SEROEPIZOOTIC SURVEY OF LEPTOSPIROSIS IN HORSES

Marija Stankevičienė[1], Jūratė Buitkuvienė[2], Neringa Bartaševičiūtė[1], Rasa Adomkienė[1], Jurgita Statkevičiūtė[1]

[1]Lithuanian University of Health Sciences, Veterinary Academy
Tilžės str. 18, Kaunas marija.stankevičienė@lsmuni.lt, 8~671 67 236
[2]National Food and Veterinary Risk Assessment Institute, J. Kairiūkščio str. 10, Vilnius, (8~5) 278 0473

Abstract. Leptospirosis is a common infectious disease which is wide spread all over the world. 440 of blood serum samples were tested to determine the spread of this contagious disease amongst equine family animals (horses, Shetland ponies, donkeys) in Lithuania. The survey was carried out in 2011 – 2015 period, blood samples were tested by MA test. There were found that 18.63% of investigated horses are serologicaly positive for leptospirosis The highest percentage 19.6% of positive samples were from stud farms' horses. Antibodies against serogroups of *L. canicola* (33.0%), *L. copenhagen* (26.1%) and *L. grippotyphosa* (20.9%) were identified in analysed samples. Most positive results (31.8%) were found among horses located in Panevezys County. Mares consisted 69.5% of all the positive reacted horses.

Keywords: *Leptospira spp*., horses, MAT

Introduction. Leptospirosis is a common infectious disease which is wide spread all over the world. The infection is transmitted by spirochetes invading through mucous membranes or broken skin, in contact with infected animals, their urine or other body fluids (Adler et al., 2010; Pikalo et al., 2016).

Many animals, especially rodents, may become infected by leptospirosis, the disease is even prevalent among people. There were recorded 12 people infected with leptospirosis during this year in Lithuania and 4 cases of infection in 2015 (ULAC data). The dominant serovars may vary depending on the source of infection and location, in which is prevalent (Hammond et al. 2014).

Equine leptospirosis is characterized by uveitis, kidney or liver dysfunction (Yan et al., 2010). Leptospirosis can cause abortion, still birth or neonatal disease (Hamond et al., 2015; Timoney et al., 2011). Clinical symptoms for sick horses are pyrexia, anorexia, haematuria, jaundice. However, most horses rarely show clinical signs, if does, symptoms are mild. (Hamond et al., 2012).

Equine leptospirosis can lead to depression and lack of strength. It is important particularly for sport horses. Infected horses can poorly reach the best results in equine sport. Even 89.5% of poorly competing horses were seropositive for leptospirosis (Hamond et al., 2012; Hamond et al., 2014; Pikalo et al., 2016).

It is not surprising that most horse leptospirosis cases are diagnosed in Brazil, where the disease is prevalent among people. In country, with appropriate conditions for the spread of infection consists of the rainy season and the high ambient temperature, more than 70% of horses are seropositive (Hamond et al., 2014; Jorge et al., 2011; Pinna et al., 2007). In Argentina 57.2% out of 561 tested horses were seropositive, in Tropical Islands – 61% (Hamond et al., 2014).

At the same time, leptospirosis is widespread in not tropical countries, there were found many cases of leptospirosis infection in Canada, Switzerland and Poland (Hamond et al., 2014). The higher number of infected are more common where horse population is greater: in stud farms, in stables between sport and leisure horses, than individually kept horses (Pikalo et al., 2016).

Leptospirosis were identified in cattle and pigs in Lithuania (Stankevičienė et al., 2013; Šiugždinienė et al., 2007). There are no proven scientific data about leptospirosis in equine population in Lithuania.

Methods and materials. 440 samples of horse, Shetland ponies and donkeys blood serum were tested. Prevalence of leptospirosis infection in equine population was analyzed. In research used blood samples were presented from equines kept in 6 counties. Results and data, of already carried out samples detecting leptospirosis, were analyzed.

Diagnostic of leptospirosis was performed using microscopic agglutination test (MAT) with alive cultures of leptospira (detecting antibodies against specific serogroups).

The study was carried out using standard method – microscopic agglutination test, in accordance to O.I.E Manual of Standards for Diagnostic Tests and Vaccines, Chapter 2.2.12. Leptospirosis, Serological tests, 2014 methodology.

Reaction was performed with alive cultures of leptospires, detecting antibodies against *L. pomona, L. copenhageni, L. grippotyphosa, L. canicola, L. saxoebing, L. sejroe.*

For production of required media, used Leptospira Medium Base EMJH and Leptospira Enrichment EMJH components manufactured in Difco Laboratories (USA).

Sodium chloride 0.85% solution, used in study, was made in the laboratory, which includes sodium chloride manufactured by Carl Roth GmbH + Co. KG (Germany) mixed with deionized water.

Leptospira strains were cultivated in a liquid medium for Leptospiras, which was prepared with distilled water. For medium preparation inoculate 2.3 g of Leptospira Medium Base EMJH reagent in 900 ml of distilled water and autoclave. The pH value of medium should be 7.2 to 7.6. Following autoclaving, aseptically inoculate 100 ml of Leptospira Enrichment EMJH and prepared medium

was ready to dispense into sterile tubes. Tubes with Leptospiras were incubated in a thermostat at ±29 ºC ±1 ºC temperature. Cultures were incubated at least 4 days, but not more than 8 days. In reaction used live cultures with density of approximately 2 × 108 Leptospires per milliliter. Leptospira used as antigen. Culture density was determined by counting the cells directly using a bacterial counting chambers and dark-field microscopy.

The reaction was performed in micro plates, for same sample both 1:10, 1:50 and 1:100 dilutions are prepared.

Samples were incubated at 30 ± 1°C for 1.5 - 4 hours. After incubation, the reaction was examined with dark-field illumination. It was observed whether agglutination has occurred. Agglutination was assessed in four pluses. The reaction was considered to be positive, if occurred agglutination, in 1:100 dilutions, assessed by 2 pluses (50 %).

Laboratory research carried out in National Food and Veterinary Risk Assessment Institute, serological tests compartment results were analyzed in Lithuanian University of Health Sciences, Veterinary Academy.

The obtained data processed with "Microsoft Excel 2016" calculators and SPSS 20.0 statistical package.

Results. 82 (18.64%) samples of blood serum from 440 tested have responded positively during the research.

Positive samples were found in four of the six surveyed counties. Positively responded horses were surveyed 2013 – 2014 in Vilnius, Panevezys County, 2011–2013 in Kaunas County, 2013 in Utena County. Antibodies against Leptospira found in 31.8% of samples from Panevezys region, slightly less found in Utena County – 19.7%, in Vilnius and Kaunas Counties about 16,0%. None positive results were found in Alytus and Siauliai Counties. Comparing positively responded cases in Vilnius and Panevėžys Counties, the difference between the numbers was statistically significant (p = 0.032).

The greatest number of positive samples was in year 2013 and 2014, accordingly, 50/144 (34.7%) and 27/164 (16.6%) of tested samples. The least number of seropositive found in 2011 - 2012 (Fig. 1).

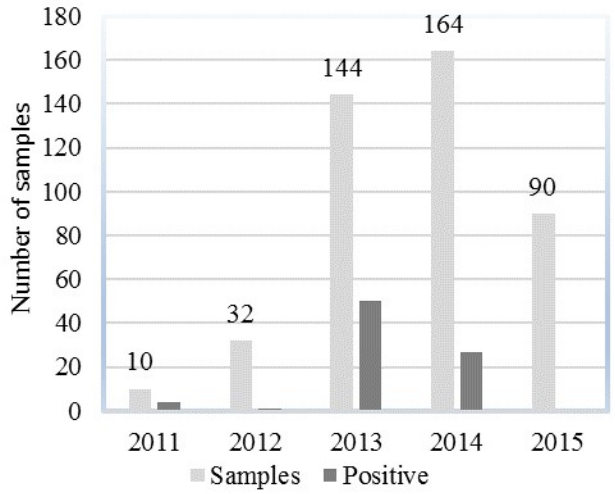

Fig.1. **Number of seropositive samples**

After statistical analysis of the data, the difference between positive samples in 2012 and 2013 is - statistically significant (p = 0.000365).

Serum samples were taken from the biggest Lithuanian stud farms, as well as individually kept horses, and all types of equines from zoo (Shetland ponies, donkeys). At stud farms number of horses varied widely, from 50 to 140 in the herd, and more. Approx. 1 – 10 horses were kept individually in private barns / stables.

From the all samples tested, the least number of serologicaly positive samples were taken from the zoo equids. 5 out of 39 tested serum samples were positive (12.8%). 30 out of 161 (18.6%) positive blood serum samples were from surveyed individually kept horses. The greatest percentage of positive samples were detected from the horses kept in stud farms. 47 out of 240 horses surveyed from stud farms had antibodies against *Leptospira*, which is 19.6% of obtained samples (p>0.05).

The analysis showed that, antibodies against one or a few different leptospira strains may be identified from one horse serum sample. For instance, in one stallion's blood sample, surveyed in 2013, according to MAT results antibodies against leptospira were found: titer 1:100/2+ *L. canicola*, titer 1:50/2+ *L .copenhageni* and titer 1:50/3+ *L. grippotyphosa*. It means that samples of mentioned stallion were seropositive against *L. canicola,* while only serologically responded against *L. copenhageni* and *L. grippotyphosa*.

At the time of investigations commonly found serogroups were: *L. canicola, L. copenhagen, L. grippotyphosa, L. sejroe, L. saxoebing, L. pomona*. The other serogroups of leptospires in horses blood serum were not detected in this study (Fig. 2).

After statistical data analysis, the difference between samples with antibodies against *L. canicola* and *L. pomona* serogroups were observed statistically significant (p = 0.024).

Seropositive results were found in horses, ponies and donkeys from all surveyed equids. Serologicaly positive samples clearly prevailed among horses 93.9%, while ponies (Shetland ponies) concluded 3.7%, followed by donkeys just 2.4%.

After statistical data analysis, the difference between seropositive different equid animals was observed statistically significant (p <0.001).

Following the performed research, it appears that, during the period, agents of leptospirosis have been found in blood samples of females - mares / female donkeys, males - stallions / donkeys and geldings.

Geldings concluded the lowest part 4.9% from 82 seropositive animals, the majority 69.5% of positive samples were among females (Fig. 3). Marked differences were visible in the graph, in prevalence of infection according to animal gender (p = 0.0026).

Discussion and conclusions. In nowadays horses, donkeys and other equines, all over Europe are used for work, sport, as companion animals, in therapeutic medicine or even for consumption. According to the data from SE Agricultural and Rural Business Centre on the 1st of October 2016 in Lithuania 16,945 horses were kept by

8,407 owners. Most of the horses kept in district municipality of Vilnius - 1447, followed by Alytus district municipality – 724, Prienai district municipality (nesivadina county) - 612, Pagegiai municipality - 588, Zarasai district municipality - 505, Utena district municipality - 484 (VIC data).

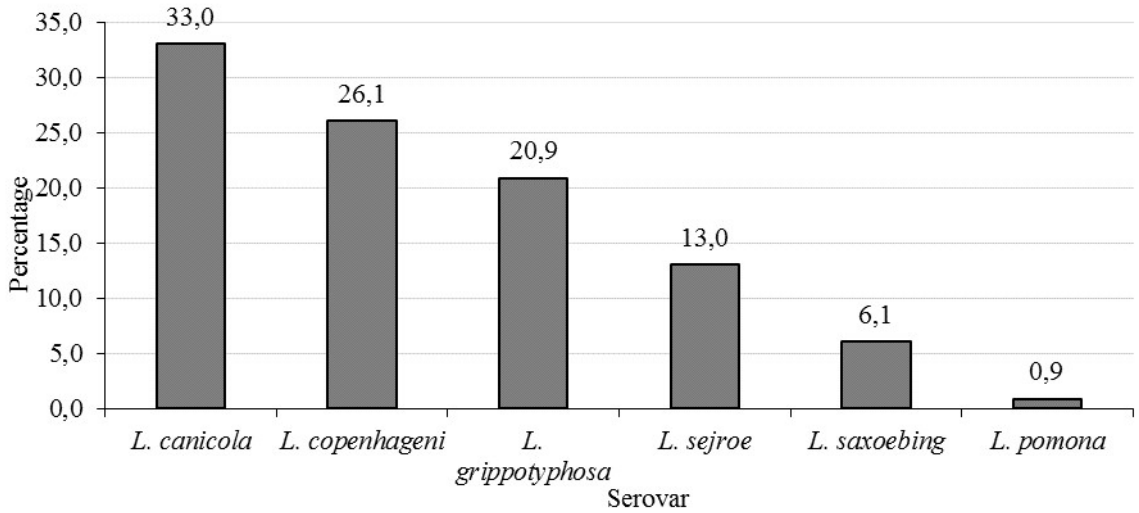

Fig 2. **Prevalence of Leptospira serogroups in horse population**

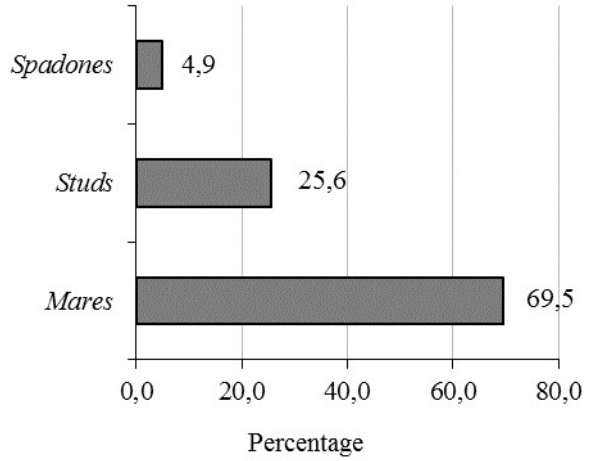

Fig 3. **Sex distribution in leptospiral seropositive horses**

This study was conducted in 2011 - 2015 on 440 horses' blood serum samples using MAT method to determine seroprevalence of leptospira infection. It can be stated that serologicaly positive samples consist 18.6% accordingly to study carried out.

NFVRAI analysis of the data showed that the most explored samples with antibodies against Leptospira were in pig population, in horse population seropositive samples rate was the highest - 42.8% also in the same year (Stankevičienė et al., 2013). In recent years, the number in surveyed horses strongly increased due to neglect in certain farms. The perfect conditions to multiply for leptospirosis agents are poor housing conditions, moisture, unbalanced diet, fasting, big invasion of rodents in farm premises and weak animal health (Verma et al., 2013). It could be noted, that, where the number of horses kept is greater, more positive cases are found. This may be influenced by the import of horses from foreign countries, individually kept and horses from stud farms participating together in open competitions (Hamond et al., 2012).

Equine leptospirosis research, performed by NFVRAI, showed that the bacteria is common in stud farms, private farms and zoo (equivalent to 19.6%, 18.6% and 12.8%). Noticeable tendency of leptospirosis relatively frequently found in stud farms (Pikalo et al., 2016). *Leptospiras* are widely spreaded by animal secretions and contaminated environment in large farms (Hamond et al., 2014).

Estimated correlation between some of the serogroups and individual animal species, for example serogroup copenhageni is associated with rats, canicola - with dogs, serotype hardjo - with cattle (Jocienė et al., 2005). Among Lithuanian equines prevalent serogroups are *L. canicola, L. copenhageni, L. grippotyphosa, L. sejroe, L. saxoebing, L. pomona*. Most of samples were found with antibodies against *L. canicola* (33.0%).

During 1997–98 cross-sectional study, from 2017 horses, was carried out to determine the seroprevalence of different serovars of *Leptospira spp.* and their association with clinical disease and host factors in Swedish horses. The seroprevalence were for serovar bratislava (16.6%) (Båverud et al., 2009). 2001 Iranian city of Ahvaz, was tested for leptospira spread among horses and donkeys. Seropositive were 27.9% out of 61 tested serum samples of horses and donkeys. The greatest part included *L. ballum* (23.8%) and *L. canicola* (14.3%) (Hajikolaei et al., 2005). United States of America included *L. pomona, L. canicola, L. icterohaemorrhagiae, L. grippotyphosa* and *L. hardjo* serogroups in the most dangerous list, because it is causing diseases for dogs, pigs, cattle and horses (Smythe, 2008).

The prevalence of the disease is very much dependent on the country and its public approach to health care. During heavy rain season many reports are received from India, Indonesia, Thailand and Sri Lanka. Particularly a

lot of positive samples of leptospirosis has been obtained in tropical regions. In southern Brazil 80.8% of tested horses were seropositive (Finger et al., 2014).

Analysis of positive blood serum samples showed that serologicaly positive were all observed equine family animals: horses, donkeys and ponies in Lithuania. Still, most antibodies against the pathogen found in horses (93.9%). Such situation could result in different number of animal populations' representatives' sampled and number of animals. According to 2001 study in Ahvaz, incidence occurred 12% higher among donkeys comparing with horses (Hajikolaei et al., 2005).

Comparing seropositive equides distribution by sex, mostly antibodies against *Leptospira* had mares / female donkeys (69.5%) comparing with stallions / male donkeys. Similar results were obtained in year 2001 (Hajikolaei et al., 2005).

Providing these conclusions:

1. Serologicaly positive consist 18.6% of investigated horses and most of positive samples 31.8 % found among horses in Panevezys County.
2. At the period of study mostly found antibodies were against *L. canicola* – 33.0%, *L. copenhageni* – 26.1% and *L. grippotyphosa* - 20,9% serogroups.
3. Samples of mares with antibodies against Leptospira presented 69.5% from all the positive samples.
4. The highest percentage of seropositive samples identified in stud farms, 19.6% of obtained samples, had antibodies against Leptospira.

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Received 08 November 2016
Accepted 06 December 2016