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Bull Vet Inst Pulawy 56, 489-493, 2012
DOI: 10.2478/v10213-012-0086-1

OCCURRENCE OF LEPTOSPIROSIS IN DOMESTIC ANIMALS REARED ON EXPOSED OR NON-EXPOSED TO FLOOD AREAS OF EASTERN POLAND

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Received: July 3, 2012 *Accepted: December 3, 2012*

Abstract

Blood serum samples collected from randomly selected groups of 32 pigs and 41 cows reared in farms belonging to the rural community "A" located in eastern Poland and exposed to the Vistula river floods, and serum samples from groups of 41 pigs and 40 cows from farms belonging to the rural community "B" located also in eastern Poland but not in the area exposed to floods, were examined by the microscopic agglutination test for the presence of antibodies against 18 *Leptospira* serovars. The percentage of serum samples presenting positive results with at least one serovar were higher in pigs and cows from community "A" comparing to community "B" (34.4% *vs*. 4.9% and 26.8% *vs.* 15.0%, respectively). In the case of pigs, the difference was statistically significant (P=0.0015). The reactions with 12 *Leptospira* serovars (Australis, Bataviae, Bratislava, Canicola, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Poi, Cynopteri, Grippotyphosa, Celledoni,), belonging to four species (*L. interrogans, L. borgpetersenii, L. kirschneri, L. weili*) were found in the examined animals. In community "B", six reactions with one serovar and two reactions with two serovars were noted whereas in community "A" - 19 reactions with one serovar, one reaction with two serovars and two reactions with six serovars were observed. The titres in animals reared in community "A" were significantly higher (up to 25,600) compared to community "B" (up to 200, P=0.0094). The obtained results suggest that the exposure to flooding may increase the infection rate in pigs and cows from afflicted areas to some extent.

Key words: swine, cattle, flood, *Leptospira*, leptospirosis, sero-prevalence, Poland.

Leptospirosis is perceived as the most widespread zoonosis in the world. Because of an increasing incidence in humans and domestic animals, the disease is classified as a re-emerging health problem (1, 7, 11, 27, 34). Leptospires usually survive in renal tubules of many domestic and wild animals (1, 2, 12). Humans become infected most commonly through the contact with contaminated by leptospires urine of clinically or subclinically ill animals, either directly or *via* contaminated water or soil (1).

Leptospirosis in pigs is considered as an important economic and public health problem (5, 25, 30). It can cause significant losses due to abortions, stillbirths, weakly piglets, or infertility. Abortions also constitute the main reason of economic losses caused by leptospirosis in cattle. Both swine and cattle may be the source of the human occupational infections caused by leptospires in farmers, veterinarians, slaughterhouse workers, meat industry workers *etc*. (6, 8). Depending on serovar, host's immune status and many other known and unknown factors, the disease can be symptomless or takes form of a mild, flu-like illness or a severe infection able to cause serious multiorgan or systemic disorders leading to death. The mechanisms of pathogenesis of *Leptospira* are largely unknown (11). With a global climate change, extreme weather events such as cyclones and floods are expected to occur with an increasing frequency and a greater intensity and they may potentially result in an upsurge in the disease incidence, as well as, the magnitude of leptospirosis outbreaks (7, 11, 22, 34).

The aim of the study was to investigate occurrence of *Leptospira* sp. in swine and cattle reared on the territories of two rural communities of the Lubelskie province, eastern Poland: community "A" and community "B". Community "A" is situated in the western part of the province at Vistula River. The mentioned area is often exposed to the consequences of raised levels of the river (wet soil, inundations) but in summer 2010, it was afflicted by two huge floods. By contrast, community "B" is situated in the central part of

the province and it does not experience floods. The study was based on the results of serological findings.

Material and Methods

Serum samples. Blood serum samples collected from 32 randomly selected pigs and 41 cows reared on the area of the rural community "A", exposed to floods, and serum samples from 41 pigs and 40 cows reared on the area of the rural community "B" not exposed to floods, 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 to prepare the 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^{o}C under aerobic conditions. The strains were subcultured every 7 days.

Microscopic agglutination test was carried out according to OIE Manual of Standards for Diagnostic Tests and Vaccines (3), as described earlier (33). The serum samples diluted 1:50 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 end point – 50% agglutination. The samples with titres ≥100 were recognised positive.

Statistical analysis. The data were analysed by Student's *t*-test and Mann-Whitney test, with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, USA). Student's *t*-test was used for the assessment of differences between the total positive MAT reactions obtained in the communities "A" and "B" while Mann-Whitney test was used for the assessment of differences between MAT titres recorded in these communities, which demonstrated a non-parametric distribution. The value of $P \leq 0.05$ was considered positive.

Results

Swine. Positive results with at least one serovar were found distinctly more often among pigs from community "A" compared to community "B" (34.4% *vs*. 4.9%) and the difference was statistically significant (P=0.0015). The reactions with 10 serovars of *Leptospira* (Bataviae, Bratislava, Canicola, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Poi, Grippotyphosa, Celledoni,), belonging to four species (*L. interrogans, L. borgpetersenii, L. kirschneri, L. weili*) were found in the animals from community "A" (Table 1). Out of 11 positive animals, 10 pigs reacted with one serovar and one with six serovars. The latter pig reacted in very high titres with serovars: Poi (25,600), Pomona (6,400), Bratislava (3,200), Celledoni (3,200) Icterohaemorrhagiae (400), and Hardjo (400) (Table 1). The titer noted in the reaction with serovar Poi was the highest titer indicated during the study. Among pigs from group "B", only two positive results were noted. One of the mentioned animals showed reaction with serovar Australis in titer 200 and the second one reacted with serovar Bratislava in titer 100. The titres in group "A" were distinctly greater compared to group "B" but the difference was not significant because of a low numbers of positive findings in group "B" (P=0.356)

Cattle. The percentage of positive results with at least one serovar was higher in cows from community "A" compared to community "B" (26.8% *vs*. 15.0%) but the difference was not statistically significant (P=0.189). The reactions with eight serovars of *Leptospira* (Bataviae, Bratislava, Canicola, Hardjo, Hebdomadis, Pomona, Cynopteri, Grippotyphosa), belonging to two species (*L. interrogans, L. kirschneri*) were found in the animals from community "A" (Table 2). Out of 11 positive animals, nine reacted with one serovar, one with two serovars and one with six serovars. The latter cow showed high titres with serovars: Pomona (6,400), Grippotyphosa (800), Hardjo (800), and Cynopteri (400), and low titers with serovars Bataviae (100) and Canicola (100) (Table 2). In cows from group "B", six positive reactions were noted. All of them demonstrated titres 100. Four cows showed reaction with one serovar (Hebdomadis, Grippotyphosa, and Poi) and two with two serovars (Australis & Canicola, and Poi & Celledoni) (Table 2). Thus, cows from group "B" reacted with six serovars belonging to four species (*L. interrogans, L. borgpetersenii, L. kirschneri, L. weili*). The titres in the group "A" were higher compared to the group "B" and the difference was significant (P=0.0128). The difference concerning titres was also significant if pooled positive findings in swine and cattle were compared (P=0.0094).

Discussion

The prevalence of positive results found during the study in pigs coming from the area not exposed to floods was lower than those observed by Krawczyk (21) during the serological survey of randomly selected pigs in northern Poland (8%). Prevalence of positive results noted in pigs from the area afflicted by floods was, however, significantly higher than observed in both mentioned above populations of animals The levels of positive results obtained in serological surveys of swine populations reported by authors from the USA (19), England (14), Portugal (28), the Netherlands (17), and Thailand (25) ranged between 10.0% and 22.2%. They were higher than in case of results noted in Poland in pigs from areas not exposed to floods and lower than in group from the region exposed to floods.

Table 1
Results of serological (MAT) findings carried on pigs
reared on the area of two rural communities in the Lubelskie province (eastern Poland)

Community	Number of examined serum samples	Positive serum samples		
		Number	Reacting with serovar(s)	Titer(s)
A	32	1	Bataviae	400
		2	Bratislava	200
		2	Canicola	100
		1	Grippotyphosa	100
		1	Grippotyphosa	200
		2	Hebdomadis	100
		1	Hebdomadis	200
		1	Poi	25,600
			Pomona	6,400
			Bratislava	3,200
			Celledoni	3,200
			Icterohaemorrhagiae	400
			Hardjo	400
Total positive (%)		11 (34.4)		
B	41	1	Australis	200
		1	Bratislava	100
Total positive (%)		2 (4.9)		

Table 2
Results of serological (MAT) findings carried on cows
reared on the area of two rural communities in the Lubelskie province (eastern Poland)

Community	Number of examined serum samples	Positive serum samples		
		Number	Reacting with serovar(s)	Titer(s)
A	41	1	Bataviae	100
		1	Bratislava	100
		1	Grippotyphosa	1,600
		2	Grippotyphosa	200
		2	Grippotyphosa	100
		1	Hardjo	100
		1	Hebdomadis	200
		1	Pomona	1,600
			Hardjo	200
		1	Pomona	6,400
			Grippotyphosa	800
			Hardjo	800
			Cynopteri	400
			Bataviae	100
			Canicola	100
Total positive (%)		11 (26.8)		
B	40	1	Australis	100
			Canicola	100
		2	Grippotyphosa	100
		1	Hebdomadis	100
		1	Poi	100
		1	Poi	100
			Celledoni	100
Total positive (%)		6 (15.0)		

Results reported from Canada (16) and Tanzania (20) were within the range 3.2%-4.4%, being also smaller compared to the results obtained in pigs from not exposed to floods areas. An interesting case observed during the study was the swine presenting titres to six serovars. Although, some of these titres indicated a high probability, the consequence of cross reactions (*e.g*. between including serovar Poi serogroup Javanica and serogroups Celedoni and Icterohaemorrhagiae), the appearance of high titres of antibodies reacting with serovars Pomona and Bratislava can point out the infection caused by the mentioned serovars and they need to be confirmed by a direct detection of leptospires.

No reports published recently in the European countries concerning the occurrence of Leptospira infections in animals on the areas exposed to floods were found. Boqvist *et al* (4) investigated the association between the rainfall and seropositivity of *Leptospira* in outdoor reared pigs. The farms included in the study were located in the southern part of Sweden. The obtained data showed a highly significant positive association between rainfall and seropositivity to *Leptospira* in outdoor reared pigs in the same climatic zone. For each millimeter of extra rainfall, there was an increase in the odds ratio for seropositivity to serovar Bratislava and to serovar from serogroup Sejroe. There was no association between seropositivity and the temperature (4).

Pigs investigated in the study were indoor reared, mainly in small piggeries (1–20 swine). During the floods many of the animals were evacuated from farms. Moreover, the samples used for the study were collected one year after the floods, thus the majority of the examined pigs (also these with positive results in MAT) was born already after these events. Therefore, the most probable source of infection of the pigs seem to be rodents, which should be surveyed for the presence of leptospires. They will be investigated in further part of the project.

The frequency of seropositive reactions in cows found in the study was greater compared to those reported in Sweden (23), Volkrust district in South Africa (24), the Netherlands (17), and Spain (10), which ranged from 1% to 10.4%, and were similar to those reported in Portugal (28), KwaZulu-Natal province in South Africa (15), Zimbabwe (13), and USA (31), which ranged from 15.3% to 35%, and smaller compared to those reported from Brazil, North Ireland, and Mexico (8, 26, 29), which ranged from 47.6% to 62.8%. The results of other serological surveys of cattle in Poland demonstrated various levels of *Leptospira* infections. One of them (21) indicated slightly more than 5% of positive results and the second one (32), continued from 2002 to 2005, indicated positive results ranging from 0% to 3.75% in particular years. Nevertheless, none of them included samples from regions afflicted recently by floods. Out of *Leptospira* serovars mentioned by authors of earlier reports as common inducers of antibodies to cattle, the presence of reactions to serovars Hardjo, Pomona, Grippotyphosa, and Bratislava but not to serovar Tarassovi was confirmed (10, 13, 15, 17, 18, 24, 26, 28, 29, 31). It is worth mentioning the case of a cow demonstrating antibodies (of titre 6,400) to serogroup Pomona and additionally lower antibody titers to five other serogroups. Here, the observed antibodies to serogroup Grippotyphosa result from a cross reaction with serogroup Pomona; however, the indicated antibodies to other serogroups are difficult to explain.

The obtained results suggest, that exposure to flooding may increase to some extent the infection rate in pigs and cows from afflicted areas. A precise identification of infection sources and estimation of ways of *Leptospira* transfer to animals reared on areas exposed to floods needs further investigations including a direct detection of leptospires in the environment and in wild animals (mainly rodents).

Acknowledgments: This work was supported by the Polish National Centre of Science, grant N N404 265840. The authors would like to thank Mrs. Małgorzata Łucjanek for an excellent technical support.

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