Fatal leptospirosis in free-ranging Eurasian beavers (*Castor fiber* L.), Switzerland

Short running title: Fatal leptospirosis in Eurasian beavers

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Summary

Leptospirosis was first diagnosed in free-ranging Eurasian beavers (*Castor fiber* L.) in Switzerland in 2010. Pathologic, serologic, molecular and epidemiologic analyses were carried out on 13 animals submitted for necropsy from 2010 through 2014. Typical lesions included alveolar hemorrhages in the lungs, tubular degeneration and interstitial nephritis in the kidneys. Microscopic agglutination test results were positive for serogroups Icterohaemorrhagiae, Australis, Autumnalis, and Sejroe. Molecular analysis identified four distinct profiles belonging to serovar Icterohaemorrhagiae or Copenhageni. The severity and features of the lesions were consistent with a fatal disease associated with leptospires similarly to what has been reported in other animals and humans. The spatiotemporal occurrence of leptospirosis in beavers suggested an upstream spread of the bacteria and coincided with an increased incidence of leptospirosis in dogs and a case cluster in humans. However, an epidemiologic link among beaver cases and among species was not supported neither by the serologic nor molecular data.

Keywords: Eurasian beaver, leptospirosis, molecular typing, pathology, serology, Switzerland

Introduction

Leptospirosis is a bacterial disease caused by pathogenic spirochetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease worldwide and numerous mammal species can shed the agent (Adler and de la Peña Moctezuma, 2010). The infection cycle typically involves: 1) the excretion of leptospires in the environment via the urine of a carrier host; 2) the infection of a new host, first characterized by a bacteremic phase; and 3) the colonization of the renal tubules of the new host, from where leptospires can be excreted into the environment (Adler, 2015). Warm and humid conditions are especially favorable to the survival of leptospires in the environment. Hence, the incidence of leptospirosis in humans is higher in tropical regions (Ullmann and Langoni, 2011). In Europe, the number of cases decreased in the second half of the 20th century but the incidence is now expected to rise due to: 1) climatic factors and changes including global warming, heavy rainfalls, and flooding; 2) increase of rodent populations in the urban environment; 3) human population growth; and 4) increase in international travel (Dupouey et al., 2014). This is supported by the increase of the incidence of human cases in France, Germany, and the Netherlands (Dupouey et al., 2014; Pijnacker et al., 2016).

In Switzerland, leptospirosis is a reportable disease only when it affects pigs and cattle, and available data on the occurrence of infection and associated clinical signs are limited. Nevertheless, concern about the reemergence of this disease is growing. In humans, leptospirosis is mainly considered to be a tropical disease relevant for tourists, long-term travelers and migrants (Utzinger et al., 2012), however, endemic cases also occur and are likely underdiagnosed (Schreiber et al., 2015). In farm animals, seroprevalence was estimated to be 19% in cattle from 1986 through 1996 (Hässig and Lubsen, 1998) and 58.8% in horses in 2011 (Blatti et al., 2011). Eleven clinical cases were reported in pigs and 485 in cattle from 1991 through 2015 (Federal Food Safety and Veterinary Office, n.d.). In domestic dogs, leptospirosis incidence has dramatically increased over the past decade (Major et al., 2014). Wildlife is also concerned: 12.6% of various free-ranging rodent and shrew species sampled around the city of Zurich harbored leptospires in their kidney (Adler et al., 2002), and a seroprevalence of 7.9% was estimated in Alpine ibex (*Capra ibex* L.) (Marreros et al., 2011). Thus, leptospiral infection occurs in different mammal species and different habitats in Switzerland, but little is known about the distribution of the pathogens, the spectrum of susceptible species and the existence of wildlife reservoirs.

The genus *Leptospira* comprises about 20 species categorized into pathogens, saprophytic, or intermediate groups (Adler, 2015). Leptospires are also classified into >300 serovars, and closely related serovars are grouped into serogroups. However, the genetics- and serology-based classifications do not always overlap (Adler, 2015). Each serovar is usually maintained by a specific host (reservoir), in which limited clinical disease and associated pathology, if any, occurs (Adler, 2015). Small rodents, especially rats (*Rattus* sp.), are seen as the most relevant reservoir of pathogenic leptospiral serovars in Europe (Dupouey et al., 2014) but larger, water-dwelling rodent species such as the muskrat (*Ondatra zibethicus* L.) and the coypu (*Myocastor coypus* Molina, 1782) have also been shown to harbor leptospires (Aviat et al., 2009). By contrast, only a few cases of infection have been reported in the Eurasian beaver (*Castor fiber* L.). Leptospirosis was diagnosed in 3 beavers from Germany, which had been translocated to the Netherlands in 1994 and were found dead between 24 and 31 days after their release (Nolet et al., 1997). Antibodies to *Leptospira* sp. were found in 5 beavers captured in Norway but without clinical signs (Goodman et al., 2012). Furthermore, pathogenic leptospires were detected in the kidney of 4 beavers found dead in Germany but causality between death and infection could not be established, either because the cause of death was obviously noninfectious (traffic kills), or because the advanced autolysis prevented a thorough interpretation of the lesions (Woll et al., 2012). The ecological relationship between the Eurasian beaver and leptospires is therefore largely unknown.

The Eurasian beaver vanished from most of Europe due to overhunting and habitat degradation but was reintroduced to Switzerland and other European regions in the middle of the 20th century (Dewas et al., 2012). Habitat restauration in the 1990s resulted in a population increase and colonization of new sites in Switzerland, particularly in the Rhine river system which includes the Aare subsystem (Dewas et al., 2012). Health monitoring of the reintroduced beaver population remained very limited until 1997 when a standard necropsy and sampling protocol was implemented at the Centre for Fish and Wildlife Health (FIWI, University of Bern, Switzerland). From 2004 onwards, Swiss hunting services have been officially encouraged to submit dead beavers to the FIWI for a necropsy performed free of charge (Roch, 2004; Ryser-Degiorgis and Segner, 2015). Leptospirosis was diagnosed for the first time in beavers from Switzerland in 2010, and since then cases have been observed every year. Here we report 13 cases of leptospirosis in Eurasian beavers including pathologic, serologic, molecular and epidemiologic investigations.

Materials and Methods

Animals submitted for pathologic examination

Thirteen beavers suspected to be affected with leptospirosis were included in this study. The animals were submitted for necropsy to the FIWI from 2010 through 2014. Eleven of them were found dead in a river or on a river bank and two were culled because of poor body condition. There were nine males and four females. Based on body size and weight, there were three juveniles and 10 adults. Most of them were found in summer (n = 7), followed by the spring (n = 4) and the fall (n = 2). All beavers originated from the same section of the Aare river subsystem (46°45´N–47°35´N and 7°00´E–8°16´E) (Fig. 1). This study did not involve purposeful manipulation or killing of the beavers. Therefore, it was not subject to approval by an ethics committee.

Representative specimens of organs and blood samples were collected at necropsy for further analyses. Tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (HE) for histologic examination following the accredited protocols of the Institute of Pathology, University of Bern. Additional organ samples (kidney and lung in 11 cases) and blood (10 cases) were stored at –20°C for further analyses.

Histology

In a first step, tissue sections of all collected organs were screened for pathologic changes as part of the routine diagnostic work. During this first histologic examination, the most prominent changes were observed in the lungs, kidneys, and liver. These organs were therefore chosen for a second, blind comparative histologic evaluation. The presence and severity (none, mild, moderate, severe) of lesions listed in Tables 2 to 5 were assessed for each of these three organs.

Laboratory analyses

Blood samples were thawed and centrifuged at 10,000 × *g* for 10 min prior to serologic assay to reduce interference in the test due to debris. Presence of antibodies against *Leptospira* sp. was assessed by microscopic agglutination test (MAT) with a panel of 25 different serovars from 14 different serogroups (Table 1). Samples were initially screened at a dilution of 1:100. Positives were further tested after serial 1:2 dilution and the endpoint titer was recorded (World Organisation for Animal Health (OIE), 2008).

DNA was extracted from kidney and lung tissues using a commercial isolation kit (MagVet Universal Isolation Kit, Laboratoire Service International, Lissieu, France) and the robot King Fisher mL (Thermo Scientific, Illkirch, France), following the manufacturer's instructions. The presence of *Leptospira* sp. DNA was assessed using a commercially available real-time PCR kit, specific for pathogenic leptospires (TaqVet PathoLept, Laboratoire Service International, Lissieu, France), considering samples with cycle threshold (C_t) value of <45 as positive. The test was performed according to the manufacturer's instructions. For *Leptospira* species determination, positive DNA extracts underwent PCR amplification of the partial sequence of the16S RNA gene followed by sequencing of the amplicon that was carried out with the same primers used for the PCR reaction (Mérien et al., 1992; Zilber et al., 2014). Given that only *L. interrogans* was identified (see below), strain typing was performed by Multispacer Sequence Typing (MST) as previously described (Zilber et al., 2014) and compared to reference strains. This recently described method allows the determination of the infecting strain among the *L. interrogans* species with differentiation of the genotype.

Results

Macroscopic pathologic examination

Most beavers were mildly (n = 7) to moderately (n = 4) emaciated. One beaver was severely emaciated, and one was in a good body condition. All beavers showed multifocal to coalescent lung hemorrhages (Fig. 2) occasionally in association with increased lung consistency (n = 5). Multifocal hemorrhages were also present in the intestines (n = 5), kidneys (n = 3), myocardium (n = 2) and urinary bladder (n = 2, Fig. 2). Yellow coloration of the mucous membranes (Fig. 2) or inner organs, or both, was observed in five beavers (Fig. 2). Renal corticomedullary junction was grossly inapparent in two beavers (Fig. 2).

Histology

In the lung, mild to severe multifocal alveolar hemorrhage and alveolar histiocytosis were observed in all 13 beavers (Fig. 3, Table 3). Other very common findings were fibrin deposition, including the formation of hyaline membranes (n = 12) along with a small up to moderate number of infiltrating macrophages and fibroblasts into the fibrin collections, and edema (n = 11). Some animals also had variable degrees of lymphocytic (n = 7) or neutrophilic (n = 4) interstitial pneumonia. Additional findings were: perivascular accumulation of macrophages (n = 4), congestion (n = 3), type II pneumocyte hyperplasia (n = 2), and interstitial fibrosis (n = 1).

The kidney of one beaver could not be assessed due to severe autolysis. In the others, the most common findings were: moderate to severe tubular degeneration, necrosis and regeneration (n = 9, Fig. 4, Table 4), with intratubular collection of viable and necrotic neutrophils (n = 8) with or without protein casts (n = 6); lymphocytic to plasmacytic interstitial nephritis (n = 9); and moderate to severe interstitial fibrosis (n = 8). Three beavers presented all described features. Additional findings included mild to moderate hemorrhages (n = 3) and intratubular crystals (n = 2).

Seven out of the eight beavers with kidney fibrosis had concurrent moderate to severe fibrin deposition in the lungs; in the four beavers without kidney fibrosis, fibrin deposition was present in the lungs but it was only mild to moderate (Table 3, 4).

Advanced autolysis impaired histologic assessment of liver and intestinal sections in five and seven beavers, respectively. Three out of eight animals had mild hepatocellular degeneration and necrosis with gold-brown casts in the bile canaliculi (cholestasis) (Table 5). In four other animals there was a mild to moderate chronic lymphocytic periportal hepatitis (n = 2), mild granulomatous hepatitis (n = 1), or mild peribiliar fibrosis (n = 1). The small intestine of five out of six beavers with interpretable sections presented multifocal mainly submucosal hemorrhage with edema; and in one of them the hemorrhages were transmural.

Molecular and serologic analyses

Nine out of 11 beavers with PCR results had detectable DNA of pathogenic *Leptospira* sp. in at least one of the organs tested, and five of these nine beavers were PCR-positive both in the lung and the kidney (Table 2). Beavers that tested positive in both organs had a higher bacterial load in the kidney than in the lung (ΔC_t: 0.4–9.4). Three out of four beavers that were positive either in the kidney or in the lung had a lower bacterial load (C_t >30) than the other cases (C_t <30, Table 2).

Molecular typing identified genotypes of the *L. interrogans* species in all PCR-positive samples. Sequences of MST1 and MST9 loci were 100% identical for all beavers but one, from which no clear MST9 locus sequence could be retrieved. . Four different sequences were identified at the locus MST3 (Table 2), corresponding to the following strain profiles: R1 (serovar Icterohaemorrhagiae) in three beavers; Michaud (serovar Icterohaemorrhagiae) in two beavers; M20 or Wijinberg (both strains of serovar Copenhageni cannot be differentiated by MST) in two beavers; and one previously undescribed profile, which was tentatively named Aare (GenBank accession no. KY785376) and was found in one beaver. Sequence alignment showed that this new profile differed from that of strain R1 by the insertion of a C nucleotide residue (C). This new MST3 sequence was also found in the beaver with the unclear MST9 sequence. Given the identity of the MST1 and MST9 sequences, and that a different MST9 sequence would have corresponded to another new strain profile deriving from the profile Aare, we concluded that the unclear MST9 sequence was most likely the same as the others, i.e. that the concerned beaver was infected with the strain Aare. When beavers tested positive in both lung and kidney, the same strain was detected in both organs (n = 3, Table 2).

Nine beavers had positive MAT results with titers up to 3,200 (Table 2), and five of them showed positive reactions to several serovars. Five beavers showed the highest titers against strains of the serogroup Icterohaemorrhagiae, three beavers against strains of the serogroup Australis, and one beaver showed similar antibody titers against strains of the serogroup Australis and Sejroe (Table 2). Higher serologic titers were found in beavers positive to strains M20/Wijinberg or Aare (≥200) than in beavers positive to strains R1 (≤200) or Michaud (100).

Comparison between pathology and microbiological results

No clearly discriminant histologic pattern was found between lungs with positive and negative PCR results. Also, no histopathologic differences were noted among beavers infected with different *Leptospira* MST profiles, neither in the lungs nor in the kidneys.

PCR positive kidneys with histology data (n = 6) consistently had tubular degeneration, necrosis, and regeneration with mild to moderate interstitial nephritis. Of those, only two also had moderate fibrosis. By contrast, the four PCR negative kidneys all had moderate fibrosis while associated tubular changes were observed in two cases only, one of them also with moderate interstitial nephritis.

Interstitial fibrosis was present in the kidney of all five beavers with the highest antibody titers to the serogroup Icterohaemorrhagiae. By contrast, only one of five beavers seronegative to the Icterohaemorrhagiae serogroup had interstitial fibrosis.

Infection with leptospires could not be confirmed by PCR or serology in two beavers because no samples were available. These beavers showed similar lung and kidney lesions as those observed in the confirmed cases.

Spatiotemporal pattern of case occurrence

The first cases (2010–2011) appeared in sections of the Aare river, downstream from the main lakes to which they are connected. In subsequent years, the cases were found around the main lakes (2012–2013) and finally in the upstream sections of the Aare river (2014, Fig. 1). Despite being found in the same water system, the affected beavers were either separated from each other in space and time or, if they were affected during the course of the same year, they were infected by different strains.

Discussion

The most evident lesions in the beavers of this study were hemorrhages in the lungs, as described in humans and dogs with fatal leptospirosis. However, while affected humans and dogs generally show only low levels of inflammatory infiltrate with or without fibrin deposition (Dolhnikoff et al., 2007; Schuller et al., 2015), some of our beavers showed inflammatory reactions with high levels of fibrin deposition, sometimes embedding macrophages and fibroblasts. These observations suggest that some beavers experience not only acute but also subacute to chronic lung lesions, i.e., they may survive infection for relatively longer.

Kidney lesions observed in this study, in particular tubular necrosis and interstitial nephritis, were also similar to those reported in other species suffering from renal failure due to leptospirosis, such as humans and dogs (Adler, 2015). Interstitial fibrosis was present in two thirds of our beavers, indicating a subacute to chronic disease process in these animals. Although fibrosis was mostly associated with a negative PCR result in kidney tissues, these organs partly presented similar tubular changes or nephritis as seen in PCR positive kidneys. Furthermore, the corresponding beavers also displayed lung lesions due to leptospirosis, two of which had a positive PCR result in the lung, and all of them had positive MAT results. Therefore, negative PCR results in kidneys with fibrosis may either be due to a past or chronic systemic infection with clearance of the leptospires from the kidneys, or correspond to false negative reactions. The latter could result from the small portion of tissue (25 mg) used for the extraction of DNA, which may be insufficient when the leptospire load in the tissue is low, or from DNA degradation in animals undergoing advanced autolysis. Alternatively, kidney fibrosis could be a process independent from the leptospiral infection. Subacute to chronic interstitial nephritis was described in North American beavers (*Castor canadensis* Kuhl, 1820) negative for leptospirosis and proposed to be secondary to parasitism due to the eosinophilic and monocytic character of the inflammation (Stuart et al., 1978). These cells, however, were not observed in our cases, suggesting that subacute to chronic kidney lesions noted in beavers of our and the former study were likely related to different renal diseases. Other frequent findings in the cases of leptospirosis reported here included hepatocellular degeneration and multisystemic hemorrhages, which are also consistent with leptospiral infection leading to multiple organ failure (Adler, 2015).

Simultaneous positive MAT results against different serovars were probably due to cross-reactions (Levett, 2001). Part of the beavers which harbored strains of the serovars Icterohaemorrhagiae or Copenhageni showed negative MAT results for members of the Icterohaemorrhagiae serogroup but positive reactions against other serogroups, mainly the serogroup Australis but also Sejroe. Such paradoxical reactions are known to occur during the acute phase of infection because less specific antibodies are produced than during the convalescent phase; they could also be due to an anamnestic response, when the rise in antibodies is first directed against unrelated serovars from previous exposure (Levett, 2001). The absence of kidney fibrosis in most beavers with paradoxical MAT reactions indeed supports a relatively shorter disease course. In agreement with this observation, all beavers with clear MAT reaction against members of the Icterohaemorrhagiae serogroup (partly confirmed by strain analysis) had interstitial fibrosis in the kidney, thus indicating a longer disease course.

Overall, the similarity between the lesions in Eurasian beavers and those seen in other susceptible hosts supports the diagnosis of clinical leptospirosis in beavers and suggests similar pathogenesis in all concerned taxa. However, beavers seem to develop not only acute, but also subacute to chronic leptospirosis. The reason for these different disease courses is unknown.

Infection with leptospires has previously been detected in both Eurasian and North American beavers, by serology, immunohistochemistry or bacterial isolation (Goodman et al., 2012; López-Pérez et al., 2017; Nolet et al., 1997; Shearer et al., 2014; Stuart et al., 1978; Woll et al., 2012). However, infection associated with clinical disease has only been reported once. In this former report, 3 out of 22 Eurasian beavers found dead shortly after translocation were diagnosed with leptospirosis (one animal by serology, no details were given for the others). Given that more than half of these deaths were due to infectious diseases, the authors hypothesized that stress-induced immunosuppression had impacted the disease resistance of the translocated animals (Nolet et al., 1997). Our results show that fatal lesions may develop in Eurasian beavers under natural conditions. These findings are relevant in the context of beaver conservation and raise questions regarding the role of beavers in the epidemiology of leptospirosis of animals and humans. Owing to its regional extinction and reintroduction history in central Europe, the Eurasian beaver has experienced a severe genetic bottleneck, possibly leading to increased susceptibility to infectious diseases (Frosch et al., 2014). Leptospirosis is an emerging disease in Europe and considering the factors believed to trigger this emergence, it is likely that it will continue to show the same trend (Dupouey et al., 2014). This along with increasing beaver populations in Switzerland and the rest of Europe (Dewas et al., 2012) may well result in increased numbers of leptospirosis cases in Eurasian beaver in the future.

Many wildlife taxa, especially rodents, can be healthy carriers of leptospires. After infection, they might display minimal nephritis, if any, but it is uncommon for them to show any clinical signs. Nonetheless, they can shed the bacteria throughout their lives (Adler, 2015; Bharti et al., 2003). To our knowledge, only one study suggested the Eurasian beaver as a potential shedder of leptospires (Woll et al., 2012). Given the positive PCR results obtained in kidney samples in our study, it is possible that beavers temporarily shed the bacteria in urine. However, the fatal character of the disease in beavers does not support their role as long-term shedders or maintenance hosts but is rather consistent with beavers being accidental hosts (Adler, 2015). Still, our report was limited to diseased beavers and further studies should encompass a larger sample size, including asymptomatic animals, to truly assess the epidemiologic role of this species.

The detection of the first cases of leptospirosis in beavers in Switzerland in 2010 and of additional cases in the following years coincided with an increased incidence of leptospirosis in domestic dogs. There was also a marked geographic overlap between the distribution of dog and beaver cases (Major et al., 2014). Additionally, human cases were recorded in people surfing on a river close to the Aare river in 2014 (Fig. 1; Schreiber et al., 2015). These observations suggested an epidemiologic link between dogs, beavers and human cases. However, the serovars Australis and Bratislava were most commonly found in dogs (Major et al., 2014) and the serovar Grippotyphosa was detected in the human cases (Schreiber et al., 2015). Beavers were infected with different strains of the serovars Icterohemorrhagiae or Copenhageni, which indicates that the cases were not related to each other. Thus, the serologic and molecular analyses suggest the existence of several infection sources in the same geographic area, affecting several hosts in an independent manner.

Conclusions

Free-ranging Eurasian beavers are susceptible to natural infection with pathogenic leptospires and show pulmonary hemorrhages and renal tubular necrosis. The severity of the lesions suggests that beavers do not become long-term carriers of the identified strains. The variety of leptospires documented in beavers and other species indicates that multiple parallel infection cycles may be present in the environment in Switzerland.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Tables

Table 1: List of *Leptospira* serovars and strains used for the microagglutination test on Eurasian beavers diagnosed with leptospirosis from 2010 through 2014 in Switzerland.

Acronym	Serovar	Serogroup	Species	Strain
19	Icterohaemorrhagiae	Icterohaemorrhagiae	*L. interrogans*	ENVN
VER	Verdun	Icterohaemorrhagiae	*L. interrogans*	Verdun
COP	Copenhageni	Icterohaemorrhagiae	*L. interrogans*	M 20
AUS	Australis	Australis	*L. interrogans*	Ballico
BRAT	Bratislava	Australis	*L. interrogans*	Jez Bratislava
MUN	Munchen	Australis	*L. interrogans*	München C 90
AKI	Autumnalis	Autumnalis	*L. interrogans*	Akiyami A
BIM	Bim	Autumnalis	*L. kirschneri*	1051
SJ	Sejroe	Sejroe	*L. borgpetersenii*	M84
SAX	Saxkoebing	Sejroe	*L. interrogans*	Mus 24
HJ	Hardjo	Sejroe	*L. interrogans*	Hardjoprajitno
WOLF	Wolffi	Sejroe	*L. interrogans*	3705
POM	Pomona	Pomona	*L. interrogans*	Pomona
MOZ	Mozdok	Pomona	*L. kirschneri*	5621
CAN	Canicola	Canicola	*L. interrogans*	Hond Utrecht IV
GRIP	Grippotyphosa	Grippotyphosa	*L. kirschneri*	Moskva V
VAN	Vanderhoedoni	Grippotyphosa	*L. kirschneri*	Kipod 179
HEB	Hebdomadis	Hebdomadis	*L. interrogans*	Kremastos
PAN	Panama	Panama	*L. noguchii*	CZ 214 K
MAN	Mangus	Panama	*L. inadai*	TRVL/CAREC 137774
BAT	Bataviae	Bataviae	*L. interrogans*	Van Tienen
BAL	Castellonis	Ballum	*L. borgpetersenii*	Castellòn 3

PYR	Pyrogenes	Pyrogenes	*L. interrogans*	Salinem
TAR	Tarassovi	Tarassovi	*L. borgpetersenii*	Perepelitsin
CYN	Cynopteri	Cynopteri	*L. kirschneri*	3522C

Table 2: Laboratory results for Eurasian beavers from Switzerland diagnosed with leptospirosis from 2010 through 2014.

ID†	PCR						MAT‡								
	Kidney			Lung			Australis			Icterohaemorrhagiae			Autumnalis		Sejroe
	Result	Ct	Profile	Result	Ct	Profile	BRAT	MUN	AUS	19	COP	VER	BIM	AKI	SAX
1	Pos	24.9	MW	Pos	27.3	MW	400	–	–	–	–	–	–	–	400
3	Pos	32.7	R1	Neg			–	–	–	–	200	–	–	–	–
4	Neg			Pos	30.8	Aare	100	–	–	800	400	–	400	–	–
5	Pos	27.6	Mi	Pos	29.7	Mi	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	Neg			Neg			–	–	–	200	400	200	–	–	–
7	Pos	26.9	Aare§	Neg			100	800	–	3,200	3,200	–	1,600	800	–
8	Neg			Pos	31.1	R1	–	–	200	–	–	–	–	–	–
10	Neg			Neg			200	–	–	800	800	3,200	–	–	–
11	Pos	20.4	MW	Pos	24.7	MW	200	–	–	–	–	–	–	–	–
12	Pos	26.7	R1	Pos	27.1	R1	–	–	–	–	–	–	–	–	–
13	Pos	23.8	Mi	Pos	33.2	Mi	100	–	–	–	–	–	–	–	–

ID, beaver identification number; Ct, cycle threshold; Pos, Positive; Neg, Negative; MW, M20 or Wijinberg; Mi, Michaud; MAT, microagglutination test; Dashes indicates titer <100; Serovar name abbreviation are shown in Table 1; NA, no blood was available for beaver 5.

†No samples material was available for beavers 2 and 9.

‡Tested serovars with negative results only are not shown.

§No clear Multispacer Sequence Typing 9 locus sequence from this organ.

Table 3: Histologic grading of lung lesions identified in Eurasian beavers from Switzerland diagnosed with leptospirosis from 2010 through 2014.

ID	Alveolar histiocytosis	Hemorrhages	Fibrin	Edema	Interstitial pneumonia	Perivascular histiocytes	Congestion	Hyperplasia of pneumocytes type II	Interstitial fibrosis
1	++	++	++	+++	++	0	0	0	0
2	++	+	++	++	++	0	0	0	0
3	+++	+++	+++	+++	++	+++	0	0	0
4	++	+++	++	+++	+	+	0	0	0
5	++	++	++	++	++	0	0	0	0
6	++	+++	++	++	+	0	0	0	0
7	+++	+++	+++	++	++	0	++	++	0
8	+++	+++	+++	++	++	++	++	0	0
9	++	+++	+++	+++	+	+++	0	0	0
10	++	++	0	0	++	0	++	0	++
11	++	++	++	0	++	0	0	0	0
12	+++	+++	++	++	++	0	0	++	0
13	++	+++	+	++	++	0	0	0	0

ID, beaver identification number; 0, none; +, mild; ++, moderate; +++, severe.

Table 4: Histologic grading of kidney lesions identified in Eurasian beavers from Switzerland diagnosed with leptospirosis from 2010 through 2014.

ID†	Tubular degeneration, necrosis, regeneration	Neutrophilic cast	Protein cast	Hemorrhages	Interstitial nephritis	Fibrosis
1	+++	+++	++	++	++	0
2	0	0	0	0	+	+++
3	++	++	++	0	+	++
4	0	0	0	0	0	++
6	++	++	+	0	++	++
7	++	++	0	0	++	++
8	++	++	0	0	0	++
9	+++	+++	++	0	++	++
10	0	0	0	0	0	++
11	+++	0	++	0	+	0
12	+++	+++	++	++	++	0
13	+++	++	0	+	++	0

ID, beaver identification number; 0, none; +, mild; ++, moderate; +++, severe.

†detailed assessment of kidney lesions was not undertaken for beaver 5 due to severe autolysis.

Table 5: Histologic lesions in livers from Eurasian beavers from Switzerland diagnosed with leptospirosis from 2010 through 2014.

ID†	Hepatocellular degeneration	Lymphocytic hepatitis	Granulomatous hepatitis	Fibrosis
1	0	++	0	0
2	0	0	0	0
3	+	0	0	0
6	+	0	0	0
9	0	0	0	+
10	0	0	+	0
11	0	+	0	0
12	+	0	0	0

ID, beaver identification number; 0, none; +, mild; ++, moderate; +++, severe.

†detailed assessment of liver lesions was not undertaken for beavers 4, 5, 7, 8, and 13 due to severe autolysis.

Figures Legends

Figure 1: Origin of Eurasian beavers diagnosed with leptospirosis from 2010 through 2014 in Switzerland. Symbols represent the profile of *Leptospira interrogans* identified. The asterisk shows the location of a cluster of leptospirosis in humans (Schreiber et al., 2015). The numbers refer to the years when the affected beavers where found.

Figure 2: Macroscopic findings in Eurasian beavers diagnosed with leptospirosis from 2010 through 2014 in Switzerland. (a) Diffuse light yellow coloration of gingival mucosa. Diffuse opaque light to severe yellow coloration, hyperemia and hemorrhages in (b) cloacal and (c) urinary bladder mucosa. (d) Diffuse yellow to orange coloration with loss of demarcation between the medulla and cortex of the kidney. (e) Poorly collapsed lungs with multifocal to coalescent hemorrhages. (f) Multifocal hemorrhages within the intestinal wall (arrows). Scale bar indicates 1 cm.

Figure 3: Lung sections from a Eurasian beaver diagnosed with leptospirosis in Switzerland. (a) Multifocal alveolar hemorrhages. (b) Thickening of the alveolar septa with infiltration of mononuclear cells. Hematoxylin and Eosin staining; scale bar indicates 200 µm.

Figure 4: Kidney section from a Eurasian beaver diagnosed with leptospirosis in Switzerland. Necrosis of tubular epithelial cells (arrows), intratubular degenerated neutrophils (asterisks), interstitial mononuclear cell infiltrate (solid arrowheads), and fibrosis (empty arrowhead). Hematoxylin and Eosin staining; Scale bar indicates 200 µm.