Canine leptospirosis in Switzerland – A prospective cross-sectional study examining seroprevalence, risk factors and urinary shedding of pathogenic leptospires

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Abstract

Leptospirosis is an important worldwide zoonosis. While human leptospirosis remains rare in Switzerland, the incidence of canine leptospirosis is unusually high compared to other European countries. The aims of this cross-sectional study were to determine the exposure of asymtomatic dogs to pathogenic *Leptospira* in Switzerland, to characterise risk factors associated seropositivity and to determine the prevalence of urinary shedding. Sampling was stratified to cover the whole of Switzerland. Sera were tested by the microscopic agglutination test for antibodies against a panel of 12 serovars. Urine was tested for pathogenic *Leptospira* using a LipL32 real-time PCR. Of 377 sera, 55.7% (95% CI 51.2-60.7) showed a reciprocal MAT titre of ≥1:40 and 24.9% (95% CI 20.7-29.4) of ≥1:100 to at least one serovar. Seropositivity (MAT ≥1:100) was most common to serovars Australis (14.9%; 95% CI 11.4-18.6) and Bratislava (8.8%; 95% CI 6.1-11.7), followed by Copenhageni (6.1%; 95% CI 4-8.5), Canicola (5%; 95% CI 2.9-7.2), Grippotyphosa (4.5%; 95% CI 2.7-6.9), Pomona (4%; 95% CI 2.1-6.1), Autumnalis (2.7%; 95% CI 1.1-4.5) and Icterohaemorrhagiae (1.6%; 95% CI 0.5-2.9). In unvaccinated dogs (n=84) the prevalence of a MAT titre ≥100 was 17.9% (95% CI 10.7-27.4), with a similar distribution of reactive serovars. Variables associated with seropositivity (≥1:40) to any serovar included age (OR 1.29/year (95% CI: 1.1-1.5) and bioregion with higher risks in Northern Alps (OR 14.5 (95% CI 2.2-292.7), Central Plateau 12.3 (95% CI 2.0-244.1) and Jura (OR 11.2 (95% CI 1.7-226.7) compared to Southern Central Alps. Dogs living with horses were significantly more likely to have antibodies to serovar Bratislava ((OR 4.68 (95% CI 1.2-17.2)). Hunting was a significant risk factor for seropositivtiy to serovar Grippotyphosa (OR 8.03 (95% CI 1.6-30.8)). Urine qPCR positivity was uncommon (1/408 dogs; 0.2%; 95% CI 0-0.7). These results demonstrate that dogs in Switzerland are commonly exposed to pathogenic

Leptospira; however, the risk of dogs contributing to the spread of *Leptospira* in the environment appears low.

1. Introduction

Leptospirosis is a zoonotic disease with worldwide distribution affecting most mammalian species (Bharti et al., 2003). It is caused by infection with pathogenic *Leptospira* spp. The organism maintains itself in nature by colonising the renal tubules of reservoir or maintenance hosts from which they are shed into the environment via urine. Maintenance hosts are often rodents, but in theory any host susceptible to infection can become a chronic carrier of *Leptospira*. Due to co-evolutionary adaptation, many serovars have a preference for specific maintenance hosts (e.g. rats often carry serogroup Icterohaemorrhagiae) and cause very little clinical signs of infection in these hosts. In contrast, if a host gets infected with a non-adapted serovar (incidental host infection), acute and potentially life threatening disease can develop. Clinical manifestations in incidental hosts include fever, renal and hepatic injury, pulmonary haemorrhage and reproductive failure (Adler, 2015). In general infected maintenance hosts show leptospiruria of higher intensity and longer duration compared to incidental hosts (Chernukcha et al., 1974; Rojas et al., 2010).

Human leptospirosis is a major public health issue in many countries, including Latin America and South-East Asia, with large outbreaks linked to natural disaster and flooding (Abela-Ridder et al., 2010). In developed countries of temperate zones leptospirosis is considered a rare disease, with an increased occupational risk for professions such as farmers, slaughterhouse workers, sewage workers and veterinarians and for individuals performing outdoor activities such as fishing and water sports (Picardeau, 2013).

In dogs, risk factors for acute clinical infection and exposure in asymptomatic dogs, as well as associations of seropositivity with environmental or climatic factors have been reported from a number of different areas of the world including Europe, North and South America; Australia and New Zealand with sometimes contradicting results. A recent meta-analysis identified the following major risk factors for leptospirosis: male sex, mixed-breed, young dogs (<1 year), working dogs, flooding occurrence in the habitat of the dog and urban environment (Azocar-Aedo and Monti, 2016). Other possible risk factors are swimming, drinking from outdoor water and exposure to wild animals (Ghneim et *al.*, 2007).

The epidemiology of leptospirosis in Switzerland is incompletely understood. While 10-20% of mice, moles and shrews captured in the city of Zürich showed PCR-based evidence of renal colonisation with *Leptospira* spp, more specific information on serovars present in the environment and their relevant maintenance hosts is currently not available. While the prevalence and incidence of leptospirosis in humans is unknown due to low awareness of the disease and lack of an official notification system, a cluster of acute leptospirosis in young, previously healthy persons was recently identified in Switzerland and traced back to a surfing spot on the river Reuss in canton Aargau (Schreiber et al., 2015).

A dramatic increase in the incidence of canine leptospirosis has been documented in Switzerland between 2003 and 2012 with a peak incidence of 28/100.000 dogs in canton Aargau in 2012 (Major *et al.,* 2014). In that study over 75% of dogs, presented to a veterinary university hospital developed leptospiral pulmonary haemorrhage syndrome, a severe and often lethal form of leptospirosis, which has previously been considered rare. In this cohort of dogs 80% showed serologic evidence of infection with serovars Bratislava, belonging to serogroup Australis (Major et al., 2014). The natural reservoir for members of this serogroup in

Switzerland has not yet been identified. While horses are considered an important maintenance host for serovar Bratislava (Arent et al., 2015), it has been suspected that this serovar can be equally carried by asymptomatic dogs thus potentially acting as a source of infection for other domestic and wild animals and for humans (Ellis, 2010).

Urinary shedding of pathogenic *Leptospira* has been reported in 1.5-8% of dogs not suspected to have leptospirosis (Harkin et al., 2003; Rojas et al., 2010; Llewellyn et al., 2016). Dog ownership has been identified as a risk factor for human leptospirosis in Germany (Jansen et al., 2005), Barbados (Douglin et al., 1997) and Nicaragua (Trevejo et al., 1998) suggesting transmission of *Leptospira* spp from dogs to humans.

We hypothesised that there is a high level of exposure to pathogenic *Leptospira* in dogs in Switzerland. Furthermore we hypothesised that urinary shedding of leptospires is common in dogs without clinical signs of leptospirosis and that dogs may therefore contribute to environmental contamination.

The aims of this present study were: 1) to estimate the level of exposure to pathogenic *Leptospira* sp. by determining the prevalence of anti-leptospiral serum antibodies, 2) to determine the prevalence of urinary shedding of pathogenic leptospires and 3) to determine risk factors for exposure to pathogenic leptospires including age, sex, breed, geographic area, vaccination status and lifestyle in dogs not suspected to have leptospirosis in Switzerland.

2. Material and Methods

2.1. Ethical approval

Ethical approval was granted by the Federal Food Safety and Veterinary Office for the study protocol (Project BE9/15). Written owner consent was obtained before enrolment of dogs in the study.

2.2. Study design, recruitment, inclusion and exclusion criteria

The study was designed as a cross-sectional prevalence study representing the whole of Switzerland. Sampling was stratified based on the number of dogs per canton according to the Swiss national registry for dogs (ANIS database, Animal Identity Service AG, Bern, Switzerland). The sample size was calculated to estimate the true prevalence and a significant difference between two proportions assuming a prevalence of 10%, test sensitivity and specificity of 95%, a power of 85% and confidence levels of 0.05. The estimated prevalence was based on the seroprevalence reported in previous studies using reciprocal MAT cut off titres of 1:10 (Arent et al., 2013; Schuller et al., 2015) and 1:100 (Davis et al., 2008; Llewellyn et al., 2016).

Dogs were recruited at the Vetsuisse Faculty Bern and Zürich, as well as via 30 private practices throughout Switzerland between April and December 2015. Private practices were recruited during in-house conferences at the Vetsuisse Faculty Bern and Zürich. In areas with low participation, additional practices were contacted via phone and invited to participate.

Dogs presented for routine check-ups, vaccination or problems unrelated to leptospirosis were eligible for inclusion. Dogs had preferably not been vaccinated against leptospirosis during the previous 16 weeks and were excluded from urine real time PCR (qPCR) examination if they had received antibiotics within the past 4 weeks. Repeated MAT testing was advised in cases with reciprocal titres of ≥1:40 in order to document titre dynamics.

2.3. Sampling and Sample Handling

2.3.1. Stability of leptospiral DNA in canine urine with and without stabilizer

In preparation of the study the stability of leptospiral DNA in canine urine during simulated shipping at ambient temperature with and without a stabiliser (AssayAssure®**;** Sierra Molecular Corporation; USA) was tested. Fresh voided urine from dogs without evidence of urinary tract infections as shown by absence of clinical signs, normal dipstick results, inactive sediment and negative bacterial culture were collected via free catch. Samples were pooled, split into 4 ml aliquots and spiked with *Leptospira interrogans* serovar Autumnalis at $1x10^4$/ml, $1x10^3$ and $1x10^2$/ml. The strain *L. interrogans* serovar Autumnalis Akiyami was chosen due to its intermediate detection limit (5 Genome equivalents per reaction) determined in a previous study (Stettler, 2015). Stabiliser was added to the test group at a concentration of 1:11 according to the manufacturer's instructions. The spiked samples were incubated at room temperature for 4, 24 or 48 hours and then processed for DNA extraction and real time PCR as described below. All spiked samples incubated for 4 and 24 hours showed amplification of the target gene *lipl32* regardless of the addition of stabiliser and incubation time. However, after incubation of 48 hours no positive amplification could be yielded without the addition of stabiliser **(Suppl. Table 1).** Based on these positive results, the stabiliser was used for all urine samples in this study.

2.3.2. Urine samples

Urine samples were collected via free catch unless cystocentesis was indicated for medical reasons. Four ml of urine were immediately transferred into polypropylene tubes prefilled with 0.36 ml of stabiliser (ratio 1:11).

2.3.3. Blood samples

Blood was collected into tubes without anticoagulant. Blood was either sent to the laboratory uncentrifuged or left to clot for 30 min before centrifugation at 2,000 x g for 10 minutes and subsequent serum separation.

2.3.4. Shipping and pre-analytical sample handling

Urine, whole blood or serum samples were shipped at ambient temperature to the Department of Veterinary Bacteriology of the Vetsuisse Faculty Bern, which is the national reference laboratory for animal leptospirosis in Switzerland. DNA extraction was performed within 48 h of sampling. Serum and DNA extracts were either immediately analyzed or frozen at -20°C until further analysis.

2.4. Questionnaire

Owners were encouraged to fill in a questionnaire addressing the following points: age, sex, breed, neuter status, occurrence of a febrile illness in the dog or owner in the past year, treatments in the past 4 weeks, life style including residential environment (urban, countryside, farm, presence or absence of a garden), whether the dog is mainly kept indoor or outdoor, contact to other pets, activities including drinking from puddles, swimming in natural waters, hunting, walking in the woods, contact with wild animal species and knowledge of confirmed cases of leptospirosis in the vicinity **(Supp. Figure 1).** The date of last vaccination and type of vaccine were either checked directly on the vaccination booklet by the veterinarian, or owners were asked by phone to read aloud the vaccination record.

2.5. Microscopic agglutination testing (MAT)

Sera were examined for the presence of antibodies against pathogenic *Leptospira* by microscopic agglutination test (MAT) according to OIE standards (Office International des Epizooties OIE, 2008) at the Institute of Veterinary Bacteriology which hosts the Swiss National Reference Laboratory for animal leptospirosis and is accredited by the Swiss Accreditation Service (SAS) for both techniques, MAT and real-time PCR. Quality management is according to DIN EN ISO/IEC 17025 with regular participation in the proficiency testing schemes for serology and real-time PCR of the National Serology Reference Laboratory (Australia) and Vetqas AHPA (UK).

For MAT testing, live cultures of twelve reference strains of *Leptospira* belonging to 10 serogroups were used in this study **(Table 1).** Sera were initially screened for agglutination of *Leptospira* at a dilution of 1:25. Reactive sera were titrated in a serial two-fold dilution to determine the end-point titre defined as the highest serum dilution at which at least 50 % agglutination occurs. All samples were tested by the same person to avoid inter-observer variability.

2.6. DNA extraction from urine and Real time PCR

Urine samples were centrifuged at 10 000 rpm at 4°C for 10 min. Subsequently, the supernatant was discarded and the pellet resuspended in 100 µL lysis buffer (containing 180 ml pyrogen-free water, 20 ml TrisHCl 1M pH 8.5, 100 µL Tween 20 and 48 mg Proteinase K (Sigma-Aldrich Co, St. Louis, Missouri, USA) and incubated for 60 min at 60°C, followed by 15 min at 97° on an Eppendorf thermomixer comfort (Vaudaux-Eppendorf AG, Basel, Switzerland). The lysates were stored at -20°C.

The real-time PCR targeted the gene encoding leptospiral major outer membrane protein LipL32 which is only present in pathogenic *Leptospira* spp. Primers and probe were according to (Villumsen et al., 2012) using the following primers: LipL32F (5'-AGA GGT CTT TAC AGA ATT TCT TTC ACT ACC T-3'), LipL32R (5'- TGG GAA

AAG CAG ACC AAC AGA-3') and LipL32-P (5'-FAM-AAG TGA AAG GAT CTT TCG TTG C-MGB-3'). The reactions were carried out on a 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA) with the cycling settings recommended by the manufacturer in a total reaction volume of 25 µL containing: 12.5 µL TaqMan® Universal PCR Master Mix, No AmpErase® UNG at a concentration of 100 µM, a 1 µM concentration of each forward and reverse primer, a 80 nM concentration of the probe, 0.5x TaqMan® Exogenous Internal Positive Control (IPC) mix, 0.5x IPC template (all reagents Applied Biosystems, Foster City, CA, USA), and 2.5 µL of template. DNA from *Leptospira* interrogans serovar Icterohaemorrhagiae strain RGA was included as a positive control and pyrogen free water as a negative control. Values above the threshold of 0.06 at cycle times <40 were considered positive.

2.7. Statistical analyses

The statistical analyses were performed using R 3.2.2 (2016, R Core Team, Vienna, Austria) and IBM SPSS Statistic Version 23 for Windows.

The prevalence of positive urine qPCR and MAT seropositivity were calculated with a confidence interval of 95%. Because of the likely interference of recent vaccination with seroprevalence, subgroup analysis was performed for the group of unvaccinated dogs (n=84) and the group of dogs not vaccinated within the past 16 weeks (n=255).

Risk factor analysis was restricted to MAT seropositivity as outcome measure because only one dog tested positive on qPCR. MAT positivity was defined as positive reaction to at least one serovar included in the 12 serovar panel at reciprocal titres of ≥1:40 (ALL40) or ≥1:100 (ALL100). Two instead of one cut-off titres were chosen in the absence of a consensus to what represents an ideal cut-off titre to document exposure in a population of dogs not suspected to have leptospirosis.

In a second step MAT seropositivity was defined as seropositivity at a cut-off of off ≥1:100 for the serovars Australis (AUS100), Bratislava (BRA100), Grippotyphosa (GRI100), Canicola (CAN100) and Copenhageni (COP100), which were the serovars that sera most commonly reacted with. Variables included in the risk factor analysis are shown in **Table 2.**

For analysis of geoclimatic factors on seroprevalence, the Swiss Territory was categorized into six bioregions according to a classification proposed by the Swiss Federal Office for the Environment (BAFU) (Gonseth, 2001). The six regions were Jura, Central Plateau, Northern Alps, Central Western Alps, Central Eastern Alps and Southern Alps; each bioregion presenting similar geological, geomorphological and climatic conditions **(Figure 1)**.

A graphical overview of dogs included in the different steps of the analysis is shown in **Figure 2**. Univariate analysis was performed using $\chi 2$ or Fisher's exact test for categorical variables and univariate logistic regression for continuous variables. A value of $p \leq 0.20$ was considered as the critical level of significance for a variable to be entered in a full multivariable logistic regression model. Subsequently, for each outcome a manual likelihood-ratio-test backward selection of exposure variables was used to determine the best fitting model and to determine independent potential risk or confounding factors associated with *Leptospira* seropositivity. Risk factors were maintained in the model at a p-value ≤ 0.05.

The assumption of independence of the data was potentially violated, since some dogs were owned by the same owner and thus were clustered. To correct for dependency, we explored extending the model by generalised estimating equations (GEE) to control for clustering, by expanding the standard errors (SE) ('robust SE') and increasing the p-values. An independence and exchangeable pattern was used as correlation structure (Liang, 1986). However, the SE hardly changed and therefore

the results of unadjusted multivariable logistic regression models were reported. The final models were evaluated on goodness of fit using the Hosmer-Lemeshow test. Influential observations were detected using Pearson residuals, hat matrix (leverage) and delta-betas (Hosmer, 2004).

Risk factor analysis was performed using the whole study population without exclusion of vaccinal serogroups (Icterohaemorrhagiae, Canicola, Australis, Grippotyphosa). To test for the potential effect of vaccination on MAT seropositivity, the variable “days since vaccination” was introduced into the analysis. For unvaccinated dogs a value had to be generated in order to incorporate them into the model and 700 days was regarded a period after which antibodies were present due to exposure to leptospires and not due to vaccination.

2.8. Mapping

Maps were created using QGIS 2.14 (QGIS Development Team, 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project)

3. Results

3.1. Study population

The sample size to estimate a true prevalence and to identify risk factors with sufficient statistical power was estimated to be 229 and 536 dogs, respectively.

The majority of dogs included in this study were privately owned pet dogs. Eleven dogs were owned by the military, but lived in individual homes with their handlers. 21 dogs lived in two different rehoming kennels. Of the 377 dogs tested by serology, 21.1% lived in urban areas and 73.1% in rural environments including 16.9% farm dogs.

Dogs originated from 20 of the 26 Swiss cantons. The geographical distribution of the sampled dogs across Switzerland is shown in **Figure 3.**

The age of the dogs ranged from 4 months to 15 years (median 5 years IQR 2-9). Of the 423 dogs of known breed, 341 (80.6%) were purebred from 102 different breeds and 82 dogs (19.3%) were mixed-breeds. The most common breeds were Malinois (8.3%), Labrador Retriever (7.8%), French Bulldogs (4%), Golden Retrievers (3.5%) and Jack Russel Terrier (3.3%). The overrepresentation of Malinois dogs was due to the inclusion of military dogs as well as dogs of this breed undergoing genetic screening. Gender was known in 97.8% of dogs and was equally distributed with 204 (45.3%) female dogs (57 entire/146 neutered) and 246 (54.7%) male dogs (139 entire/107 neutered).

Of the 377 dogs tested by MAT, 373 had a known vaccination history: 43 dogs (11.6%, 95% CI 40.6-50.7) were vaccinated using a bivalent vaccine including serovars Canicola, and Icterohaemorrhagiae, 246 (66.3%, 95% CI 61.2-70.9) with a quadrivalent vaccine including serovars Canicola, Grippotyphosa, Icterohaemorrhagiae and Pomona and 84 (22.1%, 95% CI 18.1-26.4) were not vaccinated. The median period between vaccination and test was 253 days (IQR 189.5-475.5).

3.2. Urinary shedding

qPCR of urine was performed for 408 dogs without clinical suspicion of leptospirosis. Only one dog had a positive PCR result. This dog lived on a farm in Canton Vaud and reportedly had a habit of chasing rats. The dog showed a MAT titre of 1:40 to serovar Bratislava and 1:20 to serovars Australis, Canicola, Hardjo and Autumnalis. The dog had been vaccinated with a bivalent anti-leptospiral vaccine 93 days before sampling.

In 12 dogs qPCR was repeated 2-4 weeks after initial testing because of MAT titres ≥1:40 to one or several serovars. All the repeated urine samples tested negative.

3.3. Seroprevalence and Serovars

Due to the potential effect of vaccination on MAT seroreactivity the overall seroprevalence as well as the seroreactivities to the individual serovars are shown for the overall population as well as according to the vaccination status of the dogs in **Figure 4 A-D.** While reciprocal tires of ≥1:40 and ≥1:100 were chosen as cut offs for risk factor analysis, the prevalence of MAT seroreactivity was calculated for titres between ≥1:20 and ≥1:3200 and are shown in **Tables 3-4 and Suppl. Tables 3-4.**

3.3.1. Overall population

Of 377 sera, 55.7% (95% CI 51-60.7) showed a reciprocal MAT titre of ≥1:40 and 24.9% (95% CI 20.7-39.4) of ≥1:100 to at least one serovar.

At ≥1:100, seropositivity was most common to serovars Australis (14.9%; CI 11.4-18.6) and Bratislava (8.8%; CI 6.1-11.7), folowed by Copenhageni (6.1%; CI 4-8.5), Canicola (5%; CI 2.9-7.2), Grippotyphosa (4.5%; CI 2.7-6.9), Pomona (4%; CI 2.1-6.1), Autumnalis (2.7%; CI 1.1-4.5) and Icterohaemorrhagiae (1.6%; CI 0.5-2.9).

At ≥1:40 the reactivity pattern was slighlty different with Australis, Canicola, and Copenhageni being the most commonly reactive serovars, followed by Bratislava, Gripotyphosa, Icterohaeorrahgiae and Pomona.

In order to better understand the differences in reactivity, co-reactiviy patterns were also analysed **(Table 5).** The number of dogs with MAT reactivity to more than one serogroup was higher at a cut-off titre of ≥1:40 than at a cut-off titre of ≥1:100. The most common serogroup co-reactivities or double exposures at a MAT cut-off of

357 ≥1:100 were serogroup Australis and Icterohaemorragie (40%), Australis and
358 Grippotyphosa (28.6%), Australis and Pomona (28.6%) and Canicola and
359 Icterohaemorrhagie (25.7%).
360
361 ***3.3.2. Dogs not vaccinated in the past 16 weeks.***
362 Of 335 sera, 49% (95% CI 43.6-54) were reactive at a MAT titre of ≥1:40 and 20.6%
363 (95% CI 16.4-25.1) of ≥1:100 to at least one serovar. Seropositivtiy (MAT ≥1:100) was
364 most common to serovars Australis (11.3%; CI 8.1-14.9), Bratislava (6.9%; CI 4.2-9.6),
365 Canicola (4.5%; CI 2.4-6.9), Copenhageni (3.9%; CI 2.1-6.3), Grippotyphosa (3.6%; CI
366 1.8-5.7), Pomona (3.3%; CI 1.5-5.4), Autumnalis (2.4%; CI 0.9-4.2) and
367 Icterohaemorrhagiae (1.5%; CI 0.3-3).
368
369 ***3.3.3. Unvaccinated dogs.***
370 In unvaccinated dogs (n=84) the overall seroprevalence was 44% (CI 33.3-54.8) at a
371 reciprocal MAT titre of ≥1:40 and 17.9% (CI 10.7-27.4) at a reciprocal MAT titre of
372 ≥1:100. The distribution of serovars was largely similar to the distribution in vaccinated
373 dogs with Australis (9.5%; CI 3.6-16.7), Bratislava (8.3%; CI 2.4-15.5) being the most
374 frequent serovars, followed by Copenhageni (3.6%; CI 0-8.3), Grippotyphosa (3.6%; CI
375 0-8.3), Canicola (3.6 CI 0-8.3), Pomona (2.4%; CI 0-0.6) and Autumnalis (2.4%; CI 0-
376 6).
377
378 **3.3.4. MAT titre dynamics**
379 Despite the fact that in the study protocol retesting was advised in dogs with
380 reciprocal MAT titres ≥1:40, only 11 dogs were retested after 2-3 weeks; one reason
381 being that many dogs were sampled for this study immediately prior to vaccination

and thus no meaningful information could be derived from repeat MAT testing due to the interference of vaccinal titres. One dog showed seroconversion (BRA: 1:400 to 1:1600) and one dog showed persistently high titres (BRA: 1:800 to 1:1600; AUT: 1:1600 to 1:1600), which can be consistent with recent infection. Four dogs showed decreasing titres and five dogs the same or a slightly higher titre as before.

3.4. Risk factor analysis and multivariable models

The prevalence of anti-leptospiral serum antibodies by sex, gender, neuter status, vaccination status, region and lifestyle is reported in **Suppl Table 2.**

3.4.1. Univariate analysis

The association of 17 variables for the outcomes MAT seropositivity at titres ≥40 or ≥100 to any serovar or ≥100 to individual serovars were assessed via univariate analysis. The 10 variables with significant ($p \leq 0.2$) associations with the different outcomes in the univariate analysis are shown in **Table 6**.

3.4.2. *Multivariable analysis*

The fitted models after backwards selection based on the likelihood ratio test are displayed in **Tables 7 and 8.**

Dogs living in the bioregions Jura, Central Plateau. Northern Alps and Central Eastern Alps had significantly higher odds of being seropositive for any serovar compared to the bioregion Southern Alps.

The odds of seropositivity to any serovar significantly increased with age at both cut off titres ≥1:40 and ≥1:100. The interaction between “Age” and “Days since vaccination” was significant for both cut off titres, meaning that the effect of age on seropositivity differed according to the amount of days between vaccination and test.

Being castrated significantly increased the odds of seropositivity against serovar Australis (MAT ≥1:100). The odds of seropositivity with the serovar Bratislava (MAT≥ 1:100) significantly increased when the household owned a horse.

Hunting significantly increased the odds to be seropositive for serovar Grippotyphosa. For the serovars Canicola and Copenhageni no risk factors remained significant.

The variables that remained significant in the final logistic regression models were fitted in GEE models to correct for potential dependency (clustering) in the data. However, the SE hardly changed and therefore the results of unadjusted multivariable logistic regression models were reported. The models showed no evidence of lack of fit based on the Hosmer-Lemeshow tests and there was no strong leverage. All observations were kept in the models.

4. Discussion

4.1. Seroprevalence

The results of this stratified cross-sectional study demonstrate that exposure to pathogenic *Leptospira* is common in dogs not suspected to have leptospirosis in Switzerland. Of 377 dogs tested, 24.9% showed seroreactivity to one or several serovars of a 12 serovar MAT panel at a cut-off titre of ≥100 and 55.7% of dogs showed reactivity at a titre of ≥40. The seroprevalence of anti-leptospiral antibodies in asymptomatic dogs reported in the literature ranges between 6% in dogs in Ireland using a MAT cut-off of ≥1:10 (Schuller et al., 2015), 11.4% in Greece (Burriel et al., 2003), 17% in dogs in southern Germany (Llewellyn et al., 2016), 17.1% in Washington State, 21.5% in Poland (Krawczyk, 2005) and 49% in kenneled dogs in Italy using all a cut off ≥1:100 (Scanziani et al., 2002). In comparison, the overall seroprevalence of 24.9% in Switzerland does not appear exceedingly high, especially

in view of the very high incidence of acute disease in this region in the past years. A possible explanation is that albeit dogs do not seem to get more commonly exposed to pathogenic *Leptospira*, serovars in circulation may be particularly virulent causing acute and severe disease in those dogs that do get infected. Another potential explanation could be that we are already seeing a change in epidemiology of canine leptospirosis in Switzerland due to the introduction of new quadrivalent vaccines, by the end of 2014. These vaccines contain serovars belonging to the serogroups Australis and Grippotyphosa in addition to serogroups Canicola and Icterohaemorrhagiae and thus potentially provide a broader spectrum of protection.

4.2. Reactive serogroups

Serovars Australis and Bratislava, both belonging to serogroup Australis, were associated with about half of the reactive MATs, regardless of the vaccination status of the dogs. These findings correlate with the fact that based on serological data, members of serogroup Australis are an important cause of acute clinical infection in dogs in Switzerland (Major et al., 2014). However, canine sera also reacted with a broad range of other serovars including Copenhageni, Canicola, Grippotyphosa, Pomona, Autumnalis and Icterohaemorrhagiae indicating that *Leptospira* belonging to these serovars are in circulation in the environment. Studies are underway to examine the role of wild animals as reservoir hosts for specific serogroups in Switzerland. Serovar Bratislava has been isolated from hedgehogs in Ireland (Hathaway et al., 1983). As hedgehogs are frequent visitors in private gardens, and thus can come into close contact with residing dogs, the role of hedgehogs as reservoir host for serovars belonging to serogroup Australis in Switzerland should be further investigated.

4.3. Risk factors

460 In order to assess areas of increased risk, the prevalence amongst 6 different
461 biogeographic regions was compared. This bioregion classification is based on the
462 flora and fauna found in different areas of Switzerland and reflects the significant
463 climatic variations between lower Switzerland (Plateau), with low to moderate
464 altitudes (230- 960 m NN), the Jura with medium to high altitudes (360-1300 m NN),
465 the northern and southern slopes of the Alps and the high alpine areas with altitudes
466 of up to 4600 m NN (Gonseth, 2001). This classification does not take into account
467 the potentially significant regional variations in microclimate, as well as other factors
468 of importance to the transmission of *Leptospira* such as the presence of stagnant
469 water and the density of wildlife populations.
470 The stratification of sampling was performed independently of the bioregion
471 classification and was based on the numbers of registered dogs per canton.
472 However, the stratification naturally reflected the significant regional differences in
473 density of the canine dog population, with the highest population density in the
474 Plateau area (around 75% of the human population and more than 50% of the canine
475 population) and the lowest in the central alpine regions. As a consequence, the
476 estimates of seroprevalence in the Alpine regions of the country were based on much
477 smaller numbers than in the Plateau and Jura regions, potentially leading to a higher
478 margin of error for the lower populated areas.
479 The prevalence of anti-leptospiral serum antibodies varied significantly between
480 different bioregions. While the seroprevalence in the western high alpine regions
481 (6.7%) and the south side of the Alps (10%) was low, the seroprevalence in the
482 Northern half of the country which includes the Plateau, Jura and Northern Alps,
483 ranged between 25.2% and 28.8% and odds ratios for seropositivity in these areas
484 were high. The higher seroprevalence in bioregions with lower altitudes is biologically
485 plausible due to less extreme climatic conditions throughout the year. Periods of

extreme cold or extreme hot can be associated with reduced availability of surface water and reduced seropositivity rates, because leptospires are killed by freezing or desiccation (Adler, 2015). In addition to a milder climate, lower Switzerland has a large number of lakes and rivers, which could facilitate indirect transmission of *Leptospira*. However, none of the variables such as “swimming” and “drinking from puddles” assessing exposure to surface water as potential risk factors for seropositivity remained significant in the multivariate analysis.

The higher seroprevalence in this cohort of dogs not suspected to have leptospirosis in the Jura and Plateau area correlates with previous studies describing the geographic distribution of dogs with acute clinical infection in Switzerland with 75.5% of dogs originating from the Plateau area (Major et al., 2014). A cluster of acute disease in humans was recently described and could be traced back to a surfing spot in canton Aargau, which is also situated in the plateau area (Schreiber et al., 2015). Based on these studies, the plateau region which includes numerous lakes and rivers appears to carry this highest risk of infection in Switzerland.

Dogs had 4.7 times higher odds of being seropositive to serovar Bratislava (MAT ≥1:100) if the owner also owned a horse. While horses can get infected with a wide range of leptospiral serovars, globally, reactivity to serovar Bratislava is most common and the horse is considered a maintenance host for this serovar (Ellis et al., 1983; Baverud et al., 2009; Adler, 2015; Arent et al., 2015). In a recent serologic survey from Switzerland, 58.5% of 615 tested horses showed MAT seropositivity to at least one serogroup, indicating current or recent infection. The most frequent reactive serovars were Pyrogenes (22.6%), Canicola (22.1%), Australis (19.2%) and Bratislava (15.9%) (Blatti et al., 2011). Horses could therefore be the source of exposure for dogs to serovar Bratislava. However, serovar Bratislava has also been isolated from other animals including badgers (Hathaway et al., 1983), hedgehogs,

512 rats and sheep (Arent et al., 2016). Therefore other factors such as spending more
513 time in nature and potentially increased contact with reservoir hosts while
514 accompanying the owner on excursions on horseback could be equally important
515 factors for dogs to get exposed to this serovar.
516 Dogs had 8 times higher odds of having a positive titre to serovar Grippotyphosa
517 (MAT ≥1:100) if they were hunting. This association may be explained by increased
518 contact to environmental sources and reservoir hosts of *Leptospira* and correlates
519 with the higher odds for acute leptospirosis in dogs exposed to wild animals (Ghneim
520 *et al*., 2007). Hunting as a risk factor for leptospirosis is also supported by human
521 data showing a high seroprevalence of leptospirosis in human hunters (Deutz et al.,
522 2003a; Deutz et al., 2003b).
523 In this cohort, the odds of MAT seropositivity increased with increasing age. A
524 correlation between age and MAT seropositivity has been shown in many other
525 studies in horses (Kitson-Piggot and Prescott, 1987; Baverud et al., 2009; Blatti et al.,
526 2011) and dogs (Harland et al., 2013). This relationship is plausible as infection
527 related antibodies can persist for years and the probability of having come in contact
528 with an infectious agent increases over time.
529 More difficult to explain is the fact that being castrated was a significant risk factor for
530 the seropositivity to serovar Australis (MAT ≥1:100). This finding is in contrast to
531 previous studies showing that uncastrated dogs are overrepresented in cohorts of
532 animals with acute clinical leptospirosis which lead to the hypothesis that sexually
533 intact animals manifest more often risky behaviours like sniffing around and marking
534 their territories (Ward et al., 2002; Major et al., 2014). However, due to the
535 confounding influence of vaccination on this variable, the association between neuter
536 status and seropositivity to serovar Australis should not be over interpreted.

A significant association between the living environment and seroprevalence was not found in this cohort of dogs. While and increased seroprevalence was found in some studies for dogs living in rural compared to urban areas (Ghneim et al. 2006), a recent meta-analysis identified a higher risk in urban environments (Azocar-Aedo & Monti, 2016). This is not surprising, as wildlife reservoirs can be found with high density in both urban and rural environments and represent a likely source of infection in both settings. Risk factors for exposure to pathogenic *Leptospira* identified in other studies include being male or living in a kennel (Scanziani et al., 2002), a higher risk of infection in mixed and working breeds (Harland et al., 2013; Azocar-Aedo and Monti, 2016) as well as in dogs used to swimming and drinking from outdoor sources of water (Ghneim *et al.,* 2007). None of these risk factors was confirmed in this cohort of dogs.

4.4. MAT, co-reactivities, potential confounding effects of vaccination

In the past the MAT has been widely used to determine previous exposure to *Leptospira* in domestic and wild animals and in humans (Adler, 2015). With this test, the presence of anti-leptospiral IgG and/or IgM is determined based on agglutination of live *Leptospira* species after incubation with patient serum at various dilutions. The MAT relies not only on inclusion of a representative panel of *Leptospira* spp., but its interpretation also has an element of subjectivity, which can explain the significant variability noted when MAT results from the same patient are compared across different laboratories (Miller et al., 2011). MAT has limited sensitivity with regards to the identification of the infecting serogroup in acute infection as patient IgM, which predominates in early infection, has lower binding specificity than IgG (Chernukha et al., 1976). The use of MAT can therefore be justified for seroprevalence studies. However, cross-reactivity between different serogroups can occur leading to MAT

563 reactivity at lower dilutions. In the absence of a consensus of what represents an
564 ideal cut off titres for MAT positivity, the decision was made to use two different cut
565 offs (≥1:40 and ≥1:100) to calculated seroprevalence in this present study. As
566 expected, co-reactivities were more common at lower titres in this cohort. Most
567 common co-reactivities were those between serogroups contained in the current
568 vaccines and could therefore be related to vaccination. The MAT does not
569 discriminate between vaccination titres and titres due to exposure, thus adding
570 further difficulty to the interpretation of canine tests, as a large proportion of dogs are
571 vaccinated with either bivalent or quadrivalent whole cell anti-leptospiral vaccines.
572 Despite all these limitations we decided to use MAT to estimate the seroprevalence
573 in dogs not suspected to have leptospirosis in Switzerland and to apply statistical
574 methods to minimise the effect of vaccination on prevalence results. In the absence
575 of specific data regarding antibody titres after vaccination with a quadrivalent vaccine
576 available on the European market at the time of the planning of the study, the results
577 of a study describing the dynamics of antibody titres after vaccination with anti-
578 leptospiral vaccines available on the US market were used to define inclusion criteria
579 with regards to vaccination. In this study the majority of dogs developed positive MAT
580 titers immediately after vaccination, a minority of them remained seropositive by
581 week 15 (Martin et al., 2014). On the basis of these results, we decided to select
582 dogs vaccinated at least 16 weeks (112 days) before testing. Due to the multicentre
583 design of this study, this inclusion criterion was however violated in 10% of dogs.
584 Subgroup analysis between vaccinated and unvaccinated dogs, as well as dogs not
585 vaccinated in the past 112 days showed that albeit seroprevalence was slightly lower
586 if recently vaccinated dogs were excluded, there was good correlation with regards to
587 the reactive serogroups, in particular if considering the MAT titres ≥1:100.Therefore
588 the decision was made to perform risk factor analysis on the entire cohort, regardless

589 of vaccination status and to introduce the variable “days since vaccination” as a risk
590 factor in the model to account for the possible confounding effect of vaccination. This
591 variable only remained significant as an effect modifier for the associations between
592 age and MAT seropositivity and for the association between castration and MAT
593 seropositivity. We are therefore confident that the presented risk factors reflect risk
594 factors associated with natural infection and not vaccination.
595 In order to describe titre dynamics in dogs with MAT titres ≥1:40 follow up testing 1-2
596 weeks later was advised. However, a large proportion of dogs was tested
597 immediately prior to vaccination precluding that information of relevance to this study
598 could be retrieved from retesting. Therefore only a small proportion of dogs was
599 retested and results consistent with recent infection was found in 18% (2/11) of these
600 dogs.
601

602 **4.5. Urinary shedding**

603 One of the hypotheses tested in this study was that dogs contribute to the
604 maintenance and spread of pathogenic *Leptospira* in the environment by shedding
605 *Leptospira* in their urine. In that regard urine samples from 408 dogs were tested by
606 qPCR. Care was taken to preserve leptospiral DNA during shipment by use of a
607 stabiliser, which had shown to preserve leptospiral DNA for at least 48 h post
608 sampling. However, only 1/408 samples tested positive. In this dog contact to small
609 rodents was the likely source of infection however and material was insufficient for
610 molecular typing of the serovar or species level. Based on these results we
611 concluded that urinary shedding by asymptomatic dogs is uncommon. This could be
612 explained by the fact that dogs, albeit being an important incidental host for
613 serogroup Australis in Switzerland, does not appear to be an efficient maintenance
614 host for any of the locally prevalent strains, not included in the current vaccines.

615 However, the influence of intermittent shedding and lack of detection due to pre-
616 analytical or analytical factors also need to be considered and could potentially lead
617 to a large underestimation of the actual level of renal carriage of pathogenic
618 *Leptospira* in the canine population (Gay et al., 2014).
619
620 **5. Conclusions**
621 In conclusion, the results of this study show that dogs in Switzerland are commonly
622 exposed to pathogenic *Leptospira* spp. with exposure being most common to serovars
623 belonging to serogroup Australis. The Swiss Jura and plateau regions carry the highest
624 risk of infection. Contact to horses and hunting were significant risk factors associated
625 with seroprositivity to serogroups Bratislava and Gripotyphosa respectively. Based on
626 our findings vaccination of dogs with quadrivalent vaccines containing serogroups
627 Australis and Gripotyphosa in addition to serogroups Icterohaemorrhagiae and
628 Canicola is advised. The risk of dogs contributing to the spread of pathogenic
629 *Leptospira* in the environment appears low.
630
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634 who participated in the study. We would also like to thank Valentine Jaquier und
635 Isabelle Brodard for excellent technical assistance with the serological and molecular
636 analyses. This study was presented as an oral abstract at the 26th annual congress of
637 the European College of Veterinary Internal Medicine (ECVIM-CA) in Gothenburg
638 (9.9.-10.9.2016).

Tables

Table 1. Panel of 12 *Leptospira* spp. used as live antigens for Microscopic Agglutination testing (MAT).

Genomospecies	Serogroup	Serovar	Strain
L.interrogans	Australis	Australis	Ballico
	Australis	Bratislava	Jez-Bratislava
	Autumnalis	Autumnalis	Akiyami
	Canicola	Canicola	Hond Utrecht IV
	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
	Icterohaemorrhagiae	Copenhageni	M20
	Pomona	Pomona	Pomona
	Pyrogenes	Pyrogenes	Salinem
	Sejroe	Hardjo	Hardjoprajitno
L.borgpetersenii	Ballum	Ballum	Mus127
	Tarassovi	Tarassovi	Perepelitsin
L. kirschneri	Grippotyphosa	Grippotyphosa	Moskva V

645 **Table 2.** Frequency of variables collected via questionnaire for dogs tested for anti-
646 leptospiral serum antibodies via MAT.

Variable	Category	n	%	95% CI
Vaccine	Not vaccinated	84	22.5%	18.2-26.5
	Bivalent	43	11.5%	8.3-14.7
	Quadrivalent	246	66%	61.1-71
Sex	Female	170	46.1%	41.2-51.2
	Male	199	53.9%	48.8-58.8
Castrated	No	168	45.7%	40.6-50.7
	Yes	200	54.3%	49.3-59.4
Other animals in the household	No	107	31.2%	26.3-36.1
	Dog	117	34.1%	29.1-39.1
	Cat	59	17.2%	13.2-21.2
	Dog and Cat	32	9.3%	6.3-12.4
	Livestock +/-Dog/cat	5	1.5%	0.2-2.7
	Horses +/- Dog/cat	23	6.7%	4.1-9.4
Bioregion	Jura	51	13.5%	10.1-16.7
	Central Plateau	230	61%	56.2-65.8
	Northern Alps	66	17.5%	13.8-21.5
	Central western Alps	15	4%	2.1-6.1
	Central Eastern Alps	5	1.3%	0.3-2.7
	Southern Alps	10	2.7%	1.3-4.5
Surrounding	Shelter	21	5.8%	3.6-8.3
	Urban	49	13.6%	10-17.2
	Rural	98	27.1%	23-31.9
	Farm	61	16.9%	13-20.8
	Urban + Garden	27	7.5%	5-10.2
	Rural + Garden	105	29.1%	24.4-33.8
Contact to acutely ill animals	No	162	47.9%	42.3-53.3
	Yes	28	8.3%	5.6-11.5
	I do not know	148	43.8%	38.2-49.1
Previously diagnosed and treated for Leptospirosis	No	330	97.6%	95.9-99.1
	Yes	8	2.4%	0.9-4.1
Fever this past year (dog)	No	305	90.2%	87-93.5
	Yes	33	9.8%	6.5-13
Fever this past year (owner)	No	270	81.1%	77.2-85.3
	Yes	63	18.9%	14.7-22.8
Outside	<50% of time	259	75.7%	71.3-80.1
	>50% of time	83	24.3%	17.6-31.3
Spends time in forests	No	31	9.2%	6.2-12.4
	Yes	307	90.8%	87.6-93.8
Drinking from puddles	No	104	30.9%	26.1-35.9
	Yes	233	60.1%	64.1-73.9
Swimming	No	110	32.7%	27.7-37.8
	Yes	226	67.3%	62.2-72.3
Contacts to wild animals	No	190	56.2%	50.6-61.5
	Yes	94	27.8%	23.4-32.5
	I do not know	54	16%	12.1-20.1
Hunting	No	326	96.4%	94.4-98.2
	Yes	12	3.6%	1.8-5.6

Table 3. Number of individuals, relative prevalence and 95% C.I. per *Leptospira* serovar for all dogs (n=377). Tested serovars: Australis (AUS), Autumnalis (AUT), Ballum (BAL), Bratislava (BRA), Canicola (CAN), Copenhageni (COP), Grippotyphosa (GRI), Hardjo (HAR), Icterohaemorrhagie (ICT), Pomona (POM), Pyrogenes (PYR) and Tarassovi (TAR).

MAT Titer		AUS	BRA	COP	CAN	GRI	ICT	POM	PYR	TAR	AUT	BAL	HAR
1:20	n	110	20	46	75	16	27	30	14	7	37	47	1
	%	29.2	5.3	12.2	19.9	4.2	7.2	8	3.7	1.9	9.8	12.5	0.3
	C.I.	24.7-33.7	3.2-8	9-15.4	15.6-24.1	2.1-6.4	4.8-9.8	5.3-10.9	1.9-5.8	0.5-3.4	6.9-12.7	9.3-15.9	0-0.8
1:40	n	68	21	46	75	26	30	22	4	1	17	3	1
	%	18	5.6	12.2	19.9	6.9	8	5.8	1.1	0.3	4.5	0.8	0.3
	C.I.	14.1-22	3.2-8	9.3-15.4	15.6-24.1	4.5-9.5	5.3-10.6	3.7-8.2	0.3-2.1	0-0.8	2.7-6.6	0-1.9	0-0.8
1:100	n	35	13	12	12	8	3	9	1	1	5	-	-
	%	9.3	3.4	3.2	3.2	2.1	0.8	2.4	0.3	0.3	1.3	-	-
	C.I.	6.6-12.2	1.9-5.6	1.6-5	1.6-5	0.8-3.7	0-1.9	1.1-4.2	0-0.8	0-0.8	0.3-2.7	-	-
1:200	n	12	9	8	7	8	2	4	2	-	2	-	-
	%	3.2	2.4	2.1	1.9	2.1	0.5	1.2	0.5	-	0.5	-	-
	C.I.	1.6-5	1.1-4.2	0.8-3.7	0.5-3.4	0.8-3.7	0-1.3	0.3-2.1	0-1.3	-	0-1.3	-	-
1:400	n	4	6	3	-	-	1	1	-	-	2	-	-
	%	1.1	1.6	0.8	-	-	0.3	0.3	-	-	0.5	-	-
	C.I.	0.3-2.1	0.5-2.9	0-1.9	-	-	0-0.8	0-0.8	-	-	0-1.3	-	-
1:800	n	1	4	-	-	1	-	1	-	-	-	-	-
	%	0.3	1.1	-	-	0.3	-	0.3	-	-	-	-	-
	C.I.	0-0.8	0.3-2.4	-	-	0-0.8	-	0-0.8	-	-	-	-	-
1:1600	n	3	1	-	-	-	-	-	-	-	1	-	-
	%	0.8	0.3	-	-	-	-	-	-	-	0.3	-	-
	C.I.	0-1.9	0-0.8	-	-	-	-	-	-	-	0-0.8	-	-
1:3200	n	1	-	-	-	-	-	-	-	-	-	-	-
	%	0.3	-	-	-	-	-	-	-	-	-	-	-
	C.I.	0-0.8	-	-	-	-	-	-	-	-	-	-	-

Table 4. Number of individuals, relative prevalence and 95% C.I. per *Leptospira* serovar for unvaccinated dogs (n=84). Tested serovars: Australis (AUS), Autumnalis (AUT), Ballum (BAL), Bratislava (BRA), Canicola (CAN), Copenhageni (COP), Grippotyphosa (GRI), Hardjo (HAR), Icterohaemorrhagie (ICT), Pomona (POM), Pyrogenes (PYR) and Tarassovi (TAR).

MAT Titer		AUS	BRA	COP	CAN	GRI	ICT	POM	PYR	TAR	AUT	BAL	HAR
1:20	n	25	2	10	11	1	3	5	5	3	7	9	1
	%	29.8	2.4	11.9	13.1	1.2	3.6	6	6	3.6	8.3	10.7	1.2
	C.I.	20.2-40.4	0-6	6-20.2	6-21.4	0-3.6	0-8.3	1.2-11.9	1.2-11.9	0-8.3	3.6-14.3	4.8-17.9	0-3.6
1:40	n	9	4	6	8	2	3	2	-	1	5	1	-
	%	10.7	4.8	7.1	9.5	2.4	3.6	2.4	-	1.2	6	1.2	-
	C.I.	4.8-17.9	1.2-9.5	2.4-13.1	3.6-16.7	0-6	0-8.3	0-6	-	0-3.6	1.2-11.9	0-3.6	-
1:100	n	4	2	1	2	-	-	1	-	-	-	-	-
	%	4.8	2.4	1.2	2.4	-	-	1.2	-	-	-	-	-
	C.I.	1.2-9.5	0-6	0-3.6	0-6	-	-	0-3.6	-	-	-	-	-
1:200	n	4	3	2	1	3	-	-	-	-	1	-	-
	%	4.8	3.6	2.4	1.2	3.6	-	-	-	-	1.2	-	-
	C.I.	1.2-9.5	0-8.3	0-6	0-3.6	0-8.3	-	-	-	-	0-3.6	-	-
1:400	n	-	1	-	-	-	-	1	-	-	-	-	-
	%	-	1.2	-	-	-	-	1.2	-	-	-	-	-
	C.I.	-	0-3.6	-	-	-	-	0-3.6	-	-	-	-	-
1:800	n	-	1	-	-	-	-	-	-	-	-	-	-
	%	-	1.2	-	-	-	-	-	-	-	-	-	-
	C.I.	-	0-3.6	-	-	-	-	-	-	-	-	-	-
1:1600	n	-	-	-	-	-	-	-	-	-	1	-	-
	%	-	-	-	-	-	-	-	-	-	1.2	-	-
	C.I.	-	-	-	-	-	-	-	-	-	0-3.6	-	-

658

659 **Table 5.** Number of individuals positive against one, two or more serogroups at a

660 MAT Titre of ≥1:40 and MAT ≥1:100.

661

N° of positive Serogroups	MAT ≥1:40 n (%)	MAT ≥1:100 n (%)
1	21 (23.1)	58 (62.4)
2	30 (33.0)	19 (20.4)
3	25 (27.5)	8 (8.6)
4	7 (7.7)	6 (6.5)
5	7 (7.7)	1 (6.5)
6	1 (1.1)	1 (1.1)

662

663 **Table 6.** Risk factors associated with a MAT positivity.

664

Variable	ALL40	ALL100	AUS100	BRA100	GRI100	CAN100	COP100
Swimming	*						
Castrated		*	**				
Lives with other animals	*			**		*	*
Hunting					**		
Outside >50% of time							*
Bioregion	**	*					
Surroundings	*						
Forest	*						
Season	*						
Age	**	**					
Days since last vaccination	**	**	**	*			*

665

666 * Univariate analysis ($p \leq 0.2$)

667 ** Multivariate analysis ($p < 0.05$)

668 Outcomes: Seropositivity in dogs against any of the tested serovars at MAT cut-offs
669 ≥1:40 (ALL40) and ≥1:100 (ALL100), seropositivity in dogs at the MAT cut-off ≥1:100
670 for serovars Australis (AUS100), Bratislava (BRA100), Grippotyphosa (GRI100),
671 Canicola (CAN100) and Copenhageni (COP100).

672

673 **Table 7.** Statistically significant risk factors for seropositivity against any serovar at a
674 MAT cut-off ≥ 1:40 tested in a multivariable logistic regression model.

Covariate	Levels	Odds ratio	95% CI	p-value
Bioregion	6[#]	Ref.		
	1	11.17	1.69-226.72	0.034*
	2	12.43	2.04-244.12	0.023*
	3	14.51	2.23-292.71	0.018*
	4	5.53	0.63-125.11	0.17
	5	53.05	3.39-2378.75	0.012*
Age (years)		1.29	1.13-1.47	0.0001*
Days since vaccination		1.0008	0.9988-1.0028	0.42
Days since vaccination*Age		0.9995	0.9992-0.9998	0.0034*

675
676 * Statistically significant at $p < 0.05$, AIC full model = 408.25 & n=301, AIC final model
677 = 458.49 & n=347.
678 [#] The numbers correspond with the bioregions (1) Jura, (2) Central Plateau, (3)
679 Northern Alps, (4) Central Western Alps, (5) Central Eastern Alps and (6) Southern
680 Alps.
681

682 **Table 8.** Statistically significant risk factors for seropositivity against any serovar and
683 against serovars Australis, Bratislava and Grippotyphosa at a MAT cut-off ≥ 1:100
684 tested in multivariable logistic regression models. Associations were considered
685 significant if p<0.05. The Akaike information criterion (AIC) is used to indicate the
686 relative quality of the used model.

Covariate	Levels	Odds ratio	95% CI	p-value
Risk factors for seropositivity against any serovar at MAT cut-off ≥ 1:100[&]				
Days since vaccination		1.0011	0.9986-1.0034	0.39
Age (years)		1.25	1.09-1.43	0.0012*
Days since vaccination*Age		0.9995	0.9991-0.9999	0.0077*
Risk factors for seropositivity against serovar Australis at a MAT cut off ≥ 1:100[Δ]				
Castrated	No	Ref		
	Yes	1.98	1.08-3.74	0.030*
Days since vaccination		0.9969	0.995- 0.998	0.0004*
Risk factors for seropositivity against serovar Bratislava at a MAT cut-off ≥ 1:100[▾]				
Dog		1.40	0.49-4.32	0.53
Cat		2.27	0.72-7.37	0.16
Dog and Cat		2.40	0.58-9.02	0.20
Livestock +/-Dog/cat		4.21	0.20-34.77	0.23
Horses +/- Dog/cat		4.68	1.23-17.19	0.019*
Risk factors for seropositivity against serovar Grippotyphosa at a MAT cut-off ≥ 1:100[#]				
Hunting		8.03	1.64-30.82	0.004*

687

688 [&] AIC full model = 361.64 & n=319, AIC final model = 382.36 & n=347.

689 [Δ] AIC full & final model = 296.83 & n=367

690 [▾]AIC full model = 218.26 & n=342, AIC final model = 217.87, n=342

691 [#]AIC full & final model = 126.74 & n=337.
692

693

Supplemental Tables

Supplemental Table 1. Effect of stabilisier AssayAssure® on detection of leptospires in canine urine samples during simulated shipping at room temperature. Fresh voided urine from dogs were collected via free catch, pooled, and spiked with *Leptospira interrogans* serovar Autumnalis at $1x10^4$/ml, $1x10^3$ and $1x10^2$/ml. AssayAssure® was added to the test group at a concentration of 1:11 according to the manufacturer's instructions. The spiked samples were incubated at room temperature for 4, 24 or 48 hours and then processed for DNA extraction and real time PCR. At 48h the target gene *lipl32* could only be amplified from urine samples with AssayAssure®, but not from control samples.

Leptospires/ml urine		Mean C_t value[a]	
	Incubation time	Control	AssayAssure®
$1x10^4$	4h	32.303	31.244
$1x10^4$	24h	33.805	32.496
$1x10^4$	48h	-	32.781
$1x10^3$	4h	34.728	34.728
$1x10^3$	24h	36.877	35.006
$1x10^3$	48h	-	35.635
$1x10^2$	4h	39.047	37.921
$1x10^2$	24h	41.116	40.48
$1x10^2$	48h	-	39.63

[a] Mean cycle number at which the fluorescence passes the threshold

708 **Supplemental Table 2.** Prevalence of anti-leptospiral serum antibodies by sex, gender, neuter status, vaccination status, region and lifestyle.

709 Prevalence is reported with 95% Confidence Intervals (between brackets).

710

Variable	n	MAT ≥1:40	MAT≥1:100	AUS≥1:100	BRA≥1:100	GRI≥1:100	CAN≥1:100	COP≥1:100
All samples	377	55.7% (51.2-60.7)	24.9% (20.7-29.4)	14.9% (11.4-18.6)	8.8% (6.1-11.7)	4.5% (2.7-6.9)	5% (2.9-7.2)	6.1% (4-8.5)
Vaccinated								
No	84	42.7% (32.9-53.7)	17.1% (9.2-26.8)	9.8% (3.7-17.1)	8.5% (2.4-14.6)	2.4% (0.0-6.1)	3.7% (0.0-8.5%)	3.7% (0.0-8.5%)
Yes	289	59.2% (53.3-64.7)	27% (22.1-32.2)	16.6% (12.5-21.1)	9% (5.9-12.5)	4.8% (2.4-7.3)	5.5% (3.1-8.3)	6.6% (3.8-9.3)
Sex								
Female	170	55.3% (47.7-62.9)	25.9% (19.4-32.9)	17.1% (11.8-23.5)	8.2% (4.1-12.4)	2.9% (0.6-5.9)	5.3% (2.4-8.8)	5.9% (2.9-9.4)
Male	199	55.3% (48.7-62.3)	24.1% (18.1-30.2)	13.6% (9-18.6)	9.5% (6-14.1)	5.5% (2.5-8.5)	5% (2-8)	6% (3-9.5)
Castrated								
No	168	52.4% (44.6-59.5)	19% (13.1-25)	10.7% (6.5-16.1)	11.3% (7.1-16.7)	3.6% (1.2-6.5)	4.8% (1.8-8.3)	4.8% (1.8-7.7)
Yes	200	57.5% (51-64.5)	29.5% (23-36.5)	18.5% (13.5-23.5)	10.5% (6.5-15)	5% (2.5-8.5)	5.5% (2.5-9)	7% (4-20.5)
Other animals								
No	107	55.1% (45.8-64.5)	21.5% (14-29.9)	13.1% (7.5-20.6)	5.6% (1.9-11.2)	5.6% (1.9-10.3)	3.7% (0.9-7.5)	4.7% (0.9-9.3)
Dog	117	50.4% (41.9-59.8)	23.9% (16.2-31.6)	16.2% (10.3-23.1)	7.7% (3.4-12.8)	5.1% (1.7-9.4)	6% (2.6-11.1)	6.8% (2.6-12)
Cat	59	66.1% (54.2-78)	32.2% (20.3-45.8)	16.9% (8.5-27.1)	11.9% (5.1-20.3)	3.4% (0.0-8.5)	10.2% (3.4-18.6)	11.9% (5.1-22)
Dog and Cat	32	68.8% (53-84.4)	25% (12.5-40.6)	21.9% (9.4-37.4)	12.5% (3.1-25)	3.1% (0-9.4)	0%	3.1% (0-9.4)
Livestock +/-Dog/cat	5	60% (20-100)	40% (0-80)	20% (0-60)	20% (0-60)	0%	20% (0-60)	20% (0-60)
Horses +/- Dog/cat	23	47.8% (26.1-69.6)	34.8% (17.4-56.5)	8.7% (0-21.7)	21.7% (4.5-39.1)	0%	4.3% (0-13)	8.7%(0-21.7)
Bioregion								
Jura	51	52% (37.3-64.7)	27.5% (15.7-39.2)	13.7% (3.9-23.5)	13.7% (3.9-23.5)	5.9% (0-13.7)	3.9% (0-9.8)	3.9% (0-9.8)
Central Plateau	230	59.1% (53-64.8)	25.2% (19.1-30.9)	14.8% (10-19.6)	8.3% (4.8-11.7)	3.9% (1.7-6.5)	4.8% (2.2-7.8)	6.5% (3.9-10)
Northern Alps	66	56.1% (43.9-68.2)	28.8% (18.2-39.4)	19.7% (10.6-28.8)	9.1%(3-16.7)	6.1% (1.5-12.1)	6.1% (1.5-12.1)	7.6% (1.5-15.2)
Central western Alps	15	33.3% (13.3-60)	6.7% (0-20)	6.7% (0-20)	0%	0%	6.7% (0-20)	6.7% (0-20)
Central Eastern Alps	5	80% (40-100)	20% (0-60)	20% (0-60)	20% (0-60)	20% (0-60)	20% (0-60)	20% (0-60)
Southern Alps	10	20% (0-50)	10% (0-30)	0%	0%	0%	10% (0-30)	0%
Surrounding								
Shelter	21	47.6% (23.8-66.7)	19% (4.8-38.1)	9.5% (0-23.8)	4.8% (0-14.3)	0%	4.8% (0-14.3)	9.5% (0-23.8)
Urban	49	60.4% (45.8-75)	20.8% (10.4-33.3)	10.4% (2.1-18.8)	8.3% (2.1-16.7)	0%	4.2% (0-10.4)	4.2% (0-10.4)
Rural	98	65.3% (56.1-74.5)	28.6% (20.4-37.8)	19.4% (12.2-27.5)	9.2% (4.1-15.3)	6.1% (2-11.2)	6.1% (2-11.2)	6.1% (2-11.2)
Farm	61	50.8% (39.3-63.9)	26.2% (16.4-37.7)	16.4% (8.2-26.2)	9.8% (3.3-18)	4.9% (0-11.5)	3.3% (0-8.2	6.6% (1.6-13.1)
Urban + Garden	27	44.4% (25.9-63)	29.6% (14.8-48.1)	11.1% (0-25.9)	14.8% (3.7-29.6)	3.7% (0-11.1)	11.1% (0-22.2)	7.4% (18.5)
Rural + Garden	105	52.4% (43.8-62.9)	23.8% (16.2-32.4)	15.2% (8.6-21.9)	8.6% (3.8-14.3)	5.7% (1.9-10.5)	4.8% (1-9.5)	5.7% (1.9-10.5)
Contact to acutely ill animals								

No	162	56.8% (48.8-64.2)	24.1% (17.9-31.5)	14.2% (9.3-20.4)	6.2% (3.1-9.9)	4.3% (1.3-7.4)	4.9% (1.9-8.6)	4.9% (1.9-8.6)
Yes	28	85.7% (71.4-96.4)	53.6% (35.7-71.4)	42.9% (25-60.7)	17.9% (3.6-32.1)	7.1% (0-17.9)	14.3% (3.6-28.6)	14.3% (3.6-28.6)
I do not know	148	51.4% (43.3-59.5)	23% (16.2-29.7)	12.2% (6.8-17.6)	11.5% (6.8-16.9)	4.7% (2-8.1)	4.7% (1.4-8.8)	5.4% (2-9.5)
Previously diagnosed and treated for leptospirosis								
No	330	56.1% (50.9-61.5)	24.8% (20-29.4)	14.2% (10.6-17.6)	8.5% (5.8-11.2)	4.5% (2.4-6.7)	5.5% (3-7.9)	5.8% (3.3-8.2)
Yes	8	87.5% (62.5-100)	75% (50-100)	75% (50-100)	50% (12.5-87.5)	12.5% (0-37.5)	37.5% (12.5-75)	12.5% (0-37.5)
Fever this past year (dog)								
No	305	57.4% (50.8-63.6)	26.9% (21.5-32.6)	16.5% (12-21.5)	9.5% (5.8-13.2)	4.1% (2.1-6.6)	5.8% (2.9-9.1)	5.8% (2.9-9.1)
Yes	33	80% (64-92)	36% (20-56)	20% (4-36)	8% (0-20)	16% (4-32)	8% (0-20)	12% (0-28)
Fever this past year (owner)								
No	270	58.6% (52.6-65.6)	27.4% (21.9-34)	16.7% (12.1-21.9)	9.3% (6-13.5)	6% (3.3-9.3)	6.5% (3.3-9.8)	7.4% (4.2-11.2)
Yes	63	63.3% (49-77.6%)	28.6% (16.3-42.9)	16.3% (6.1-26.5)	8.2% (2-16.3)	2% (0-6.1)	4.1% (2-16.3)	2% (0-6.1)
Outside								
<50% of time	259	57.1% (42.9-67.9)	28.6% (17.9-41.1)	7.1% (1.8-14.3)	10.7% (3.6-17.9)	7.1% (1.8-14.3)	5.4% (0-12.5)	8.9% (1.8-6.1)
>50% of time	83	56.8% (50.6-62.5)	24.7% (19.3-29.7)	16.6% (12.4-20.8)	8.5% (5.4-12)	4.2% (1.9-6.9)	5% (2.7-7.7)	4.6% (2.3-7.3)
Walks in the Forest								
No	31	41.9% (25.8-61.2)	19.4% (25.8-61.2)	16% (3.2-29)	6.5% (0-16)	0%	3.2% (0-9.7)	6.5% (0-16.1)
Yes	307	58.3% (53.1-64.2)	26.7% (21.5-32.2)	15.6% (11.4-19.9)	9.8% (6.8-13)	5.2% (2.6-7.8)	5.9% (3.6-8.5)	5.9% (3.6-8.5)
Drinking from puddles/rivers								
No	104	57.7% (48.1-67.3)	27.9% (19.2-36.5)	18.3% (10.6-26)	7.7% (2.9-12.5)	2.9% (0-6.7)	3.8% (1-7.7)	5.8% (1.9-10.6)
Yes	233	56.7% (50.2-63.1)	25.3% (19.7-30.9)	14.6% (10.3-19.3)	10.3% (6.4-14.2)	5.6% (3-9)	6.4% (3.4-9.9)	6% (3-9.4)
Contacts with wild animals								
No	190	54.2% (46.8-61.1)	25.8% (19.5-32.6)	17.4% (12.1-23.2)	9.5% (5.3-14.2)	4.7% (1.6-8.4)	7.4% (3.7-11.6)	6.3% (3.2-10)
Yes	94	57.4% (47.9-67)	23.4% (14.9-31.9)	12.8% (6.4-19.1)	7.4% (3.2-12.8)	4.3% (0-8.5)	4.3% (1.1-8.5)	4.3% (1.1-8.5)
I do not know	54	64.8% (50-77.8)	31.5% (20.4-44.4)	14.8% (5.6-24.1)	13% (3.8-22.2)	5.6% (0-13)	1.9% (0-5.6)	7.4% (1.9-14.8)
Swimming								
No	110	51.8% (42.7-60.9)	28.2% (20-36.4)	18.2% (10.9-26.4)	9.1% (4.5-14.5)	3.6% (0.9-7.3)	4.5% (0.9-9.1)	3.6% (0.9-7.3)
Yes	226	59.7% (53.5-65.9)	25.2 (19.9-31)	14.6% (10.6-19.5)	9.7% (6.2-13.7)	5.3% (2.7-8.4)	6.2% (3.5-9.3)	7.1% (4-10.6)
Hunting								
No	326	56.4% (50.6-61.3)	25.8% (21.2-30.7)	15.6% (12-19.6)	9.5% (6.1-12.9)	4% (1.8-6.1)	5.5% (3.1-8)	6.1% (3.7-8.9%)
Yes	12	66.7% (41.7-91.7)	33.3% (8.3-58.3)	16.7% (0-41.7)	8.3% (0-25)	25% (0-50)	8.3% (0-25)	33% (8.3-58.3)
Season of testing								
Spring	102	48% (38.2-57.8)	26.5% (18.6-36.3)	11.8% (5.9-17.6)	7.8% (2.9-12.7)	3.9% (1-7.8)	5.9% (2-10.8)	4.9% (1-9.8)
Sommer	98	60.2% (51-70.4)	23.5% (15.3-32.6)	15.3% (8.2-22.4)	9.2% (4.1-15.3)	3.1% (0-7.1)	3.1% (0-7.1)	8.2% (3.1-14.3)
Fall	167	57.5% (49.7-65.3)	25.7% (19.8-32.3)	17.4% (12-23.4)	9.6% (5.4-14.4)	6% (3-10.2)	6% (2.4-9.6)	5.4% (1.8-9)
Winter	7	42.9% (0-71.4)	14.3% (0-42.9)	0%	0%	0%	14.3% (0-42.9)	14.3% (0-42.9)

711

Supplemental Table 3. Number of individuals, relative prevalence and 95% C.I. per *Leptospira* serovar for dogs vaccinated with a bivalent vaccine (n=43). Tested serovars: Australis (AUS), Autumnalis (AUT), Ballum (BAL), Bratislava (BRA), Canicola (CAN), Copenhageni (COP), Grippotyphosa (GRI), Hardjo (HAR), Icterohaemorrhagie (ICT), Pomona (POM), Pyrogenes (PYR) and Tarassovi (TAR).

MAT Titer		AUS	BRA	COP	CAN	GRI	ICT	POM	PYR	TAR	AUT	BAL	HAR
1:20	n	9	1	5	8	-	3	2	3	1	4	4	1
	%	20.9	2.3	11.6	18.6	-	7	4.7	7	2.3	9.3	9.3	2.3
	C.I.	9.3-32.6	0-7	4.7-20.9	7-30.2	-	0-14	0-11.6	0-16.3	0-7	2.3-18.6	2.3-18.6	0-7
1:40	n	6	3	6	9	1	5	2	-	-	3	1	-
	%	14	7	14	20.9	2.3	11.6	4.7	-	-	7	2.3	-
	C.I.	4.7-25.6	0-14	4.7-25.6	9.3-34.9	0-7	2.3-20.9	0-11.6	-	-	0-16.3	0-7	-
1:100	n	1	-	1	1	1	1	-	-	-	-	-	-
	%	2.3	-	2.3	2.3	2.3	2.3	-	-	-	-	-	-
	C.I.	0-7	-	0-7	0-7	0-7	0-7	-	-	-	-	-	-
1:200	n	-	-	3	2	1	1	-	1	-	1	-	-
	%	-	-	7	4.7	2.3	2.3	-	2.3	-	2.3	-	-
	C.I.	-	-	0-16.3	0-11.6	0-7	0-7	-	0-7	-	0-7	-	-
1:400	n	1	1	-	-	-	-	-	-	-	-	-	-
	%	2.3	2.3	-	-	-	-	-	-	-	-	-	-
	C.I.	0-7	0-7	-	-	-	-	-	-	-	-	-	-
1:800	n	-	-	-	-	-	-	-	-	-	-	-	-
	%	-	-	-	-	-	-	-	-	-	-	-	-
	C.I.	-	-	-	-	-	-	-	-	-	-	-	-
1:1600	n	1	1	-	-	-	-	-	-	-	-	-	-
	%	2.3	2.3	-	-	-	-	-	-	-	-	-	-
	C.I.	0-7	0-7	-	-	-	-	-	-	-	-	-	-

Supplemental Table 4. Number of individuals, relative prevalence and 95% C.I. per *Leptospira* serovar for dogs vaccinated with a quadrivalent vaccine (n=246). Tested serovars: Australis (AUS), Autumnalis (AUT), Ballum (BAL), Bratislava (BRA), Canicola (CAN), Copenhageni (COP), Grippotyphosa (GRI), Hardjo (HAR), Icterohaemorrhagie (ICT), Pomona (POM), Pyrogenes (PYR) and Tarassovi (TAR).

MAT Titer		AUS	BRA	COP	CAN	GRI	ICT	POM	PYR	TAR	AUT	BAL	HAR
1:20	n	74	16	31	58.56	15	21	23	6	3	26	33	-
	%	30.1	6.5	12.6	22.8	6.1	8.5	9.3	2.4	1.2	10.6	13.4	-
	C.I.	24.8-36.2	3.7-9.8	8.5-16.7	17.9-28	3.3-9.3	5.3-12.2	5.7-13	0.8-4.5	0-2.8	6.9-14.6	9.3-17.9	-
1:40	n	53	14	33	57	23	20	18	4	-	9	1	-
	%	21.5	5.7	13.4	23.2	9.3	8.1	7.3	1.6	-	3.7	0.4	-
	C.I.	16.7-26.8	2.9-8.9	9.3-17.9	17.9-2	5.7-13	4.9-11.8	4.1-11	0.4-3.3	-	1.6-6.1	0-1.2	-
1:100	n	30	11	9	9	7	2	8	1	1	5	-	-
	%	12.2	4.5	3.7	3.7	2.8	0.8	3.3	0.4	0.4	2	-	-
	C.I.	8.5-16.3	2-6.9	1.6-6.1	1.2-6.1	0.8-4.9	0-2	1.2-5.7	0-1.2	0-1.2	0.4-1	-	-
1:200	n	8	6	3	4	4	1	4	1	-	-	-	-
	%	3.3	2.4	1.2	1.6	1.6	0.4	1.6	0.4	-	-	-	-
	C.I.	1.2-5.7	0.8-4.5	0-2.8	0.4-3.3	0.4-3.3	0-1.2	0.4-3.3	0-1.2	-	-	-	-
1:400	n	3	4	3	-	-	1	-	-	-	2	-	-
	%	1.2	1.6	1.2	-	-	0.4	-	-	-	0.8	-	-
	C.I.	0-2.8	0.4-3.3	0-2.8	-	-	0-1.2	-	-	-	0-2	-	-
1:800	n	1	3	-	-	1	-	1	-	-	-	-	-
	%	0.4	1.2	-	-	0.4	-	0.4	-	-	-	-	-
	C.I.	0-1.2	0-2.8	-	-	0-1.2	-	0-1.2	-	-	-	-	-
1:1600	n	2	-	-	-	-	-	-	-	-	-	-	-
	%	0.8	-	-	-	-	-	-	-	-	-	-	-
	C.I.	0-2	-	-	-	-	-	-	-	-	-	-	-
1:3200	n	1	-	-	-	-	-	-	-	-	-	-	-
	%	0.4	-	-	-	-	-	-	-	-	-	-	-
	C.I.	0-1.2	-	-	-	-	-	-	-	-	-	-	-

Figure legends

Figure 1. Biogeographic regions of Switzerland**.** The six biogeographic regions of Switzerland and the number of MAT seropositive dogs per total tested at a cut-off ≥ 1:40 and ≥ 1:100 are shown.

Figure 2. Graphical overview of stdy population and statistical analyses MAT: Microscopic agglutination test; RT-PCR: *LipL32* real time PCR

AUS: Australis; CAN: Canicola, GRI: Grippotyphosa, ICT: Icterohaemorrhagie

Figure 3. **The geographical distribution of 377 dogs tested for serum antibodies against *Leptospira* spp via MAT.** Geocoding was performed based on the zip code of the owner's home address. Seropositivity was defined as positive MAT titre at ≥1:100.

Figure 4 Relative prevalence (%) of anti-leptospiral serum antibodies overall and per *Leptospira* serovar at reciprocal titres of ≥1:40 and ≥1:100.

Tested serovars: Australis (AUS), Autumnalis (AUT), Ballum (BAL), Bratislava (BRA), Canicola (CAN), Copenhageni (COP), Grippotyphosa (GRI), Hardjo (HAR), Icterohaemorrhagie (ICT), Pomona (POM), Pyrogenes (PYR) and Tarassovi (TAR).

Supplemental Figure 1: Questionnaire filled in by the owners of dogs enrolled in the study.

Supplemental Figure 2. The 26 cantons of Switzerland and their corresponding number of seropositive dogs per total tested at a cut-off of MAT≥1:40 and MAT≥1:100.

Legend to suppl Figure 2

AG	Aargau	NW	Nidwalden
AR	Appenzell Ausserrhoden	OW	Obwalden
AI	Appenzell Innerrhoden	SG	Sankt Gallen
BL	Basel-Land	SH	Schaffhausen
BS	Basel-Stadt	SZ	Schwyz
BE	Bern	SO	Solothurn
FR	Fribourg	TG	Thurgau
GE	Genève	TI	Ticino
GL	Glarus	UR	Uri
GR	Graubünden	VS	Valais
JU	Jura	VD	Vaud
LU	Luzern	ZG	Zug
NE	Neuchâtel	ZH	Zürich

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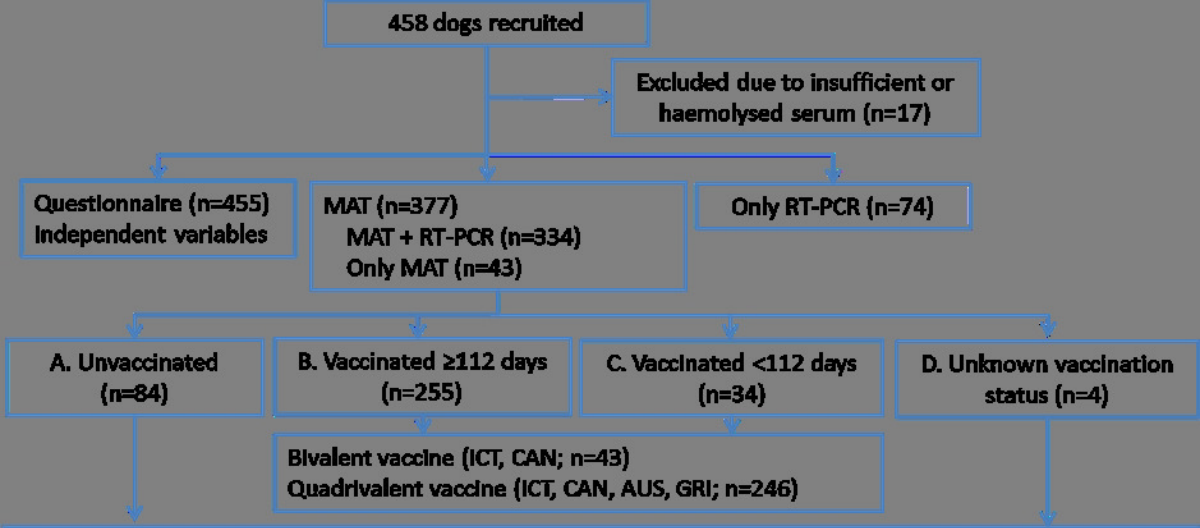
876

Group	n	MAT ≥40 % (95% CI)	MAT ≥100 % (95% CI)
Overall population	377	55.7% (51-60.7)	24.9% (20.7-39.4)
Not vaccinated	84	42.7% (32.9-53.7)	17.2% (10-27)
Vaccinated > 16 weeks ago	335	49% (43.6-54)	20.6% (16.4-25.1)
Bi-valent vaccine	42		
Quadrivalent vaccine	246		
Vaccinated < 16 weeks ago	42		

Kommentar [SS(1]: Numbers don't add up

Group	n	AUS	BRA	COP	CAN	GRI	HAR	ICT	POM	PYR	TAR	AUT	BAL
Overall population	377	14.9 (11-19)	8.8(6-12)	6.1(4-8.5)	5(3-7)	4.5(3-7)		1.6(1-3)	4(2-6)			2.7 (1-5)	
Not vaccinated	84	9(4-17)	8(3-16)	3.8(1-11)	3 (1-12)	3.4(1-10)			2.3(0-8)			2.3(0-6)	
Vaccinated > 16 weeks ago	335	11.3(8-15)	6.9(4-10)	3.9(2-6)	4.5 (2-7)	3.6(2-6)		1.5(0-3)	3.3(2-5)			2.4(1-4)	
Bi-valent vaccine	42												
Quadrivalent vaccine	246												
Vaccinated < 16 weeks ago	42												

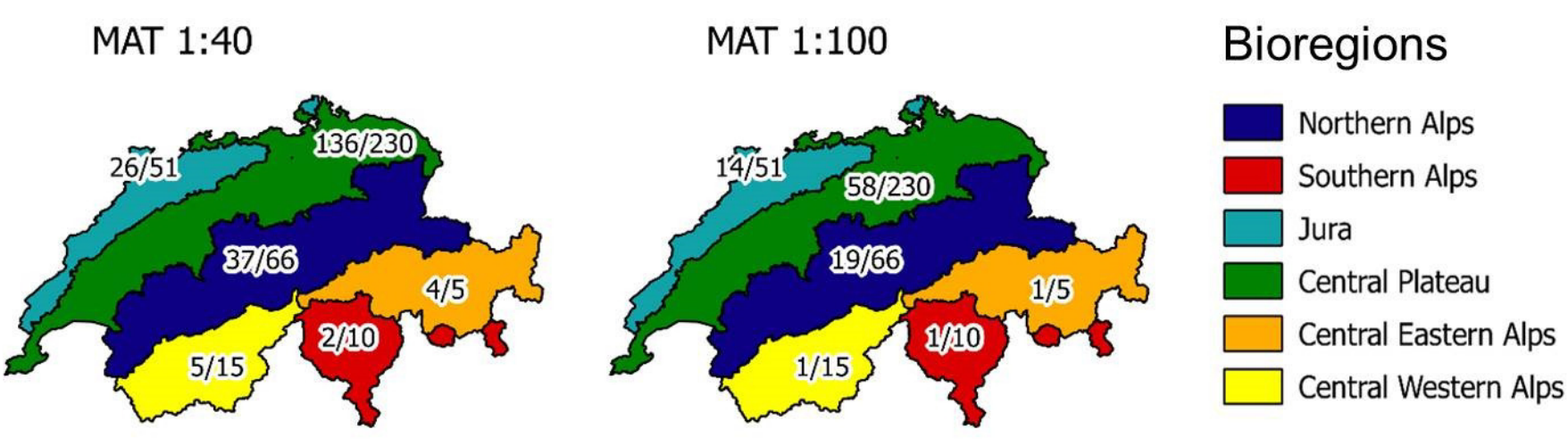


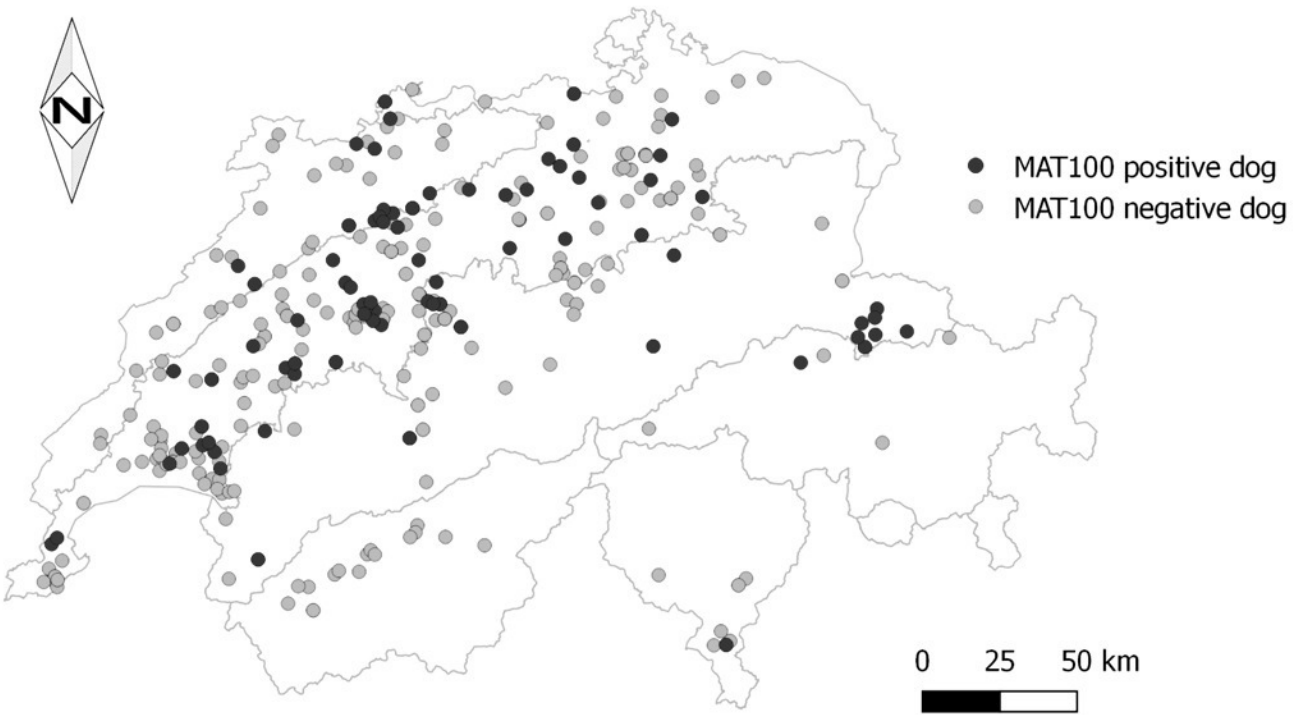
Prevalence

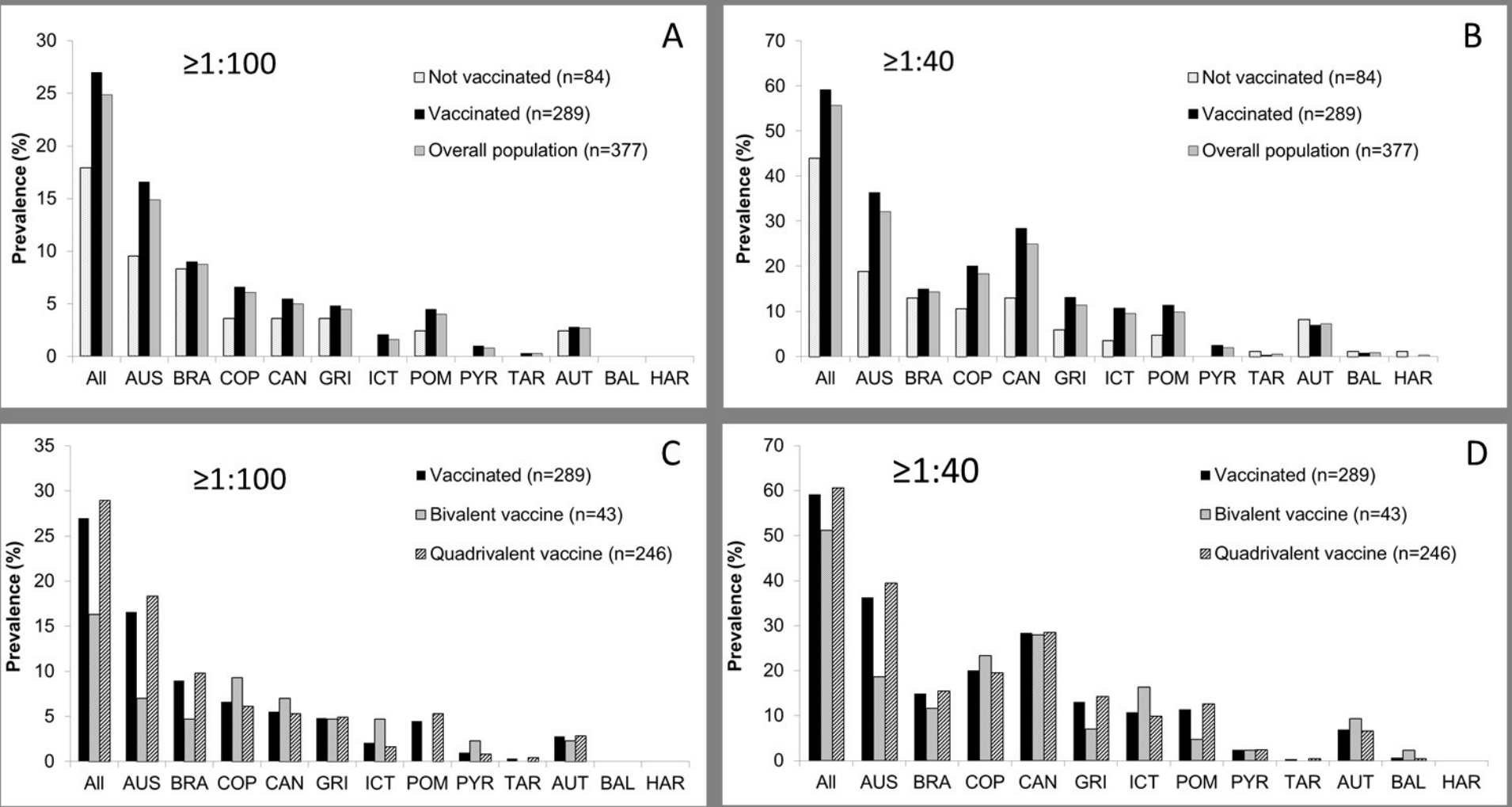
- MAT positivity: Calculated for overall population (A-D) and for subgroups A and B
- RT-PCR positivtiy: Calculated for overall population

Risk factor analysis

- **Outcome measures**
  - MAT positivity at ≥1:40 and ≥1:100 to any of 12 serovars tested
  - MAT positivity at ≥1:100 to individual serovars
- **Univariate analysis**
  - «Days since vaccination» introduced as independant variable to assess effect of vaccination on MAT positivity
  - Assessed association between outcome measures and each individual independent variable
  - Variables with likelyhood ratio test p value p≤0.2 entered into multivariate analysis
- **Multivariate analysis**
  - Six variables remained signficant after backward selection


MAT 1:40
26/51
136/230
37/66
4/5
2/10
5/15
MAT 1:100
14/51
58/230
19/66
1/5
1/10
1/15
Bioregions
Northern Alps
Southern Alps
Jura
Central Plateau
Central Eastern Alps
Central Western Alps


N
MAT100 positive dog
MAT100 negative dog
0
25
50 km


A
≥1:100
Not vaccinated (n=84)
Vaccinated (n=289)
Overall population (n=377)
Prevalence (%)
0
5
10
15
20
25
30
All AUS BRA COP CAN GRI ICT POM PYR TAR AUT BAL HAR
B
≥1:40
Not vaccinated (n=84)
Vaccinated (n=289)
Overall population (n=377)
Prevalence (%)
0
10
20
30
40
50
60
70
All AUS BRA COP CAN GRI ICT POM PYR TAR AUT BAL HAR
C
≥1:100
Vaccinated (n=289)
Bivalent vaccine (n=43)
Quadrivalent vaccine (n=246)
Prevalence (%)
0
5
10
15
20
25
30
35
All AUS BRA COP CAN GRI ICT POM PYR TAR AUT BAL HAR
D
≥1:40
Vaccinated (n=289)
Bivalent vaccine (n=43)
Quadrivalent vaccine (n=246)
Prevalence (%)
0
10
20
30
40
50
60
70
All AUS BRA COP CAN GRI ICT POM PYR TAR AUT BAL HAR