Schweiz. Arch. Tierheilk.
© 2011 by Verlag Hans Huber, Hogrefe AG, Bern
Blatti S., Overesch G., Gerber V., Frey J., Hüssy D., Band 153, Heft 10, Oktober 2011, 449–456
DOI 10.1024/0036-7281/a000247

Seroprevalence of *Leptospira* spp. in clinically healthy horses in Switzerland

S. Blatti[1], G. Overesch[1], V. Gerber[2], J. Frey[1], D. Hüssy[1]

[1]Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance (ZOBA), Institute of Veterinary Bacteriology and [2]Equine Clinic, Department of Clinical Veterinary Medicine, University of Bern, Switzerland

Abstract

A retrospective, cross-sectional study was conducted to determine the leptospiral seroprevalence in clinically healthy horses in Switzerland. A representative sample of 615 horse sera was examined by microscopic agglutination test for the presence of antibodies against 15 *Leptospira* spp. serovars. In total, 58.5 % (n = 360) of the horses were positive for one or more of the antigens analysed, with 20.3 % of them showing titres ≥ 400. The most prevalent serovar was Pyrogenes (22.6 %), followed by serovars Canicola (22.1 %) and Australis (19.2 %). Older horses, mares, ponies and animals spending increased time on pasture exhibited significantly higher prevalence rates ($p < 0.05$). Moreover, the prevalence was higher in summer and autumn ($p = 0.003$). The high seroprevalence in healthy horses indicates that they are often exposed to or infected with *Leptospira* spp. without developing signs of disease. Therefore, other laboratory and clinical data should always be taken into consideration when interpreting serological test results for *Leptospira* spp.

Keywords: *Leptospira* spp., seroprevalence, horse, Switzerland, risk factors

Seroprävalenz von *Leptospira* spp. bei klinisch gesunden Pferden in der Schweiz

Zur Bestimmung der Seroprävalenz von Leptospiren bei klinisch gesunden Pferden in der Schweiz wurde eine retrospektive Querschnittstudie durchgeführt. Eine repräsentative Stichprobe von 615 Pferdeseren wurde mittels mikroskopischem Agglutinationstest auf Antikörper gegen 15 *Leptospira* spp. Serovare untersucht. Insgesamt waren 58.5 % (n = 360) der Pferde für eines oder mehrere der analysierten Antigene positiv, und 20.3 % davon hatten Titer ≥ 400. Das häufigste Serovar war Pyrogenes (22.6 %), gefolgt von den Serovaren Canicola (22.1 %) und Australis (19.2 %). Ältere Pferde, Stuten, Ponies und Tiere, die mehr Zeit auf der Weide verbrachten, wiesen signifikant höhere Prävalenzraten auf ($p < 0.05$). Zudem war die Prävalenz im Sommer und im Herbst höher ($p = 0.003$). Die hohe Seroprävalenz bei gesunden Pferden weist darauf hin, dass sie häufig gegenüber *Leptospira* spp. exponiert oder mit diesen infiziert sind ohne Krankheitszeichen zu entwickeln. Deshalb sollten bei der Interpretation von serologischen Testresultaten für *Leptospira* spp. immer auch andere Laborwerte und klinische Daten berücksichtigt werden.

Schlüsselwörter: *Leptospira* spp., Seroprävalenz, Pferd, Schweiz, Risikofaktoren

Introduction

Leptospirosis is a worldwide zoonotic disease caused by any of the pathogenic serovars within the genus *Leptospira*. The disease affects virtually all mammalian species (Adler and de la Peña Moctezuma, 2010). In horses, acute, chronic and subclinical infections do occur. Clinical leptospirosis is mostly associated with recurrent uveitis, renal and hepatic dysfunction, stillbirth and abortion (Office International des Epizooties OIE, 2005). Infected animals can become long-term carriers and thus an important source of infection for other animal species and humans by shedding the bacteria in their urine (Adler and de la Peña Moctezuma, 2010).

Serological data from many parts of the world indicate that horses are susceptible to a wide variety of *Leptospira* serovars maintained by other animals sharing the same environment. Geographical variations in the seroprevalence of particular serovars are documented in numerous regional studies. The knowledge of the prevalent serovars in a particular region is essential to understand the epidemiology and to include the relevant serovars in the routine diagnostic panel. Further, it is important for the development of new vaccines as immunity is serovar-specific (Adler and de la Peña Moctezuma, 2010).
The aim of the present study was to determine the seroprevalence of different serovars of *Leptospira* spp. in clinically healthy horses in Switzerland. For this purpose, a representative sample of horse sera was analysed by microscopic agglutination test (MAT). This method has the advantages that it can be applied to sera from any animal species and that the range of antigens can be expanded or reduced as required. Moreover, the MAT is the most commonly used assay in seroprevalence studies of *Leptospira* spp. performed in other countries, and results are therefore comparable. A further goal was to evaluate whether there was an association between seropositive animals and potential risk factors.

Animals, Material and Methods

Animals and Sampling procedure

A cross-sectional study was performed on serum samples collected from December 2006 to September 2008 to determine the seroprevalence of 15 different serovars of *Leptospira* in healthy horses in Switzerland. Sampling calculations revealed that a minimum sample of 596 randomly selected animals was required to achieve a diagnostic detection limit of 0.5 % prevalence with a certainty of 95 %, given the population of 85'000 horses in Switzerland (WinEpiscope v2, Module Disease Detection). Blood samples were collected and processed as described previously (Kaiser et al., 2009). A total of 615 samples were included in this study, 238 sera of indigenous horses and 377 sera of horses imported from neighbouring countries. All horses were deemed healthy on the day of sampling. For each horse, a questionnaire was completed regarding potential risk factors.

Serological analysis by MAT

The sera were examined for the presence of antibodies against *Leptospira* by MAT according to OIE standards (Office International des Epizooties OIE, 2008). Live cultures of 15 *Leptospira* reference serovars of the species *interrogans*, *kirschneri* and *borgpetersenii* were included in this study (Tab. 1). The sera were initially screened at a dilution of 1:100. Those with a positive reaction were titrated in a serial two-fold dilution to determine the end-point titre, defined as the reciprocal of the highest dilution of serum at which at least 50 % agglutination occurs (Office International des Epizooties OIE, 2008). A titre of ≥ 100 was considered positive, i.e. indicating past exposure to leptospires (Levett, 2001). All samples were tested by the same person to avoid inter-observer variability. The purity of the antigens was checked regularly by inoculation into thioglycolate broth and subsequent subculture on blood agar.

Table 1: *Leptospira* spp. reference serovars (strains) used in the study[1].

Leptospira species	Serovar	Strain
L. interrogans	Australis	Ballico
	Pomona	Pomona
	Canicola	Hond Utrecht IV
	Icterohaemorrhagiae	RGA
	Hardjo	Hardjoprajitno
	Bataviae	Swart
	Bratislava	Jez-Bratislava
	Autumnalis	Akiyami
	Hebdomadis	Hebdomadis
	Pyrogenes	Salinem
L. kirschneri	Grippotyphosa	Moskva V
L. borgpetersenii	Tarassovi	Perepelitsin
	Sejroe	M 84
	Ballum	Mus 127
	Javanica	Veldrat Batavia 46

[1]All reference strains were obtained from the Royal Tropical Institute (KIT), Amsterdam, The Netherlands

Preliminary experiments

The MAT was standardised with respect to the density and the age of the bacterial cultures, as these factors are known to influence the sensitivity of the test (Terpstra et al., 2003). In our experiments, highest titre levels were obtained using 17 to 22 days old cultures adjusted to a density of 0.5 McFarland (data not shown). Thus, for ensuring a maximum and constant sensitivity, cultures of this quality were utilised throughout the study. Exceptionally, cultures at a density of only 0.4 McFarland

Table 2: Seroprevalence of 15 different *Leptospira* spp. serovars in 615 healthy horses in Switzerland.

Leptospira spp. serovar	Number of positive horses	Seroprevalence (%)	95 % CI
Pyrogenes	139	22.6	19.5–26.1
Canicola	136	22.1	19.0–25.6
Australis	118	19.2	16.3–22.5
Bratislava	98	15.9	13.3–19.1
Autumnalis	64	10.4	8.2–13.1
Grippotyphosa	54	8.8	6.8–11.3
Icterohaemorrhagiae	49	8.0	6.1–10.4
Pomona	38	6.2	4.5–8.4
Tarassovi	38	6.2	4.5–8.4
Bataviae	27	4.4	3.0–6.3
Javanica	6	1.0	0.4–2.2
Hebdomadis	5	0.8	0.3–2.0
Ballum	2	0.3	< 0.01–1.3
Hardjo	1	0.2	< 0.01–1.0
Sejroe	1	0.2	< 0.01–1.0
Overall[1]	360	58.5	54.6–62.4

[1]positive for one or more serovars

were used when they did not grow to higher concentrations. The horse serum samples used for the seroprevalence study had been frozen to –80 °C and thawed three times for other use before they were examined. In order to analyse the effect of repeated freezing and thawing of sera on the MAT results, preliminary studies were undertaken, which showed that freeze-thawing of the sera up to four times did not affect their titres (data not shown).

Statistics

The overall seroprevalence and the percentages for the individual serovars were calculated with 95 % confidence intervals (95 % CI) by the modified Wald method (www.graphpad.com). The Chi-square test (Preacher, 2001) was used to assess associations between seropositivity and potential risk factors. Values of $p \leq 0.05$ were considered statistically significant.

Results

Seroprevalence

Out of 615 horses, 360 (58.5 %) were positive for at least one of the 15 serovars tested. The highest percentages of positive results were found for serovar Pyrogenes (22.6 %), followed by serovars Canicola (22.1 %), Australis (19.2 %), Bratislava (15.9 %), Autumnalis (10.4 %) and Grippotyphosa (8.8 %). The prevalence of each serovar investigated in the study population is presented separately in Table 2. Among the seropositive horses, 60 % (n = 216) were tested positive for more than one serovar, and only 40 % (n = 144) presented antibodies to just one serovar. The most prevalent serovars that were found concurrently in many individuals were Pyrogenes and Canicola as well as Australis and Bratislava (Fig. 1).

Regarding only the highest titre of all tested serovars per serum, 79.7 %, (n = 287) of the positive horses showed values ranging from 100 to 200. The other 20.3 % (n = 73) had even higher titres: 15.6 % (n = 56) had a titre of 400, 3.6 % (n = 13) had a titre of 800 and 1.1 % (n = 4) had

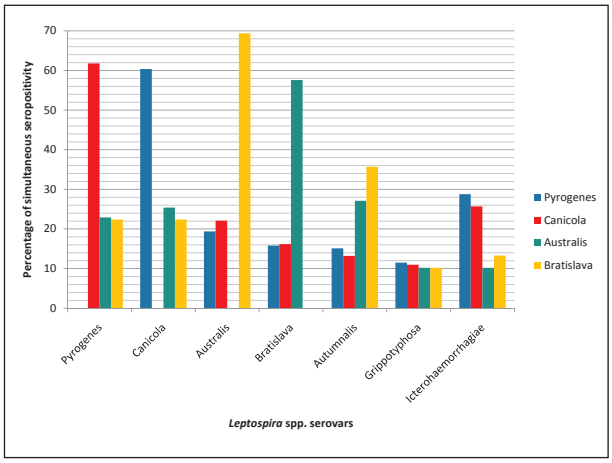

Figure 1: Percentages of simultaneous seropositivity of serovars Pyrogenes, Canicola, Australis and Bratislava and the seven most prevalent serovars.

a titre of 1600. The majority (59.6 %, n = 59) of all titres ≥ 400 were observed for serovars Australis (22.2 %), Canicola (20.2 %) and Pyrogenes (17.2 %).

Potential risk factors

The seroprevalences of *Leptospira* spp. among healthy horses in Switzerland according to potential risk factors are listed in Table 3. With respect to the country of origin, no significant difference (p = 0.417) in prevalence was found between indigenous horses (62.2 %) and imported horses from Germany (55.9 %), France (54.3 %), Italy (61.3 %) and Austria (72.7 %). Hence, for analyses of further risk factors, all horses were combined as one group.

Comparison of different age groups revealed a significant increase in seroprevalence with age (p = 0.006), from 49.6 % in horses up to 5 years old to 67.8 % in those older than 15 years. In terms of sex, mares showed a significantly higher seroprevalence than males (p = 0.001). However, there was no difference between stallions and geldings (p = 0.943). In addition, no significant association (p = 0.720) between seroprevalence and breed was detected. Pertaining to height, ponies had significantly more often (p = 0.028) antibodies against the tested panel of *Leptospira* serovars (74.4 %, n = 32) than horses (57.3 %, n = 324).

A significant rise in seropositivity (p = 0.005) with increasing duration spent on pasture per day was observed. Gathering with other horses had no effect on the prevalence rate: horses participating in events like exhibitions and competitions were not more frequently seropositive (p = 0.975) than horses never attending such occasions. Regarding the month of blood withdrawal, seasonal variation in seropositivity was apparent, with the prevalence being higher in summer and autumn, and lower in winter and spring (p = 0.003).

Discussion

The objectives of the present study were to determine the seroprevalence of 15 serovars of *Leptospira* spp. in clinically healthy horses in Switzerland and to identify risk factors associated with seropositivity. For this purpose, a representative sample of 615 horse sera was examined by MAT, the reference method and the most widely used test to detect *Leptospira*-specific antibodies of both IgM and IgG classes (Office International des Epizooties OIE, 2008). After infection, antibodies can persist for years or even during the entire lifespan of an animal (Levett, 2001; Terpstra et al., 2003). The panel of serovars analysed in this study included locally occurring serovars found in dogs, cattle, swine and horses (Institute of Veterinary Bacteriology, 2009), as well as serovars discovered in horses elsewhere in the world (Ellis et al., 1983; Hashimoto et al., 2007; Jung et al., 2010).

In our study, 58.5 % of the horses were positive for one or more of the 15 serovars analysed. The high seroprevalence in healthy horses suggests that horses are often in contact or infected with *Leptospira* without developing clinical signs. This is most likely a consequence of an exposure to host-adapted *Leptospira* spp., leading to an unapparent infection in their hosts (Terpstra et al., 2003). As none of the horses suffered from clinical leptospirosis in the past, persisting titres originating from a previous disease are unlikely. Positive titres due to vaccination can be excluded because vaccination of horses against leptospirosis is not practised in Switzerland and its neighbouring countries. Our findings are consistent with a survey of healthy horses in New York State, USA (Barwick et al., 1998), where an overall prevalence of 56 % was detected. In Paraná, Brazil (Hashimoto et al., 2007), an even higher seroprevalence (66.9 %) was found, whereas in Korea (Jung et al., 2010) and Portugal (Rocha et al., 2004) lower prevalence rates of 25 % and 8 %, respectively, were revealed. As not all studies include the same panel of serovars, the results are not fully comparable. Based on the experience of our laboratory (Institute of Veterinary Bacteriology, 2009) and a study carried out in France (Gaumont, 1986), the percentage of seropositivity in healthy horses was much higher than in healthy animals of other species such as cattle, swine or dog, which are mostly negative at a cut-off titre of 100. Exposure to leptospires may elicit a stronger immune response in horses than in other animals.

The most frequent serovar found in our study was Pyrogenes, followed by serovars Canicola, Australis and Bratislava. In many surveys of healthy horses in Europe, North America and Mongolia (Gaumont, 1986; Kitson-Piggot and Prescott, 1987; Barwick et al., 1998; Rocha et al., 2004; Odontsetseg et al., 2005; Baverud et al., 2009), serovars Bratislava and/or Australis, both belonging to the same serogroup, were most prevalent. However, in the investigation conducted in Brazil, they were only rarely observed (Hashimoto et al., 2007). The horse has been suggested to be a maintenance host for serovar Bratislava (Ellis et al., 1983; Kitson-Piggot and Prescott, 1987; Rocha et al., 2004; Baverud et al., 2009), being infected without developing any signs of disease. Maintenance hosts can carry particular pathogenic strains of *Leptospira* in their kidneys for long periods and shed them in their urine, thereby being an important source of infection for other susceptible species (Levett, 2001; Adler and de la Peña Moctezuma, 2010).

The lowest prevalences in our study were recorded for serovars Sejroe (0.2 %) and Hardjo (0.2 %). This is in accordance with the findings of numerous other reports from Europe, North and South America (Kitson-Piggot and Prescott, 1987; Barwick et al., 1998; Rocha et al., 2004; Hashimoto et al., 2007; Baverud et al., 2009). Conversely, in Korea, Sejroe was found to be the predominant serovar with a prevalence of 19.2 % (Jung et al., 2010).

Table 3: Seroprevalence of *Leptospira* spp. in 615 healthy horses in Switzerland according to potential risk factors.

Potential risk factor	Number of tested horses	Number of positive horses	Seroprevalence (%)	95 % CI
Country of origin	**615**			
Germany	195	109	55.9	48.9–62.7
Austria	11	8	72.7	42.9–90.8
France	140	76	54.3	46.0–62.3
Italy	31	19	61.3	43.8–76.3
Switzerland	238	148	62.2	55.9–68.1
Age[1]	**610**			
1–5 years	117	58	49.6	40.7–58.5
6–15 years	319	182	57.1	51.6–62.4
16–30 years	174	118	67.8	60.5–74.3
Sex[1]	**613**			
Stallion	46	24	52.2	38.1–65.9
Gelding	292	154	52.7	47.0–58.4
Mare	275	181	65.8	60.0–71.2
Breed[1]	**608**			
Swiss Warmblood	213	132	62.0	55.3–68.2
Islandic horse	39	22	56.4	41.0–70.7
Hannoveraner	36	21	58.3	42.2–72.9
Oldenburg	21	14	66.7	45.2–83.0
Selle Français	57	30	52.6	39.9–65.0
Other breeds	242	137	56.6	50.3–62.7
Height at the withers[1]	**608**			
Horses (> 148 cm)	565	324	57.3	53.2–61.4
Ponies (≤ 148 cm)	43	32	74.4	59.6–85.2
Duration of daily pasturing[1]	**460**			
0–4 h	184	96	52.2	45.0–59.3
> 4–12 h	142	83	58.5	50.2–66.2
> 12 h	134	94	70.1	61.9–77.3
Gathering with other horses[1]	**536**			
Yes	413	241	58.4	53.5–63.0
No	123	72	58.5	49.7–66.9
Season of blood withdrawal[1]	**605**			
Winter (December–February)	178	97	54.5	47.2–61.6
Spring (March–May)	297	167	56.2	50.5–61.8
Summer (June–August)	105	74	70.5	61.1–78.4
Autumn (September–November)	25	17	68.0	48.3–82.9

[1]For several horses, information about some potential risk factors was lacking in the questionnaires

Antibodies to serovar Pyrogenes, which was the most commonly found serovar in horses in Switzerland (22.6 %), were also detected in horses in Brazil (8 %) and Portugal (7 %). Rocha et al. believe that seropositivity to serovar Pyrogenes results from cross-reacting antibodies against other *Leptospira* serovars because a strain of serovar Pyrogenes has actually never been isolated in Europe from horses or other animal species (Rocha et al., 2004). Another possibility is that the horse acts as a maintenance host for serovar Pyrogenes as previously postulated for serovar Bratislava.

The prevalence of serovar Grippotyphosa, the most often implicated serovar in equine recurrent uveitis in Switzerland (Tömördy et al., 2010), was 8.8 %. Although *Leptospira* Grippotyphosa seems to be less frequent than many other serovars, it must be considered as an important *Leptospira* serovar causing clinical infections and complications in horses.

Among the seropositive horses in our survey, a substantial percentage (60 %) reacted positively to several serovars. Seropositivity to various serovars is most probably due to cross-reactivity, which is a common feature among antigenically related serovars of *Leptospira* (Levett, 2001; Terpstra et al., 2003), but it can also result from multiple infection with different serovars. In contrast, antibodies against other bacteria usually do not cross-react to a considerable extent with *Leptospira* spp. (Office International des Epizooties OIE, 2008). Regarding the most prevalent serovars in this study, simultaneous seropositivity, in all likelihood resulting from cross-reactions, was mainly observed for serovars Australis and Bratislava as well as for serovars Pyrogenes and Canicola, cross-reactions thus occurring not only within but also among different serogroups. One fifth of the samples tested positive had end-point titres ≥ 400, although none of the horses had signs of leptospirosis. Equal or even higher percentages were found in previous studies (Barwick et al., 1998; Hashimoto et al., 2007). This fact makes it difficult to assess the clinical relevance of seropositive results, even if the titres are high. Therefore, other laboratory and clinical data should always be taken into consideration when interpreting serological test results for *Leptospira* spp.

Our analyses revealed that seroprevalence increased with age as already seen in previous studies (Kitson-Piggot and Prescott, 1987; Baverud et al., 2009). This is plausible because the probability of having come into contact or being infected with *Leptospira* spp. increases with time. The duration of daily pasturing was positively associated with seropositivity, whereas gathering with other horses on special events had no significant influence on the prevalence rate. Thus, seropositivity to *Leptospira* spp. is

Séroprévalence de *Leptospira* spp. chez des chevaux cliniquement sains en Suisse

Dans le but de déterminer la séroprévalence des leptospires chez les chevaux sains en Suisse, une étude transversale rétrospective a été effectuée. Un échantillon représentatif de 615 sérums de chevaux a été examiné avec un test d'agglutination microscopique quant à des anticorps contre 15 sérotypes de *Leptospira* spp. Au total, 58.5 % (n = 360) des chevaux présentaient des anticorps face à un ou plusieurs des antigènes analysés et 20.3 % d'entre eux avaient des titres ≥ 400. Le sérotype le plus fréquent était Pyrogenes (22.6 %) suivi des sérotypes Canicola (22.1 %) et Australis (19.2 %). Les chevaux âgés, les juments, les poneys et les animaux passant beaucoup de temps en prairie, présentaient des taux de prévalence significativement plus élevés ($p < 0.05$). En outre, la prévalence était plus élevée en été et en automne ($p = 0.003$). La séroprévalence élevée chez les chevaux sains montrent qu'ils sont fréquemment exposés ou infectés par *Leptospira* spp. sans développer de signe de maladie. En conséquence, l'interprétation de résultats sérologiques quant à *Leptospira* spp. doit toujours tenir compte des autres résultats de laboratoire et des signes cliniques.

Sieroprevalenza della *Leptospira* spp. nei cavalli clinicamente sani in Svizzera

È stato condotto uno studio trasversale retrospettivo per determinare la sieroprevalenza di leptospire nei cavalli clinicamente sani in Svizzera. Un campione rappresentativo di 615 sieri equini sono stati analizzati via il test di agglutinazione microscopica per gli anticorpi contro 15 sierotipi di *Leptospira* spp. Complessivamente, il 58.5 % (n = 360) dei cavalli è risultato positivo per uno o più degli antigeni analizzati e il 20,3 %, mostrava un titolo ≥ 400. Il sierotipo più comune era Pirogene (22.6 %), seguito dai sierotipi Canicola (22.1 %) e Australis (19.2 %). I tassi di prevalenza nei cavalli anziani, nelle giumente, nei pony e negli animali che hanno passato più tempo al pascolo risultavano significativamente più alti ($p < 0.05$). Inoltre, la prevalenza in estate e in autunno era maggiore ($p = 0.003$). L'alta sieroprevalenza nei cavalli sani indica che essi sono spesso esposti alla *Leptospira* spp. o infettati da questa senza però svilupparne sintomi. Pertanto, nell'interpretazione dei risultati dei test sierologici per la *Leptospira* spp. bisogna considerare anche e sempre altri valori di laboratorio e dati clinici.

more likely a result of exposure to fields and meadows contaminated by the urine of other animal species such as rodents, the primary reservoir hosts for most *Leptospira* serovars (Adler et al., 2002; Office International des Epizooties OIE, 2005; Aviat et al., 2009; Adler and de la Peña Moctezuma, 2010), rather than direct contact with other potentially shedding horses.

The significantly higher seroprevalence of blood samples collected during summer and autumn is most likely due to longer survival and infectivity of leptospires in the environment in warm and humid climate conditions. In Switzerland, the highest precipitation amounts and the highest monthly average temperatures are recorded in summer (MeteoSwiss, 2010). Hence, during summertime horses are at higher risk to get in contact or infected with leptospires or, in case of repeated exposure, to be boostered. Concerning the higher seroprevalence found in mares compared to stallions and geldings, it must be considered that a significantly higher percentage of the mares belonged to a higher age category (> 10 years, $p = 0.011$) and spent > 12 h on pasture per day ($p < 0.001$). Thus, the apparent sex-related difference might be a result of the combined influence of other factors such as those mentioned above. In contrast to the findings in Sweden (Baverud et al., 2009), ponies displayed a significantly higher prevalence rate than horses. The most striking disparity regarding risk factors was the daily pasturing time: significantly more ponies spent > 4 h outside ($p < 0.001$). Therefore, we speculate that the higher seroprevalence in ponies is mainly due to an elevated probability of contact or infection with *Leptospira* spp. during pasturing.

In summary, our survey reports serological evidence of widespread exposure to different serovars of *Leptospira* spp. in healthy horses in Switzerland. The high background level of exposure in the horse population must be considered when interpreting serological test data, and positive results have diagnostic value solely in conjunction with a consistent clinical picture. The importance of the horse acting as carrier and shedder of *Leptospira* spp. remains to be investigated.

Acknowledgments

We are grateful to S. Ackermann and A. Thomann for excellent technical support and to Prof. M. Doherr for helpful advice on statistics.

References

Adler B., de la Peña Moctezuma A.: *Leptospira* and leptospirosis. Vet. Microbiol. 2010, 140:287–296.

Adler H., Vonstein S., Deplazes P., Stieger C., Frei R.: Prevalence of *Leptospira* spp. in various species of small mammals caught in an inner-city area in Switzerland. Epidemiol. Infect. 2002, 128:107–109.

Aviat F., Blanchard B., Michel V., Blanchet B., Branger C., Hars J., Mansotte F., Brasme L., De Champs C., Bolut P., Mondot P., Faliu J., Rochereau S., Kodjo A., Andre-Fontaine G.: *Leptospira* exposure in the human environment in France: A survey in feral rodents and in fresh water. Comp. Immunol. Microbiol Infect. Dis. 2009, 32:463–476.

Barwick R. S., Mohammed H. O., Atwill E. R., McDonough P. L., White M. E.: The Prevalence of Equine Leptospirosis in New York State. J. Equine Sci. 1998, 9: 119–124.

Baverud V., Gunnarsson A., Engvall E. O., Franzen P., Egenvall A.: *Leptospira* seroprevalence and associations between seropositivity, clinical disease and host factors in horses. Acta Vet. Scand. 2009, 51:15.

Ellis W. A., O'Brien J. J., Cassells J. A., Montgomery J.: Leptospiral infection in horses in Northern Ireland: serological and microbiological findings. Equine Vet. J. 1983, 15:317–320.

Gaumont R.: [Study of the presence of anti-leptospira agglutinins in domestic animals in France]. Bull. Off. Int. Epizoot. 1986, 66:833–848.

Hashimoto V. Y., Goncalves D. D., Silva F. G., Oliveira R. C., Alves L. A., Reichmann P., Muller E. E., Freitas J. C.: Occurrence of antibodies against *Leptospira* spp. in horses of the urban area of Londrina, Paraná, Brazil. Rev. Inst. Med. Trop. Sao Paulo 2007, 49:327–330.

Institute of Veterinary Bacteriology, University of Bern: Annual Report, 2009.

Jung B. Y., Lee K. W., Ha T. Y.: Seroprevalence of *Leptospira* spp. in clinically healthy racing horses in Korea. J. Vet. Med. Sci. 2010, 72:197–201.

Kaiser A., Meier H. P., Doherr M. G., Perler L., Zanoni R., Gerber V.: [Serological and clinical proof of freedom from Equine Infectious Anemia (EIA) in imported and domestic horses in Switzerland]. Schweiz. Arch. Tierheilk. 2009, 151:165–170.

Kitson-Piggot A. W., Prescott J. F.: Leptospirosis in horses in Ontario. Can. J. Vet. Res. 1987, 51:448–451.

Levett P. N.: Leptospirosis. Clin. Microbiol. Rev. 2001, 14:296–326.

MeteoSwiss, Federal Office of Meteorology and Climatology, Zürich, Switzerland, 2010 [on-line: http://www.meteosuisse.admin.ch/web/en/climate/swiss_climate/tabellen.html].

Odontsetseg N., Boldbaatar D., Mweene A. S., Kida H.: Serological prevalence of *Leptospira interrogans* serovar Bratislava in horses in Mongolia. Vet. Rec. 2005, 157:518–519.

Office International des Epizooties OIE: Leptospirosis. In: Technical Factsheets of the Center for Food Security and Public Health. Iowa State University, Ames, IA, U.S.A., 2005 [on-line: http://www.cfsph.iastate.edu/Factsheets/pdfs/leptospirosis.pdf].

Office International des Epizooties OIE: Leptospirosis. In: The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual). Ed. OIE, Paris, France, 2008, 251–264.

Preacher K. J.: Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence, 2001 [on-line: http://quantpsy.org].

Rocha T., Ellis W. A., Montgomery J., Gilmore C., Regalla J., Brem S.: Microbiological and serological study of leptospirosis in horses at slaughter: first isolations. Res. Vet. Sci. 2004, 76:199–202.

Terpstra W.J., World Health Organization WHO, International Leptospirosis Society ILS: Human leptospirosis: Guidance for diagnosis, surveillance and control. 2003.

Tömördy E., Hässig M., Spiess B. M.: The outcome of pars plana vitrectomy in horses with equine recurrent uveitis with regard to the presence or absence of intravitreal antibodies against various serovars of *Leptospira interrogans*. Pferdeheilkunde 2010, 26:251–254.

Corresponding author

Daniela Hüssy
Institut für Veterinär-Bakteriologie, Abt. ZOBA
Vetsuisse-Fakultät Universität Bern
Länggassstrasse 122
CH-3012 Bern
Tel.: + 41 (0)31 631 24 35
Fax: + 41 (0)31 631 26 34
E-mail: daniela.huessy@vetsuisse.unibe.ch

Received: 9 March 2011
Accepted: 7 June 2011