, Grippotyphosa, – *L. weili* serovar Celledoni, – and *L. biflexa* serovar Patoc kept in the Department of Swine Diseases of the National Veterinary Research Institute in Pulawy were used for preparation of MAT. The strains were obtained from FAO/WHO Reference Laboratory for Leptospirosis of the Royal Tropical Institute in Amsterdam. Leptospires were cultivated in EMJH (Ellinghausen McCullough Johnson and Harris) liquid medium at 30°C in aerobic conditions. The strains were subcultured every 7 days.

Microscopic agglutination test. This test was carried out according to the OIE Manual of Standards for Diagnostic Tests and Vaccines [25], as described previously [26]. The serum samples were mixed with an equal volume of each of the *Leptospira* serovars. Serum dilution (including added antigen) used during preliminary examination was 1:100. For samples reacting in the preliminary examination with one or more serovars, series of twofold dilutions were prepared to titre the end point – 50% agglutination. The samples with titres ≥100 were recognized as positive.

Statistical analysis. The data were analysed by Student's t-test and Pearson's test for correlation, with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

The results are summarized in Table 1. In the group of persons from community 'A', 3% of serum samples showed the presence of anti-*Leptospira* antibodies. In community 'B', not affected by flood, the seroprevalence was 9.2%, being insignificantly higher than in community 'A' (p=0.0764).

Reactions with 10 serovars of *Leptospira* spp. belonging to 3 species (*L. interrogans, L. borgpetersenii, L. kirschneri*) were found in the examined communities. From both communities, a total of 12 persons who presented positive results in MAT, 9 of them showed positive reactions with one serovar, 2 with two serovars and 1 with three serovars (Tab. 1). Altogether, there were indicated 2 serum samples reacting with serovar Australis and 2 with serovar Zanoni, 5 samples, each reacting with one of following serovars: Bataviae, Bratislava, Canicola, Grippotyphosa, Hebdomadis, 1 serum reacting with serovars Sejroe and Hardjo, 1 reacting with serovars Hebdomadis and Autumnalis and 1 showing reactions with serovars Hebdomadis, Hardjo and Sejroe. Among the positive samples, 4 sera presented titer 100, 4 showed titer 200, 3 demonstrated titer 400 and 1 – titer 800.

Table 1. Comparison of serological reactions with various serovars of *Leptospira* spp. indicated by the use of MAT among inhabitants of two rural communities in the Lublin Province of eastern Poland

Community	Number of examined serum samples	Positive serum samples			Sex of positive reactant(s)	Age of positive reactant(s)
		Number	Reacting with serovar(s)	Titer(s)		
A	100	1	Bataviae	100	F	42
		1	Bratislava	100	F	18
		1	Hardjo	800	M	70
			Sejroe	400		
B	98	2	Australis	200	F, F	39, 70
		1	Canicola	400	F	85
		1	Grippotyphosa	100	M	56
		1	Hebdomadis	100	F	79
		1	Hebdomadis	200	F	56
			Autumnalis	100		
		1	Hebdomadis	200		
			Sejroe	200	F	52
			Hardjo	100		
		2	Zanoni	400	F, M	57, 61

M=Male, F=Female

For both examined communities (n=198), a significant positive correlation was found between the prevalence and titer of seropositive response and age of the examined people (r=0.145, p=0.042). No significant differences were found between the prevalence of positive reactions in males and females (p>0.05).

DISCUSSION

In Poland, the most important endemic area of leptospirosis is Lower Silesia, an area where epidemics of 'marsh fever', often associated with floods, propagation of rodents and/or performing of agricultural works, have been described since the 19th century up to the second half of the 20th century [13, 14, 27, 28, 29]. In the southern part of the Lublin Province, epidemics of leptospiral 'marsh fever' were noted in the years

1954-1955 [14]. In 1955, a scientific expedition consisting of experts from medicine, veterinary medicine and biology was organized to elucidate the etiology, pathogenesis and environmental aspects of the epidemic [30, 31]. Samples for serological and microbiological investigations were collected from over 400 hospitalized persons. Additionally, over 2,500 small mammals were caught in area affected by the epidemic [32, 33]. The animals were subjected to serological and microbiological examination for presence of *Leptospira* spp., the results of which indicated serovar Grippotyphosa as the most widespread etiological agent of the disease. The second serovar often found in humans and small mammals was Sejroe.

In the years 1955 and 1956, a total of 608 serum samples were collected from inhabitants of the epidemic region who were ill or suspected of being ill with leptospirosis. Antibodies for 11 serovars of Leptospira were found in 60.9% of these samples. Almost 69% of positive samples reacted with serovar Grippotyphosa and 15% with serovar Sejroe [34]. Serological findings of 4,322 domestic animals (cattle, horses, pigs, sheep, hens, geese, ducks) from this region, also carried out in 1955 -1956 indicated 13.3% of positive results. Among positive samples, 31 % reacted with serovar Grippotyphosa, 28% with serovar Icterohaemorrhagiae, 23.4% with serovar Bovis, and the remaining 17.6% with serovars: Pomona, Canicola, Sejroe, Mitis, Australis, and Saxkoebing. In 1957-1958, only a few cases of human leptospirosis were noted in the southern part of Lublin Province [33]. It was not until twenty years later that a group of 10 leptospirosis cases were described in the eastern part of the province [35]. No extensive epidemics of leptospirosis have occurred in Poland since the 1970s, not even after a big flood that afflicted Lower Silesia in 1997. The seroepidemiology of the disease is continuously monitored by the central laboratory in Wroclaw [29] and was the subject of separate studies in various parts of the country [36, 37, 38]; however, considering the scale of the monitoring and the frequency of mentioned studies, the threat of a leptospirosis epidemic in Poland can be quickly identified and eliminated.

The prevalence of seropositive farmers noted in the present study in community 'A', experienced after a flood was low (3%), and insignificantly smaller compared to farmers from community 'B' who were not affected by flood (9.2%). These, rather unexpected, results could be explained by the proper ensuring of the inhabitants against health consequences of the flood, and most probably also by the properties of the natural constuents of the environment – soil, water, fauna – which favoured the circulation of leptospiras in community 'B'.

The prevalence values obtained in the presented study are higher compared to those reported by Juszczyk *et al.* [36] who found in the farmers living in the Wielkopolskie Province (western Poland) 1.2% of seropositive reactions to *Leptospira*. These are lower compared to the data of Cybulska *et al.* [37] who obtained 11% of seropositive reactions in the farmers living in the north-central part of Lublin Province. Our results are also lower compared to those reported by Krawczyk [38] who found 13.8% of seropositive reactions among the rural inhabitants of Kujawsko-Pomorskie Province exposed to infected animals, and 1.5% among those without such contact. Compared to similar studies performed in Europe, the results of the presented study are higher compared to those obtained in the Netherlands by Elbers *et al.* [19] (0.5%), and lower compared to those obtained in Northern Ireland by Stanford *et al.* [22] (8.1%), and in Italy by Nuti *et al.* [23] (10-12%) and Fenga *et al.* [24] (23.5%).

In conclusion, the results of the presented study suggest that there is only a slight, if any, hazard of a leptospirosis epidemic after the flood that affected eastern Poland in 2010. However, although the general epidemiological situation of leptospirosis in eastern Poland does not seem of great concern, further research is stll needed.

Acknowledgements

This study was supported by the Polish National Centre of Science, Grant No. N N404 265840. The authors express their thanks Mrs. Małgorzata Łucjanek for excellent technical support.

REFERENCES

1. Adler B, de la Peña Moctezuma A. Leptospira and leptospirosis. Vet Microbiol. 2010; 140: 287-296.
2. Adler B, de la Peña Moctezuma A. Leptospira. **In:** Gyles CL, Prescot JF, Songer JG, Thoen CO. Pathogenesis of bacterial infections in animals. 4th ed. Willey-Blackwell, 2010. p. 572-547.
3. Monahan A, Miller I, Nally J. Leptospirosis risk during recreational activities. J Appl Microbiol. 2009; 107: 707-716.
4. Faine S. Leptospira and leptospirosis. 1st ed. CRC Press, Boca Raton; 1994.
5. Dutkiewicz J, Cisak E, Sroka J, Wójcik-Fatla A, Zając V. Biological agents as occupational hazards – selected issues. Ann Agric Environ Med. 2011; 18: 286-293.
6. Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. J Biosci. 2008; 33: 557-69.
7. Cruz 2009 Cruz LS, Vargas R, Lopes AA. Leptospirosis: a worldwide resurgent zoonosis and important cause of acute renal failure and death in developing nations. Ethn Dis. 2009; 19(Suppl 1): S1-37-41.
8. Evangelista KV, Coburn J. Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. Future Microbiol. 2010; 5: 1413-1425.
9. Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalization of leptospirosis: worldwide incidence trends. Int J Infect Dis. 2008; 12: 351-357.
10. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin Microbiol Infect. 2011; 17: 494-501.
11. Dutkiewicz J, Śpiewak R, Jabłoński L, Szymańska J. Biological Agents of Occupational Risk. Classification, Exposed Professional Populations, Measurements, Prevention. Ad Punctum, Lublin; 2007 (in Polish).
12. Lau CL, Smythe LD, Craig SB, Weinstein P. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Trans R Soc Trop Med Hyg. 2010; 104: 631-638.
13. Zwierz J. Marsh fever in Silesia. Pol Tyg Lek. 1951; 6: 1510-1515 (in Polish).
14. Zwierz J. Leptospiroses. Edition 2. PZWL, Warsaw 1964 (in Polish).
15. Schoonman L, Swai ES. Risk factors associated with the seroprevalence of leptospirosis, amongst at-risk groups in and around Tanga city, Tanzania. Ann Trop Med Parasitol. 2009; 103: 711-718.
16. Onyemelukwe NF. A serological survey for leptospirosis in the Enugu area of eastern Nigeria among people at occupational risk. J Trop Med Hyg. 1993; 96: 301-304.
17. Cacciapuoti B, Nuti M, Pinto A, Sabrie AM. Human leptospirosis in Somalia: a serological survey. Trans R Soc Trop Med Hyg. 1982; 76: 178-182.
18. Sharma S, Vijayachari P, Sugunan AP, Natarajaseenivasan K, Sehgal SC. Seroprevalence of leptospirosis among high-risk population of Andaman Islands, India. Am J Trop Med Hyg. 2006; 74: 278-283.
19. Elbers AR, Vecht U, Osterhaus AD, Groen J, Wisselink HJ, Diepersloot RJ, Tielen MJ. Low prevalence of antibodies against the zoonotic agents *Brucella abortus*, *Leptospira* spp., *Streptococcus suis* serotype II, hantavirus, and lymphocytic choriomeningitis virus among veterinarians and pig farmers in the southern part of The Netherlands. Vet Q 1999; 21: 50-54.
20. Bokhout BA, Peterse DJ, Koger PL, Terpstra WJ. The occurrence of hardjo-positive dairy cows in the Northern Netherlands. A prospective serological survey. Tijdschr Diergeneeskd 1989; 114: 123-130 (in Dutch).

21. Campagnolo ER, Warwick MC, Marx HL Jr, Cowart RP, Donnell HD Jr, Bajani MD, Bragg SL, Esteban JE, Alt DP, Tappero JW, Bolin CA, Ashford DA. Analysis of the 1998 outbreak of leptospirosis in Missouri in humans exposed to infected swine. J Am Vet Med Assoc. 2000; 216: 676-682.
22. Stanford CF, Connolly JH, Ellis WA, Smyth ET, Coyle PV, Montgomery WI, Simpson DI. Zoonotic infections in Northern Ireland farmers. Epidemiol Infect. 1990; 105: 565-570.
23. Nuti M, Amaddeo D, Crovatto M, Ghionni A, Polato D, Lillini E, Pitzus E, Santini GF. Infections in an Alpine environment: antibodies to hantaviruses, leptospira, rickettsiae, and *Borrelia burgdorferi* in defined Italian populations. Am J Trop Med Hyg. 1993 ;48: 20-25.
24. Fenga C, Spatari G, Cacciola A, Ioli A. Serologic investigation on the prevalence of *Leptospira* spp. infection in occupationally exposed subjects. Med Lav. 2004; 95: 441-446 (in Italian).
25. Bolin CA. Leptospirosis. In: OIE Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals. Office International des Epizooties 2008, pp. 251-264.
26. Wasiński B, Pejsak Z. Occurrence of leptospiral infections in swine population in Poland evaluated by ELISA and microscopic agglutination test. Pol J Vet Sci. 2010; 13: 695-699.
27. Korczyńska A, Suchowiak J. An epidemic of marsh fever in the Wroclaw Province in 1971. Przegl Epidemiol. 1973; 27: 563-568 (in Polish).
28. Hałat Z, Korczyńska A, Kasiński J. Leptospirosis outbreak in the Wrocław Province in 1974. Przegl Epidemiol. 1976; 30: 491-495 (in Polish).
29. Litarska U, Knap JP, Zieba J. Leptospirosis laboratory of WSSE in Wrocław (1947-2005). Comment on its activity. Przegl Epidemiol. 2006; 60: 213-224 (in Polish).
30. Parnas J. The organization and methods of investigations on swamp fever in the province if Lublin. A field trial in 1955-1957. Przegl Epidemiol. 1958; 12: 1-4 (in Polish).
31. Uziak S. The results of soil investigations in foci of swamp fever in rural areas of the Lublin province. Przegl Epidemiol. 1958; 12: 43-49 (in Polish).
32. Cechowicz W, Hryniewicz H. Zoological and ecological investigations on small mammals in natural foci of leptospirosis in the Lublin province. Przegl Epidemiol. 1958; 12: 25-28 (in Polish).
33. Parnas J, Dąbrowski T, Łazuga K, Koślak A, Paroszkiewicz M. Microbiological investigations on leptosirosis inn small mammals and in domestic animals in the district of Tomaszów Lubelski. Przegl Epidemiol. 1958; 12: 29-34 (in Polish).
34. Łazuga K. Microbiological and serological investigations on leptospirosis in the province of Lublin (1956-57). Przegl Epidemiol. 1958; 12: 5-7 (in Polish).
35. Patorska-Mach E, Mierzejewska I, Kornaś B. Leptospiroses in the Chełm province. Przegl Epidemiol. 1978; 32: 323-329 (in Polish).
36. Juszczyk J, Lewkowicz H, Adamek J, Furmaniuk J, Klemczak K, Szkaradkiewicz A, Kiczka W. Occurrence of antibodies against Listeria, Bedsonia, Leptospira and Brucella in workers of animal husbandry farms in the Poznań region. Przegl Epidemiol. 1984; 38: 11-18 (in Polish).
37. Cybulska M, Doleżko-Marciniak H, Hencner Z. Serologic investigations for leptospira infections in humans in the Province of Lublin in the years 1966-1971. Pol Tyg Lek. 1973; 28: 733-735 (in Polish).
38. Krawczyk M. Estimation of transmission hazard of *Leptospira* sp. infections in 2 groups of people. Przegl Epidemiol. 2004; 58: 207-212 (in Polish).