

Prevalence of *Leptospira* spp. in Lithuanian wild boars (*Sus scrofa*)

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Abstract: *Leptospira* is a bacteria responsible for a widespread zoonosis that affects both humans and animals. Leptospirosis is a challenging pathology to diagnose and treat since its signs are unspecific and symptoms vary greatly. Disease seems to be highly prevalent in environments where reservoir animals such as rats and small mammals are common. Even though leptospirosis in humans in Lithuania is rare, it remains a disease of significance in Europe. Information on reservoir animals and prevalence of *Leptospira* in wild animals in Lithuania is lacking. The aim of this country-wide study was to evaluate the seroprevalence of *Leptospira* in wild boars in Lithuania. Hunted animals were collected from ten counties that represented the boar population in the country. Sera of 451 collected boars was evaluated for 8 *Leptospira* serovars using the microscopic agglutination test. Seropositivity was observed in 102 (22.6%) boars. Overall, 194 positive reactions occurred. Boars older than 2 years were affected by more serovars and were more seropositive than younger boars ($p < 0.05$). Highest number of positive reactions was observed in Panevėžys (87.9%) and Vilnius (69.1%) county. The results of this study might indicate that the wild boar is a reservoir animal of *Leptospira* and plays a role in its transmission in Lithuania.

Keywords: *Leptospira*; wild boar; Lithuania; serology.

1. Introduction

Leptospira was first described in 1907 and has been studied ever since [1]. Spirochetes are the causatives of a zoonotic Weil's disease in humans and leptospirosis infection in animals, that manifests in a wide variety of clinical presentations [1-4]. In humans, leptospirosis has been recognized as an important cause of febrile disease, renal failure and jaundice [5-8]. In dogs, all organ systems can be affected from acute kidney injury to ophthalmologic, dermatologic and reproductive tract involvement [4,9]. In farm animals, abortions and infertility cases are as common as reduced production of milk, resulting in economic losses [6,10,11]. Reservoir animals, such as rats, mice, opossums, bats, or even cattle are usually asymptomatic, but play an important role in transmission of *Leptospira*, shedding the bacteria in their urine [2,6,12,13]. Multiple risk factors have been established for contracting leptospirosis. The most significant one is being in close contact with reservoir animals, especially rats [1,13,14]. Equally as important is the occupational hazard as *Leptospira* thrives in certain locations such as humid soils and water bodies. Being a hunter, butcher, farmer, veterinarian or working in agriculture increases the risk of being in close contact to the causative of the disease [2,7,15,16]. Recent seroepidemiological study of hunters in Austria has concluded that as much as 10% of hunters [17] and three

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fourths of hound dogs in Japan [18] were seropositive to *Leptospira*. Higher risk of infection was also observed in socioeconomically challenged areas [15,16,19]. It is speculated that communities affected by poverty are more likely to be in contact with rural areas that contain small mammals, rodents and other *Leptospira* reservoir animals.

The prevalence of leptospirosis in humans varies greatly on the geographical location. The United State centers for disease control and prevention have estimated that a million cases of human leptospirosis occur worldwide yearly [20]. Prevalence in Europe is considerably lower. The data of the European center for disease prevention and control (ECDC) states that 1261 cases of leptospirosis were reported in Europe in 2023, 14 of whom deceased [21]. This disease is not common in Lithuania and the last single case was recorded in 2021 [22,23]. However, information on *Leptospira*, its prevalence, possible reservoir animals and risk factors in Lithuania are lacking. The aim of this study was to evaluate the prevalence of *Leptospira* antibodies in the population of wild boars (*Sus scrofa*) in Lithuania. We hypothesized that even though human leptospirosis is rare, boars could be reservoir animals.

2. Materials and Methods

The study was approved by the Ethics Committee of the Lithuanian University of Health Sciences with the licence number 2024 – BEC3-T-034 issued on 2nd of December, 2024.

2.1. Sample collection

The study was conducted during the wild boar hunting season of the year 2021 in months from May to November in 10 different counties that represent the country. All animals were hunted with adherence to the national hunting law with the purpose of controlling the population. None of the animals were specifically hunted for conduction of this study. The collected blood samples were also used for national African Swine fever disease control and assessment. Responsible veterinary doctors from the State Food and Veterinary Service collected blood samples of hunted wild boars using sterile vacuum tubes free of anticoagulant. The samples were transported in ice-cooled containers to the National Food and Veterinary Risk Assessment Institute, Serology Unit, accompanied by documentation indicating the location, county code, gender, and age of each hunted wild boar. The samples were centrifuged at 10000 RPM, and the obtained separated serum was stored at -20°C until further analysis. For each hunted wild boar, the age group was determined by evaluating the degree of tooth eruption and wear of the lower jaw teeth. Three age groups were identified: juvenile (up to 12 months old), sub-adult (12–24 months old), and adult (over 24 months old).

2.2. Leptospiral detection

Leptospirosis was detected using the microscopic agglutination test (MAT) with live leptospiral cultures to detect antibodies against specific serogroups. The standard methodology used for this study adhered to the guidelines as proposed by the OIE (World Organization for Animal Health) Manual of Standards for Diagnostic Tests and Vaccines, Chapter 3.1.12. Leptospirosis, Serological tests [24]. The antigens used for 8 serogroup detection were as follows: *Leptospira bratislava* (strain Jez Bratislava); *L. canicola* (strain Hond Utrecht IV); *L. saxcoebing* (strain Mus 24); *L. copenhageni* (strain M-20); *L. grippityphosa* (strain Andaman); *L. pomona* (strain Pomona); *L. sejroe* (strain M 84); and *L. tarassovi* (strain Perepelicin). MAT was conducted using microplates with duplicate dilutions of 1:50 and 1:100. The reaction was assessed using a dark-field microscopy to evaluate the degree of agglutination. A four-plus scale was used. A positive reaction was defined as a 2+ agglutination (50%) in the 1:100 dilution.

2.3. Statistical analysis

The data was recorded on Microsoft Office Excel 2019. Statistical data analysis was performed using the IBM SPSS Statistics® software package (Statistical Package for Social Sciences 29 for Windows), version 29. Relationships between boar age groups, sex and county were evaluated using the Chi-square (χ^2) test. Statistically significant differences were considered when $p < 0.05$.

3. Results

In total, sera samples of 451 boars were collected and examined. Male boars were more common (342/451; 75.9%) than the females (109/451; 24.2%) in our study. Youngest boars (up to 12-month-old age) were the least common (30/451; 6.7%). About half of the boars (233/451; 51.7%) were 12- to 24-month-olds and 188/451 (41.7%) were older than 24 months. Boars were hunted in 10 counties: Vilnius (55; 12.2%), Kaunas (60; 13.3%), Klaipėda (42; 9.3%), Šiauliai (56; 12.4%), Panevėžys (33; 7.3%), Marijampolė (15; 3.3%), Telšiai (56; 12.4%), Tauragė (41; 9.1%), Alytus (37; 8.2%), and Utena (56; 12.4%). Hunting took place in the months from May to November (except for August). Almost two thirds (68.5%) of the boars were hunted in May and June.

Within the study, boar seropositivity and the number of positive reactions are described as different entities. Overall seropositivity was observed in 102 (22.6%) boars, but 194 positive reactions were observed. Of seropositive boars, 65 (63.7%) were positive for one serovar and 37 (36.3%) were positive for multiple pathogens. Of boars that were seropositive for multiple serovars, 12 (32.4%) tested positive for two serovars, 13 (35.1%) for three, 5 (13.5%) for four, 1 (2.7%) for five, 3 (8.1%) for six, 2 (5.4%) for seven, and 1 (2.7%) for eight. Seropositivity for serovars (sv.) was as follows: Copenhageni 44 (9.8%), Bratislava 42 (9.3%), Canicola 37 (8.2%), Sejroe 21 (4.7%), Tarassovi 15 (3.3%), Pomona 13 (2.9%), Grippytyphosa 12 (2.7%), Saxkoebing 10 (2.2%).

No statistical difference was observed between sexes: males were positive in 72/342 cases (21.1%) and females were positive 30/109 (27.5%) ($p > 0.05$). Within age groups, seropositivity was lowest in boars up to 12 months old (5/30; 16.7%). Boars from 12 to 24 months old were slightly more seropositive (41/233; 17.6%). Boars older than 24 months were the most seropositive (56/188; 29.8%). This finding was statistically significant ($p < 0.05$). Seropositivity for serovars Saxkoebing, Canicola and Bratislava was significantly more common in older boars ($p < 0.05$). In terms of number of positive reactions, youngest boars had 10 positive reactions (5.2%), middle age group had 73 positive reactions (37.6%) and boars older than 2 years had 111 positive reactions (57.2%). Out of all positive reactions, 57.2% belonged to boars older than 24 months. Boars older than 2 years were statistically significantly affected by more serovars and more seropositive than younger boars ($p < 0.05$). Results are summarized in Table 1.

Table 1. Seropositivity to different *Leptospira* serovars in three age groups. (Pom- Pomona; Gryp- Grypythyphosa; Sax- Saxkoebing; Sej- Sejroe; Can- Canicola; Brat- Bratislava; Copen- Copenhageni; Tar- Tarassovi). Results are displayed as n (%; Confidence Interval of 95% (CI)).

Age of boars	Number of positive reactions, n (%; CI)								Total
	Pom	Gryp	Sax*	Sej	Can*	Brat*	Copen	Tar	
Younger than 12 months	2 (1.0%, 0.1-3.7)	0 (0%, 0-1.9)	0 (0%, 0-1.9)	1 (0.6%, 0.01-2.8)	2 (1.0%, 0.1-3.7)	2 (1.0%, 0.1-3.7)	1 (0.6%, 0.01-2.8)	2 (1.0%, 0.1-3.7)	10 (5.2%, 2.5-9.3)
12 to 24 months	4 (2.1%, 0.6-5.2)	4 (2.1%, 0.6-5.2)	2 (1.0%, 0.1-3.7)	11 (5.7%, 2.9-9.9)	12 (6.2%, 3.2-10.6)	14 (7.2%, 4.0-11.8)	22 (11.3%, 7.3-16.7)	4 (2.1%, 0.6-5.2)	73 (37.6%, 30.8-44.9)

Older than 24 months	7 (3.6%, 1.5-7.3)	8 (4.1%, 1.8-8.0)	8 (4.1%, 1.8-8.0)	9 (4.6%, 2.1-8.6)	23 (11.9%, 7.7-17.3)	26 (13.4%, 9.0-19.0)	21 (10.8%, 6.8-16.1)	9 (4.6%, 2.1-8.6)	111 (57.2%, 49.9-64.3)
Total	13 (6.7%, 3.6-11.2)	12 (6.2%, 3.2-10.6)	10 (5.2%, 2.5-9.3)	21 (10.8%, 6.8-16.1)	37 (19.1%, 13.8-25.3)	42 (21.6%, 16.1-28.1)	44 (22.7%, 17.0-29.2)	15 (7.7%, 4.4-12.4)	194 (100%)

* Difference between age groups is statistically significant (p<0.05).

Seropositivity varied statistically significantly in different months and peaked in September (p<0.05). Seropositivity by month was as follows: May 14/118 (11.9%), June 24/191 (12.6%), July 42/97 (43.3%), September 6/11 (54.5%), October 16/31 (51.6%), November 0/3 (0%).

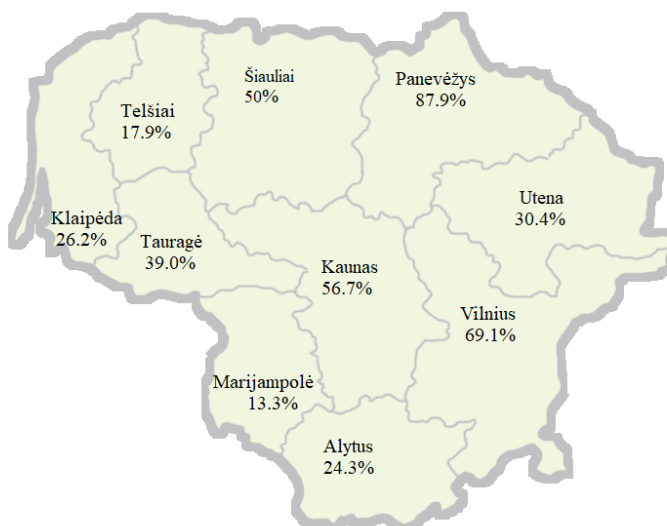


Figure 1. Percentage of seropositive reactions (%) in 10 Lithuanian counties.

Boar seropositivity within the counties was as follows: Vilnius (18/55; 32.7%), Kaunas (15/60; 25.0%), Klaipėda (7/42; 16.7%), Šiauliai (17/56; 30.4%), Panevėžys (8/33; 24.2%), Marijampolė (2/15; 13.3%), Telšiai (9/56; 16.1%), Tauragė (7/41; 17.1%), Alytus (6/37; 16.2%), Utena (13/56; 23.2%). No significant associations between distribution for serovars and county were observed. The number of positive reactions for different serovars in counties is summarized in Figure 1 and Table 2. The highest number of positive reactions was observed in Panevėžys (87.9%) and Vilnius (69.1%) county. The least number of positive reactions was found in Marijampolė (13.3%) and Telšiai (17.9%) county.

Table 2. Number of positive reactions to different Leptospira serovars in 10 different counties. (Pom- Pomona; Gryp- Grypothyphosa; Sax- Saxkoebing; Sej- Sejroe; Can- Canicola; Brat- Bratislava; Copen- Copenhageni; Tar- Tarassovi). Results are displayed as n (% , Confidence Interval of 95% (CI)).

County	Number of positive reactions, n (% , CI)								
	Pom	Gryp	Sax	Sej	Can	Brat	Copen	Tar	Total
Vilnius	3 (5.5%, 1.1-15.1)	1 (1.8%, 0.1-9.7)	1 (1.8%, 0.1-9.7)	4 (7.4%, 2-17.6)	8 (14.5%, 6.5-26.7)	7 (12.7%, 5.3-24.5)	11 (20.0%, 10.4-33.0)	3 (5.5%, 0.1-12.4)	38/55 (69.1%, 57.2-80.9)

								1.1-15.1)	55.2-80.9)
Kaunas	3 (5.0%, 1.0-13.9)	3 (5.0%, 1.0-13.9)	4 (6.7%, 1.9-16.2)	3 (5.0%, 1.0-13.9)	6 (10.0%, 3.8-20.5)	6 (10.0%, 3.8-20.5)	6 (10.0%, 3.8-20.5)	3 (5.0%, 1.0-13.9)	34/60 (56.7%, 43.2-69.4)
Klaipėda	2 (4.8%, 0.6-16.2)	0 (0%, 0-8.4)	1 (2.4%, 0.06-12.6)	3 (7.1%, 1.5-19.5)	0 (0%, 0-8.4)	3 (7.1%, 1.5-19.5)	1 (2.4%, 0.06-12.6)	1 (2.4%, 0.06-12.6)	11/42 (26.2%, 13.9-42.0)
Šiauliai	3 (5.4%, 1.1-14.9)	2 (3.6%, 0.4-12.3)	1 (1.8%, 0.04-9.6)	3 (5.4%, 1.1-14.9)	4 (7.1%, 2.0-17.3)	8 (14.3%, 6.38-26.22)	5 (8.9%, 3.0-19.6)	2 (3.6%, 0.4-12.3)	28/56 (50.0%, 36.3-63.7)
Panevėžys	2 (6.1%, 0.7-20.2)	3 (9.1%, 1.9-24.3)	1 (3.0%, 0.08-15.8)	4 (12.1%, 3.4-28.2)	5 (15.2%, 5.1-31.9)	5 (15.2%, 5.1-31.9)	4 (12.1%, 3.4-28.2)	5 (15.2%, 5.1-31.9)	29/33 (87.9%, 71.8-96.6)
Marijampolė	0 (0%, 0-21.8)	0 (0%, 0-21.8)	0 (0%, 0-21.8)	0 (0%, 0-21.8)	1 (6.7%, 0.2-32.0)	0 (0%, 0-21.8)	1 (6.7%, 0.2-32.0)	0 (0%, 0-21.8)	2/15 (13.3%, 1.7-40.5)
Telšiai	0 (0%, 0-6.4)	1 (1.8%, 0.04-9.6)	0 (0%, 0-6.4)	1 (1.8%, 0.04-9.6)	5 (8.9%, 3.0-19.6)	2 (3.6%, 0.4-12.3)	0 (0%, 0-6.4)	1 (1.8%, 0.04-9.6)	10/56 (17.9%, 8.9-30.4)
Tauragė	0 (0%, 0-8.6)	1 (2.5%, 0.06-1.9)	0 (0%, 0-8.6)	2 (4.9%, 0.6-16.5)	4 (9.8%, 2.7-23.1)	4 (9.8%, 2.7-23.1)	5 (12.2%, 4.1-26.2)	0 (0%, 0-8.6)	16/41 (39.0%, 24.2-55.5)
Alytus	0 (0%, 0-9.5)	1 (2.7%, 0.07-14.2)	1 (2.7%, 0.07-14.2)	1 (2.7%, 0.07-14.2)	2 (5.4%, 0.7-18.2)	3 (8.1%, 1.7-21.9)	1 (2.7%, 0.07-14.2)	0 (0%, 0-9.5)	9/37 (24.3%, 11.8-41.2)
Utena	0 (0%, 0-6.4)	0 (0%, 0-6.4)	1 (1.8%, 0.04-9.6)	0 (0%, 0-6.4)	2 (3.6%, 0.4-12.3)	4 (7.1%, 2.0-17.2)	10 (17.9%, 8.9-30.4)	0 (0%, 0-6.4)	17/56 (30.4%, 18.8-44.1)

4. Discussion

Leptospira is an organism responsible for a zoonosis, dangerous to both animals and humans. Species such as mice, rats, boars, deer and other mammals are thought to be reservoir animals that continue to spread the pathogen by shedding bacteria in their urine [5,25-27]. In Lithuania, wild boar population is carefully monitored as the African swine fever is a disease of increasing importance [28]. It is considered that 20-30 thousand boars are hunted yearly to limit their spread into rural areas where they could become a potential health hazard to both humans and other animals.

Multiple studies have already been performed worldwide to evaluate seroprevalence of *Leptospira* in boars and the data is variable [29- 42]. The main findings of boar *Leptospira* seroprevalence in different countries are summarized in Table 3. In Portugal, the

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seroprevalence seems to be the highest (65.4%) [36]. In Slovenia it reached 45.5% [40], in Croatia ranged between 26 to 31.9% [30,34], in Brazil- 20.5% [32], in France- 18.4% [37], in Germany- 17.7% [33], In Czech republic 16.9% [35], in Spain- 14.6% [42], In Italy varied 6-15.3% [29,31,41], in Poland- 10.4% [38] and in Sweden- 3.1% [39]. In our study it was established to be 22.6%. In the context of the current data in the European continent, the *Leptospira* seroprevalence in boars of Lithuania is considered high.

Table 3. Seropositivity to *Leptospira* in 12 different countries [29-42].

Study	Country	N. of sera	Positive samples	% of positive samples	Most common serovar
Vale-Gonçalves, 2015 [36]	Portugal	101	66	65.4	Tarassovi
Vengust G, 2008 [40]	Slovenia	437	200	45.5	Tarassovi
Cvetnic Z, 2003 [30]	Croatia	154	40	26	Pomona
Slavica A, 2010 [34]	Croatia	351	112	31.9	Australis
Fornazari F, 2011 [32]	Brazil	308	63	20.5	Hardjo
Roquelo C, 2021 [37]	France	358	66	18.4	Australis
Jansen A, 2007 [33]	Germany	141	25	17.7	Pomona
Treml F, 2003 [35]	Czech rep	307	52	16.9	Grippothyphosa
Espí A, 2010 [42]	Spain	171	25	14.6	Pomona
Cilia G, 2020 [29]	Italy	287	39	13.6	Australis
Chiari M, 2016 [41]	Italy	2101	321	15.3	Bratislava
Ebani VV, 2003 [31]	Italy	562	34	6	Bratislava
Żmudzki J, 2015 [38]	Poland	3621	377	10.4	Hardjo
Boqvist S, 2012 [39]	Sweden	386	12	3.1	Bratislava
Current study	Lithuania	451	102	22.6	Copenhageni

In this study, boar sera were tested for 8 chosen *Leptospira* serovars. However, new serovars that can potentially affect people and animals are emerging. For instance, when *Leptospira wolffii* was first detected in a dog, it was concluded, that it was a dominant species affecting 93% of tested dogs in Iran [43]. To minimize the risk of dogs becoming a host animal and shedding bacteria in a household environment, preventions are taken. In Europe and North America, vaccines are used for almost half a century and dog seroprevalence is successfully decreasing [4,44]. However, vaccination does not prevent disease completely and protocols are not uniform around the globe. Vaccines can contain from one to four different serovars, depending on the geographic location [4,45]. It was found, however, that even immunized animals can be infected if prevention is not proper [46]. Vaccinated dogs, therefore, would be susceptible to novel serovars, not found in the vaccines. Constant effort is being put to establish which serovars are the most relevant clinically and if collected evidence is sufficient to include certain *Leptospira* serovars into the vaccines [4,44,47]. Consequently, understanding the distribution of serovars within the countries is of incredible importance.

Up to date, in Lithuania, several epidemiological studies have been performed to detect *Leptospira* in small rodents, swine, horses and cattle to establish possible reservoir animals [48-51]. Only 4.4% of small rodents such as mouse and vole had Leptospiral DNA and *L. Kirschneri* was isolated [48]. Swine were mostly seropositive for serovar Bratislava and Pomona [49]. Prevalence in horses was 18.6% and they were mostly affected by sv. Canicola and Copenhageni [50]. Cattle were most seropositive for sv. Grippotyphosa and total seroprevalence for *Leptospira* was 7% [51]. Our study results indicate that boars in Lithuania are mostly affected by sv. Copenhageni and Bratislava. Based on the current literature and our findings, it is speculated that sv. Copenhageni, Bratislava, and Canicola are common serovars between reservoir animals in Lithuania [49,50].

The boar seroprevalence between sexes did not differ. However, in accordance with recent literature [34-36,41], the oldest boars (older than 24 months) were the most affected in this study. Boars older than 2 years were statistically significantly affected by more serovars and more seropositive than younger boars. This could be explained by several factors. Older individuals have a longer cumulative exposure to *Leptospira* time and the chances, therefore, are higher for older boars to have had multiple subclinical infections with different serovars. Having had close contact with the pathogen, and possibly a leptospirosis infection, allows us to detect the antibodies for certain *Leptospira* serovars in boar blood.

Leptospira detection by Polymerase chain reaction (PCR) is known for its specificity and sensitivity, but it does not allow the identification of the serovar [6,52]. Up to date, several studies were performed using boar kidneys, livers and reproduction organs [29,30,53-55]. It was established that in Croatia 8.4% of boar kidneys had *Leptospira* DNA [30], in Japan 15.2% of boar kidneys were positive [54], while in Italy the percentage varied 11.2-12.6% for kidneys and 30.3% for reproductive organs [29,53,55], demonstrating yet another *Leptospira* transmission method. In swine, artificial insemination is demonstrated to be an important risk factor for *Leptospira* contraction [46]. And in boars, subclinical genital leptospirosis was detected and isolated in testicles, epididymes, uteri, placentas and fetuses [55,56]. This newly recognized spreading mechanism should be further investigated as it could explain why older, sexually mature boars tend to have a significantly higher prevalence [34-36,41].

Panevėžys is located in northeast Lithuania, and it was the county with the highest percentage of *Leptospira* antibody positive boars (87.9%) as well as horses [50]. Possible explanations include different climatic and socioeconomic reasons. As described by Taminskas *et al.*, 2011, Panevėžys county is located in a highly humid climate: soils there are less permeable to water and the Nevėžis river sub-basin area has the second largest expanse of wet forests (wet woodlands take up 21.5% of the territory) [57]. Abundant rivers, lakes, and wetlands create ideal conditions for leptospires to thrive in water and soil as well as for the boars to habituate. In our study Marijampolė county had the least seropositive boars (13.3%). This location has significantly less wetlands and wet woodlands than Panevėžys [57].

We acknowledge that our study has several limitations. MAT is a test that has a limited specificity and sensitivity. It requires careful attention and inspection to avoid false positive or false negative examinations [2,4,6,7,27]. We only tested boar sera for 8 serovars. It is possible, that other serovars were distributed within the boar population as well but we failed to isolate them.

5. Conclusions

A hundred and two (22.6%) boars had *Leptospira* antibodies in their sera. Hunted animals were mostly positive for serovar Copenhageni (9.8%), Bratislava (9.3%) and Canicola (8.2%). Boars over 24 months old were more prevalent and affected by a higher number of serovars than younger boars. The highest number of positive reactions was observed in Panevėžys (87.9%), which is known for its highly humid soil, rich in wet woodlands. The results of our study might indicate that boars are reservoir animals of *Leptospira* and contribute to its transmission in Lithuania.

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