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Equine leptospirosis in Croatia - occurrence of subclinical infections and abortions

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ABSTRACT

Clinical leptospirosis in horses has been primarily associated with abortion, stillbirth and equine recurrent uveitis. Severe septicaemia that includes fever, jaundice and anorexia, haematuria, renal and hepatic dysfunction, respiratory distress and pulmonary haemorrhage is rare. In order to obtain a better insight into the occurrence of equine leptospirosis in Croatia a sero-survey was performed using the microscopic agglutination test (MAT) among healthy and non-healthy horses. Non-healthy horses were represented by the mares that aborted. The aim of the study was to determine the prevalence of *Leptospira* infection and related serovars, as well as to point out the present clinical disease status of equine leptospirosis in Croatia. A total of 8059 horse sera samples of different breeds, age and sex were taken in 2011 from apparently healthy animals. In the same period, 56 samples were taken from mares that had aborted, representing non-healthy animals. Out of 8059 sera samples from healthy horses, 2999 (37.2) were considered seropositive. The highest seroprevalence for healthy horses was found for sv Bratislava in 1342 (44.8%) samples, sv Pomona 546 (18.2%) and sv Icterohaemorrhagiae 448 (14.9%). Out of 56 sera samples from the mares that aborted, representing non-healthy horses, 37 (66.1%) were considered seropositive. The highest seroprevalence for mares that aborted was found for sv Bratislava in 16 (43.2%) sera samples, sv Pomona in 11 (29.7%) and sv Icterohaemorrhagiae in 4 (10.8%). The results showed that horses in Croatia are commonly exposed to *Leptospira* infections. Regarding clinical disease status, mainly subclinical infections and fewer abortions, as the only clinically apparent form of leptospirosis, are currently present in Croatia, irrespective of the level of agglutinating antibody titres.

Key words: leptospira, equine leptospirosis, seroprevalence, microscopic agglutination test

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Introduction

Leptospirosis is a zoonosis of worldwide distribution caused by pathogenic serovars (sv) within the genus *Leptospira (L.)*. The disease affects humans, domestic animals and wildlife. Clinical manifestations of leptospirosis in both humans and animals vary from a mild subacute disease to sever illness, with jaundice, renal failure and death. Infection usually results from direct or indirect transmission via the infected urine of sick or convalescent animals. Reservoir hosts of the causative agent are predominantly small rodents, which, once they are infected, asymptomatically excrete a large number of leptospires into the environment for life, making them secondary sources of infection (FAINE et al., 1999).

The first report of naturally occurring leptospirosis in horses was in 1947 in Russia (LJUBAŠENKO and NOVIKOVA, 1947), while the second one was the first report of equine leptospirosis in Croatia in 1953 (ZAHARIJA, 1953). Clinical leptospirosis in horses has been primarily associated with abortion (ELLIS et al., 1983; DONAHUE et al., 1991), stillbirth (DONAHUE et al., 1991; VEMULAPALLI et al., 2005) and equine recurrent uveitis (WOLLANKE et al., 1998; FABER et al., 2000; BRANDES et al., 2007). Severe septicaemia that includes fever, jaundice and anorexia (LJUBAŠENKO and NOVIKOVA, 1947; ZAHARIJA, 1953), haematuria (BERNARD et al., 1993), renal and hepatic dysfunction (DIVERS et al., 1992), respiratory distress (INGH van den et al., 1989) and pulmonary haemorrhages (HAMOND et al., 2011) is rare. Unfortunately, a definitive diagnosis of leptospirosis is difficult to obtain. Most diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, the complexity of the isolation procedure and long incubation period. Therefore, diagnosis of leptospirosis has often been based on serology, since antibodies are usually present in the blood about 5 to 7 days after the onset of clinical signs (FAINE et al., 1999). The standard reference method for serologic diagnosis of leptospirosis is the microscopic agglutination test (MAT), in which sera react with live antigen suspensions of various *Leptospira* serovars (ANONYM., 2004).

Serological evidence of *Leptospira* infection is common in horses. Titres to a wide variety of serovars have been reported, and although there is variation between studies, the predominant serovars reported are *L. interrogans* sv Bratislava, *L. interrogans* sv Pomona, *L. interrogans* sv Icterohaemorrhagiae and *L. kirschneri* sv Grippotyphosa (ZAHARIJA, 1953; ELLIS et al., 1983; CVETNIĆ et al., 2004; BÄVERUD et al., 2009). The occurrence of specific serovars depends on the presence and prevalence of the specific host species that act as infection reservoirs in different geographical areas.

In order to obtain a better insight into the occurrence of equine leptospirosis in Croatia, a serosurvey was performed using MAT among healthy and non-healthy horses. Non-healthy horses were represented by the mares that aborted. The aim of the study was

to determine the prevalence of *Leptospira* infection and related serovars as well as to point out the present clinical disease status of equine leptospirosis in Croatia.

Materials and methods

During 2011, blood samples were taken by local veterinary practitioners across Croatia within the framework of routine measures of leptospirosis control ordered by the Veterinary Directory, of the Ministry of Agriculture of the Republic of Croatia. A total of 8059 horse sera samples of different breeds, age and sex were taken in 2011 from apparently healthy animals. In the same period 56 samples were taken from mares that had aborted, representing non-healthy animals. They were of different breeds and age.

The microscopic agglutination test (MAT) was performed following the standard procedure (DIKKEN and KMETY, 1978; HARTSKEERL et al., 2006) against 8 *Leptospira* serovars for screening of healthy horses (Table 1) and against 12 *Leptospira* serovars for confirmation of seropositivity of healthy horses and for non-healthy horses (Table 2). These reference strains were obtained from the Royal Tropical Institute in Amsterdam, The Netherlands. The panels of antigens used in the study were chosen on the basis of the results of earlier studies of the horse (CVETNIĆ et al., 2004; MODRIĆ et al., 2004) and rodent populations in Croatia (MILAS et al., 2002; TURK et al., 2003; ŠTRITOF MAJETIĆ, 2010).

According to Croatian regulations a titre of >400 (for serovars Bratislava and Australis a titre of >200) is considered positive and indicates the suspicion of leptospirosis or possible convalescent shedding, regardless of clinical status, and it is notifiable.

Table 1. Panel of antigens for serological screening of healthy horses

No.	Serogroup	Serovar	Strain	Genomospecies
1	Grippotyphosa	Grippotyphosa	Moskva V	*L. kirschneri*
2	Sejroe	Sejroe	M 84	*L. borgpetersenii*
3	Australis	Bratislava	Jež Bratislava	*L. interrogans*
4	Pomona	Pomona	Pomona	*L. interrogans*
5	Canicola	Canicola	Hond Utrecht IV	*L. interrogans*
6	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	*L. interrogans*
7	Sejroe	Saxkoebing	Mus 24	*L. interrogans*
8	Bataviae	Bataviae	Swart	*L. interrogans*

Table 2. Panel of antigens for serological testing of non-healthy horses (in this case aborting mares)

No	Serogroup	Serovar	Strain	Genomospecies
1	Grippotyphosa	Grippotyphosa	Moskva V	*L. kirschneri*
2	Sejroe	Sejroe	M 84	*L. borgpetersenii*
3	Australis	Australis	Ballico	L. interrogans
4	Australis	Bratislava	Jež Bratislava	*L. interrogans*
5	Pomona	Pomona	Pomona	*L. interrogans*
6	Pomona	Mozdok	5621	*L. kirschneri*
7	Canicola	Canicola	Hond Utrecht IV	*L. interrogans*
8	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	*L. interrogans*
9	Tarassovi	Tarassovi	Perepelitsin	*L. borgpetersenii*
10	Sejroe	Saxkoebing	Mus 24	*L. interrogans*
11	Ballum	Ballum	Mus 127	*L. borgpetersenii*
12	Bataviae	Bataviae	Swart	*L. interrogans*

Quality control. The purity of the antigens used in the MAT was checked regularly by dark field microscopy. An evaluation of the identity of the antigens, against reference sera obtained from the Royal Tropical Institute in Amsterdam, The Netherlands, was performed three times per year. The MAT was performed by one person, to limit inter-observer variations of the results. The method has been approved for laboratory testing in veterinary medicine, according to HRN EN ISO/IEC 17025:2005:2007 by the Croatian Accreditation Agency (HAA).

Results

Out of 8059 sera samples of healthy horses, 2999 (37.2%) had agglutinating antibodies against one or more serovars of *Leptospira* and were considered seropositive. The highest seroprevalence for healthy horses were found for sv Bratislava, in 1342 (44.8%) samples, sv Pomona in 546 (18.2%) and sv Icterohaemorrhagiae in 448 (14.9%). The prevalence of other *Leptospira* serovars for healthy horses is presented in Table 3. There were 285 (9.5%) seropositive sera samples that remained undetermined since they had two or more serovars in the same titre. Titres ranged from 400 (200 for sv Bratislava and Australis) to 51200.

Out of 56 sera samples of mares that aborted, representing non-healthy horses, 37 (66.1%) had agglutinating antibodies against one or more serovars of *Leptospira* and were considered seropositive. The highest seroprevalence for mares that aborted were found for sv Bratislava in 16 (43.2%) sera samples, sv Pomona in 11 (29.7%) and sv Icterohaemorrhagiae in 4 (10.8%) sera samples. The prevalence of other *Leptospira*

serovars involved as the possible causative agent of the abortions is presented in Table 4. Titres ranged from 400 (200 for sv Bratislava and Australis) to 12800.

Table 3. Distribution of *Leptospira serovars* among seropositive (SP) samples of healthy horses

Serovar	No. of SP samples	%
Bratislava	1342	44.75
Pomona	546	18.20
Icterohaemorrhagiae	448	14.93
Grippotyphosa	203	6.77
Sejroe	108	3.60
Canicola	3	0.10
Saxkoebing	60	2.00
Australis	2	0.07
Mozdok	1	0.03
Tarassovi	1	0.03
Nondetermined	285	9.50
TOTAL	2999	

Table 4. Prevalence of *Leptospira* serovars among seropositive (SP) samples of aborting mares as non-healthy horses

Serovar	No. of SP samples	%
Bratislava	16	43.2
Pomona	11	29.7
Icterohaemorrhagiae	4	10.8
Grippotyphosa	3	8.1
Saxkoebing	2	5.4
Sejroe	1	2.7
TOTAL	37	

Discussion

Although equine leptospirosis research in Croatia started in 1951 (ZAHARIJA, 1953), our insights into the current disease status in horses in different areas of Croatia are still scanty or incomplete. In Croatia, no equine leptospirosis investigation has been carried out since 2004 (CVETNIĆ et al., 2004; MODRIĆ et al., 2004) although leptospirosis in horses, as well as in other animals and humans, has continued.

In our study, out of 8059 sera samples of healthy horses 2999 (37.2%) were seropositive. At the same time, out of 56 sera samples from mares that aborted (representing non-healthy horses) we found 37 (66.1%) seropositive samples. This study confirms that there is widespread exposure to *Leptospira* in different areas, implying that Croatia is a highly endemic country. In 2004, CVETNIĆ et al. reported a finding of 57% of *Leptospira* seropositive horses in Croatia over a ten year period having performed a survey on more than 10000 horses. Unfortunately, data on *Leptospira* infection rates and the prevalence of isolates from horses are rare in Europe and in the rest of the world. Some studies specifically performed in horses showed a seroprevalence that varied from 1% to 95%, depending on the geographic location and the serovars assessed, indicating that exposure is significantly more common than the clinical disease (VERMA et al., 1977; HATHAWAY et al., 1981; ELLIS et al., 1983; KITSON-PIGGOT and PRESCOTT, 1987). This underlines the low level of systemic inflammatory response in horses with subclinical leptospirosis (TURK et al., 2011). However, there is a permanent risk of convalescent carriers who excrete leptospires from their kidneys, with a large volume of daily urine content and high potential for contamination of the environment. Outbreaks of leptospirosis are rare and they are usually associated with incidences of flooding, as leptospires favour moist conditions.

We found sv Bratislava to be the most prevalent infective serovar of *Leptospira* in the horse population in Croatia. In spite of the fact that for most serovars of *Leptospira* horses are incidental hosts, sv Bratislava is the only *Leptospira* infection that has been postulated as being maintained by horses. This suggestion was based on the high seroprevalences in the equine population world-wide and its recovery from horses in Northern Ireland (ELLIS, 1983) and Portugal (ROCHA et al., 2004).

The second most prevalent serovar we found was Pomona in both healthy and non-healthy horses. Sv Pomona was the first Croatian isolate from clinically ill horses, reported in almost historical data (ZAHARIJA, 1953). The finding of Pomona infection is very important considering the clinical importance of *Leptospira* infections by this serovar, because it is the one most commonly associated with clinical disease in horses (HATHAWAY et al., 1981). Serovar Icterohaemorrhagiae was also found and it is exclusively connected with rats (*Rattus rattus*) as a reservoir of infection. In our investigation related to mares that aborted, in spite of the high titres of agglutinating antibodies to leptospires we could not claim that *Leptospira* was the main causative agent of the abortions. It is well known that co-infection with viral arteritis virus or equine herpesvirus, that are also causative agents of abortions in mares, could be found in some animals. Nevertheless, using 1-D and 2-D electrophoresis and immunoblotting on the sera samples of the mares that aborted, we recently showed a specific reactive antigens that caused the humoral immune system to produce IgG antibodies against *Leptospira* infection (unpublished data).

Various small rodent species serve as reservoir hosts for leptospires and exhibit potentially lifelong urinary shedding. Furthermore, in favorable environmental conditions leptospires can remain infective for as long as several weeks or months. Horses are often directly or indirectly exposed to such a contaminated environment. Horses live in different environments which usually combines pasture and a stable, sharing these biotopes with a number of small rodents. During winter, horses are fed with hay and oats, inevitably contaminated with the urine of small rodents.

Studies conducted up to now in Croatia have demonstrated that between 7.0% and 29.9% of small rodents, depending on investigated area, are leptospira carriers (BORČIĆ et al., 1982 and 1983; MILAS et al., 2002; TURK et al., 2003; ŠTRITOF MAJETIĆ, 2010). This high prevalence points out the risk of heavy soil contamination by leptospires excreted in the urine of these animals. Close interaction between humans, animals, soil and water in these regions defines a considerable hazard of leptospiral infection for the local population, as well as for outdoor-reared domestic animals, included horses. Serovars Bratislava, Lora, Saxkoebing, Bataviae from *L. interrogans*, Grippotyphosa, Mozdok and Tsaratsovo from *L. kirschneri*, and Istrica from *L. borgpetersenii*, have already been isolated from small rodents in Croatia (MILAS et al., 2002; TURK et al., 2003; ŠTRITOF MAJETIĆ, 2010). They probably all pose a threat of infection for horses, other animals and humans.

The resurgence of interest in equine leptospirosis is due to its potential to occur in both tropical and temperate climates, as well as in both developing and developed countries. Various factors that have contributed to the re-emergence of this infection include disturbances in natural ecological systems, the increase in the international horse industry and the transport of animals (sport horses) and improvements in diagnostic facilities, resulting in the better detection of these infections. Consequently, equine leptospirosis still has a great impact on both human and veterinary public health.

In conclusion, our study showed that horses in Croatia are commonly exposed to *Leptospira* infections. Regarding the clinical disease status we consider that a majority of subclinical infections and a minority of abortions, as the only clinically apparent form of leptospirosis, are currently present in Croatia, irrespective of the level of agglutinating antibody titres. It remains to investigate other possible forms of leptospirosis that are probably underreported (primarily recurrent uveitis) or unrecognized in the field, presenting a diagnostic dilemma because of its protean clinical manifestation, aberrant from the known classical patterns of the disease.

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SAŽETAK

Leptospiroza konja klinički se očituje prvenstveno pobačajima, ždrebljenjem uginule ždrebadi i mjesečnom sljepoćom. Septikemijski oblik koji se očituje groznicom, žuticom, gubitkom apetita, hematurijom, bubrežnom i jetrenom disfunkcijom, respiratornim poremećajima i krvarenjima u plućima javlja se rijetko. Kako bi dobili bolji uvid u pojavnost leptospiroze konja u Hrvatskoj provedeno je serološko istraživanje uporabom mikroskopske aglutinacije (MA) u populaciji zdravih konja, te u kobila nakon pobačaja. Cilj istraživanja bio je ustanoviti prevalenciju infekcije leptospirama i zastupljenost pojedinih serovara leptospira te ukazati na kliničku pojavnost leptospiroze konja u Hrvatskoj. Tijekom 2011. godine pretraženo je 8059 uzoraka seruma zdravih konja različitih pasmina, dobi i spola. Usporedno je pretraženo i 56 uzoraka seruma kobila zaprimljenih nakon pobačaja. Od 8059 uzoraka seruma zdravih konja protutijela za leptospire ustanovili smo u 2999 (37,2) uzoraka. Najveća je bila zastupljenost sv Bratislava u 1342 (44,8%) uzoraka, sv Pomona u 546 (18,2%) i sv Icterohaemorrhagiae u 448 (14,9%) uzoraka. Od 56 uzoraka seruma kobila koje su pobacile ustanovili smo 37 (66,1%) seropozitivnih uzoraka. Najveća je bila zastupljenost sv Bratislava u 16 (43,2%) uzoraka, sv Pomona u 11 (29,7%) i sv Icterohaemorrhagiae u 4 (10,8%). Rezultati ukazuju da su konji u Hrvatskoj vrlo izloženi infekciji leptospirama. S obzirom na kliničku pojavnost možemo zaključiti da trenutno u Hrvatskoj većina infekcija leptospirama u konja prolazi supklinički, te uz pojavu pobačaja u gravidnih kobila. Budući da je leptospiroza zoonoza, izuzetnu opasnost predstavljaju konji rekonvalescentni kliconoše koji mogu mokraćom izlučivati velike količine leptospira u okoliš te predstavljati izvor zaraze za druge životnje i ljude.

Ključne riječi: leptospira, leptospiroza konja, seroprevalencija, mikroskopska aglutinacija