TOXOPLASMA GONDII INFECTION IN THE EURASIAN BEAVER (CASTOR FIBER) IN SWITZERLAND: SEROPREVALENCE, GENETIC CHARACTERIZATION, AND CLINICOPATHOLOGIC RELEVANCE

Authors: Scherrer, Patrick, Ryser-Degiorgis, Marie-Pierre, Frey, Caroline F., and Basso, Walter

Source: Journal of Wildlife Diseases, 60(1) : 126-138

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/JWD-D-23-00077

DOI: 10.7589/JWD-D-23-00077 *Journal of Wildlife Diseases*, 60(1), 2024, pp. 126–138

Toxoplasma gondii Infection in the Eurasian Beaver (*Castor fiber*) in Switzerland: Seroprevalence, Genetic Characterization, and Clinicopathologic Relevance

Patrick Scherrer,[1,2,3] Marie-Pierre Ryser-Degiorgis,[1] Caroline F. Frey,[2] and Walter Basso[2,3]

[1] Institute for Fish and Wildlife Health, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty University of Bern, Laenggassstrasse 122, 3012 Bern, Switzerland
[2] Institute of Parasitology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty University of Bern, Laenggassstrasse 122, 3012 Bern, Switzerland
[3] Corresponding authors (email: scherrer.patrick@hotmail.com, walter.basso@unibe.ch)

ABSTRACT: *Toxoplasma gondii* is a coccidian parasite able to infect all warm-blooded animals and humans. Rodents are one of the most important intermediate hosts for *T. gondii,* but little is known about infection in beavers and its clinical relevance. Toxoplasmosis was not considered an important waterborne disease until recently, but with increased outbreaks in humans and animals this perspective has changed. Serum samples from 247 Eurasian beavers (*Castor fiber*) collected from 2002 to 2022 were tested for antibodies to *T. gondii* by a commercial ELISA. Antibodies to *T. gondii* were found in 113 (45.8%) beavers. Higher weight and proximity to urban areas were found to be significant predictors for seropositivity. Additionally, *T. gondii* DNA was detected in 23/41 brain tissue samples by real-time PCR. Histopathologic examination of brain sections revealed inflammatory changes in 26/40 beavers, mainly characterized by encephalitis, meningitis, choroid plexitis, or a combination of them. In six of these cases the lesions were in direct association with parasitic stages. With an adapted nested PCR multilocus sequence typing and in silico restriction fragment length polymorphism analysis approach, three different *T. gondii* genotypes were detected in brain samples: the clonal Type II strain (ToxoDB 1), a Type II variant (ToxoDB 3), and a novel genotype exhibiting both Type II and I alleles in a further animal. *Toxoplasma gondii* infections in beavers have epidemiologic and clinical significance. The high seroprevalence indicates frequent contact with the parasite, and as competent intermediate hosts they may play an important role, contributing to maintaining the life cycle of *T. gondii* in semiaquatic habitats. In addition, although most beavers appear to develop subclinical to chronic disease courses, acute and fatal outcomes, mainly characterized by encephalitis and generalized infection, do also occur.

Key words: ELISA, encephalitis, genotyping, toxoplasmosis.

INTRODUCTION

Beavers (Rodentia, Castoridae) are the second largest living rodents, after the capybaras (*Hydrochoerus* spp.). The family Castoridae today consists of a single genus, *Castor*, which includes two species: the Eurasian beaver (*Castor fiber*), also called European beaver, and the Canadian beaver (*Castor canadensis*). In recent centuries, the Eurasian beaver was almost completely eradicated from vast areas in Europe, except for a few thousand specimens. Almost all of today's occurrences in Europe are due to reintroduction projects in the 20th century (Angst and Fasel 2021; Zahner et al. 2021). In Switzerland, the Eurasian beaver had completely disappeared and was reintroduced in 1956. The Swiss population amounts to approximately 4,900 individuals (latest estimate 2022; Angst et al. 2023). Since the releases, two separate populations have formed in the catchment areas of the Rhone and Rhine rivers. Today, the beaver mainly inhabits water bodies in the Swiss Plateau, as well as the Alpine Rhine valley (Angst and Fasel 2021). As the largest rodent in Europe, the beaver is herbivorous and feeds mainly on herbaceous plants in summer and bark and buds of woody plants in winter (Angst and Fasel 2021).

Toxoplasma gondii (*Apicomplexa*, *Sarcocystidae*) is a protozoan parasite that occurs worldwide. Intermediate hosts are practically all warm-blooded animals, including a wide variety of mammals and birds (Dubey 2022). Definitive hosts are members of the family Felidae, which in Switzerland are

represented by the domestic cat (*Felis catus*), the European wildcat (*Felis silvestris*), and the Eurasian lynx (*Lynx lynx*). *Toxoplasma gondii* causes one of the most common zoonotic diseases worldwide—toxoplasmosis in humans—and it is estimated that one third of the human population is infected (Halonen and Weiss 2013). *Toxoplasma gondii* exhibits a great genetic variability, which may be associated with different virulence phenotypes. Initially, three clonal archetypes, I, II, and III, were recognized, and these seem to prevail in the US and Europe, but in recent years, so-called nonclonal and variant or recombinant genotypes have been found (Grigg et al. 2001; Deiró et al. 2021). Those nonclonal genotypes are sometimes associated with more severe clinical disease in humans and animals (de-la-Torre et al. 2013; Hamilton et al. 2019; Pardini et al. 2019; Casartelli-Alves et al. 2021).

Rodents are among the most important intermediate hosts for *T. gondii*, as they are frequent prey of cats, which in turn shed infectious oocysts with their feces into the environment. Additionally, *T. gondii* is thought to influence rodent behavior (e.g., reduced fear of cats), which contributes to cycle maintenance (Webster 2001; Pardo Gil et al. 2023). Response to infection depends on different factors related to the host (e.g., species, age, immune status, coinfections with other agents) and the parasite (e.g., infection dose, parasitic stage, genetic background; Hassan et al. 2019; Stelzer et al. 2019; Dubey 2022). For example, strains from the Type I lineage are considered lethal for laboratory mice, whereas for rats they do not seem to be pathogenic. Type II and III lineages are commonly less virulent to mice, whereas some new nonclonal genotypes in Brazil caused lethal infections (Pena et al. 2008).

Little is known about *T. gondii* infection and toxoplasmosis in beavers. Of the three published studies investigating seroprevalence, two studies in North America on Canadian beavers reported seroprevalences ranging from 7% to 10% (Smith and Frenkel 1995; Jordan et al. 2005), and a study from the Netherlands on Eurasian beavers documented a seroprevalence of 6% (Maas et al. 2022). There are also very few studies on the clinical relevance of *T. gondii* infection in beavers. In one Canadian beaver, systemic toxoplasmosis was found to be the cause of death (Forzán and Frasca 2004). One study from Germany isolated *T. gondii* from tissues of two beavers, and in one of these animals, histopathologic changes mainly characterized by encephalitis associated to the presence of tachyzoites were found and were consistent with the death of the animal (Herrmann et al. 2013). In recent years, *T. gondii* infections have also been detected in Swiss beavers, and encephalitis was observed in some animals (Zürcher-Giovannini 2018), raising the question of the clinical relevance of infection in this animal species and of the role of beavers in *T. gondii* epidemiology.

Until recently, unlike *Cryptosporidium* and *Giardia* spp., *T. gondii* was not considered to be a significant waterborne pathogen, but several outbreaks in humans have led to a growing concern (Bowie et al. 1997; Keenihan 2002; Minuzzi et al. 2021). Additionally, in southern sea otters (*Enhydra lutris nereis*) in California, US, mass mortalities have been associated with *T. gondii*–contaminated freshwater runoff into coastal waters (Miller et al. 2002). Other rodent species with a semiaquatic lifestyle similar to beavers are the nutria (*Myocastor coypus*) and the muskrat (*Ondatra zibethicus*). Both have been used as sentinel species in serologic studies for *T. gondii*. Factors such as proximity to cities, agricultural land, or the amount of municipal waste in waterbodies have been assumed to play an important role for infection (Hejlícek et al. 1997; Ahlers et al. 2015, 2020; Zanzani et al. 2016). Given that beavers are mainly distributed along the water bodies of the Swiss Plateau, they are often located close to settlements and agricultural land and thus in potentially oocyst-contaminated areas.

The aims of our study were to investigate the epidemiologic role of the Eurasian beaver in the life cycle of *T. gondii* as an intermediate host and the clinical relevance of infection. For this, we combined several approaches: a seroprevalence study, assessment of risk factors for seropositivity, and parasite detection

TABLE 1. Percentage of Eurasian beaver (*Castor fiber*) serum samples collected in Switzerland, 2002 to 2022, positive for antibodies against *Toxoplasma gondii* (apparent prevalence) on ELISA by variable category, and odds ratios (OR) with 95% confidence intervals (CIs) and *P* values based on univariate testing.

Variable	N_{Pos}/N_{Tot}[a]	Apparent prevalence (95% CI)	OR (95% CI)	*P* value
Sex				
Male	58/131	44.3 (35.8–52.8)	Reference	0.621
Female	55/116	47.4 (38.3–56.5)	0.90 (0.62–1.30)	
Age				
Adult	88/170	51.7 (44.3–59.3)	Reference	0.005
Juvenile	25/77	32.5 (22.0–42.9)	0.45 (0.25–0.78)	
Urban area				
Outside	47/126	37.3 (28.9–45.8)	Reference	0.007
Within	65/119	54.6 (45.7–63.6)	2.02 (1.22–3.39)	
Weight	NA[a]	NA	NA	<0.0002

[a] N_{Pos} = no. positive; N_{Tot} = no. total; NA = not applicable.

by molecular and histologic methods, as well as genetic characterization of the detected parasites.

MATERIALS AND METHODS

Study area and sample collection

Switzerland is a small country (41,285 km^2) in central Europe and is home to numerous inland waters and watercourses that flow into various seas. The Rhine and the larger Rhine tributaries (Aare, Reuss, Limmat) in the east, northeast, and Swiss Plateau, as well as the Rhone in the canton of Valais, are of particular importance for the distribution area of the beaver. We obtained samples from beaver carcasses submitted by game wardens within the frames of the general surveillance program of wildlife health in Switzerland (Ryser-Degiorgis and Segner 2015) and of a beaver health surveillance project. Surveillance was intensified from 2002 and a detailed necropsy and sampling protocol was established at the Institute for Fish and Wildlife Health (FIWI) in collaboration with the beaver consultancy office. We included 247 dead beavers found 2002–2022, from which we obtained serosanguinous fluid samples from the heart and thoracic cavity. We centrifuged these samples to obtain sera, which were then stored at −20 C until further analysis. During necropsies, we collected brain samples (n=41) whenever possible. A set of metadata was compiled including sex, age, weight, body condition, coordinates of the finding site, and proximity to urban areas. We classified beavers into two age categories based on body size, weight, and body condition (Dežkin and Safonov 2004): juveniles (0–12 mo) and adults ≥1 yr old (cutoff at 16.95 kg in good body condition). The weight was taken in kilograms with a commercial scale, except for four animals. To investigate whether beavers were found in the immediate vicinity of humans (within/outside urban areas), we compared the coordinates of the finding sites with a map provided by the mapping platform of the Swiss Confederation (Swiss Confederation 2022) depicting the urban areas of Switzerland, generated with information from the Federal Statistics Office. Because of missing coordinates, the location of two animals could be assigned on only a cantonal level.

We drew the maps with the free software QGIS3 (QGIS.org 2017). Sample composition is provided in Supplementary Material (Supplementary Table 1).

Serologic testing

We tested all 247 sera with a commercially available multispecies ELISA kit (ID Screen Toxoplasmosis Indirect, ID vet, Grabels, France), designed to detect specific antibodies against *T. gondii* in serum, plasma, or meat juice from different animal species, including dogs, cats, goats, sheep, cattle, and pigs (TOXO-MS). ELISA plates were precoated with cell culture–derived *T. gondii* tachyzoite antigen (TgSAG1/P30). The multispecies conjugate to be used was supplied in the kit. According to the manufacturer's instructions, a sample to positive ratio (S/P ratio) based on the optical density of the samples was calculated for each serum. Samples

with an S/P% ≤40% were considered negative, 40%<S/P%<50% inconclusive, and S/P% ≥50% positive. We included internal serum controls from a pig experimentally infected with *T. gondii* oocysts and from a seronegative control pig (Basso et al. 2017) in each plate.

DNA extraction and real-time (quantitative) PCR

We extracted DNA from fresh brain tissue samples as follows: 500 mg of tissue was homogenized with 900 µL Buffer ATL and 100 µL proteinase K and incubated overnight at 56 C. We then used 200 µL of the homogenate to complete further DNA extraction steps as indicated by the manufacturer (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany).

We performed a Taq-Man–based quantitative PCR (qPCR) in a CFX96 qPCR instrument (Bio-Rad Laboratories AG, Cressier, Switzerland) to detect and quantify *T. gondii* DNA from a subset of fresh brain samples (n=41). We used the protocol from previous research (Scherrer et al. 2023) to target the 529-bp repetitive genomic sequence of *T. gondii* with a specific Taq-Man–based real-time qPCR and analyzed the PCR results with the CFX manager software version 1.6 (Bio-Rad).

Histology

Since 2012 the FIWI has systematically examined brain macroscopically and collected samples for histologic examination and archiving at −20 C. Histologic specimens were fixed in 4% buffered formalin, embedded in paraffin, stained with H&E, and further examined by light microscopy. For this study we included and screened only histologic specimens from which real-time PCR results from brain tissue were available (40/41; in one animal no histologic examination was performed).

In the H&E-stained histologic specimens, we looked for hallmarks including encephalitis, meningitis or meningoencephalitis, gliosis, tachyzoite aggregations, and tissue cysts containing bradyzoites.

Nested PCR multilocus sequence testing and in silico restriction fragment length polymorphism

We performed strain typing with the widely known PCR restriction fragment length polymorphism (RFLP) method using a multilocus nested PCR (Mn-PCR) amplification of 10 known genetic markers of *T. gondii* (SAG1, SAG2 [5′-SAG2, 3′-SAG2 and alt. SAG2 amplified separately], SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico; Su and Dubey 2020). We adapted the protocol proposed by Su and Dubey (2020) by using a Multiplex PCR Kit (Qiagen) including a HotStarTaq DNA polymerase. The PCR products were purified and sequenced in both directions with the internal primers used in the previous Mn-PCR protocol (Mycrosynth, Balgach, Switzerland). To create alignments for analysis, we imported the resulting sequences into the Geneious Prime software version 2022.2.1 (Biomatters Inc. 2022). To obtain genotype specific fragment patterns we used an online program for in silico digestion of the sequences with specific enzymes (NEBcutter V2.0; Vincze et al. 2003; Castro et al. 2020). We assigned RFLP genotype numbers according to the ToxoDB database (Harb and Roos 2020).

Phylogenetic analysis

Toxoplasma gondii population structure was evaluated through DNA sequence–based analyses. To generate an unrooted phylogenetic tree, all SAG3 consensus sequences and reference sequences were aligned using MEGA X software (version 11.0.13; Kumar et al. 2018). We inferred an evolutionary history using the neighbor-joining method.

Data analyses and statistics

Apparent seroprevalence was estimated based on the results obtained by ELISA and calculated with a free online sample size tool (Kohn and Senyak 2021). Parasite detection was considered positive when *T. gondii* DNA could be amplified from thawed tissues.

For statistical analyses we used the R program (R Core Team 2022). Level of significance was set at 0.05. To identify potential risk factors for seropositivity we first performed univariate comparisons between serology results (positive vs. negative) and explanatory variables such as sex (male, female), age (juvenile, adult), urban areas (within, outside) and weight (in kilograms). When explanatory variables consisted of two categories (i.e., sex, age, urban areas) we applied Pearson's chi-square test. For continuous nonnormally distributed data (weight) we used Spearman's rank correlation. In a next step, we investigated the effects of sex, weight, and occurrence in an urban area on the serology results by fitting a generalized linear model. We did

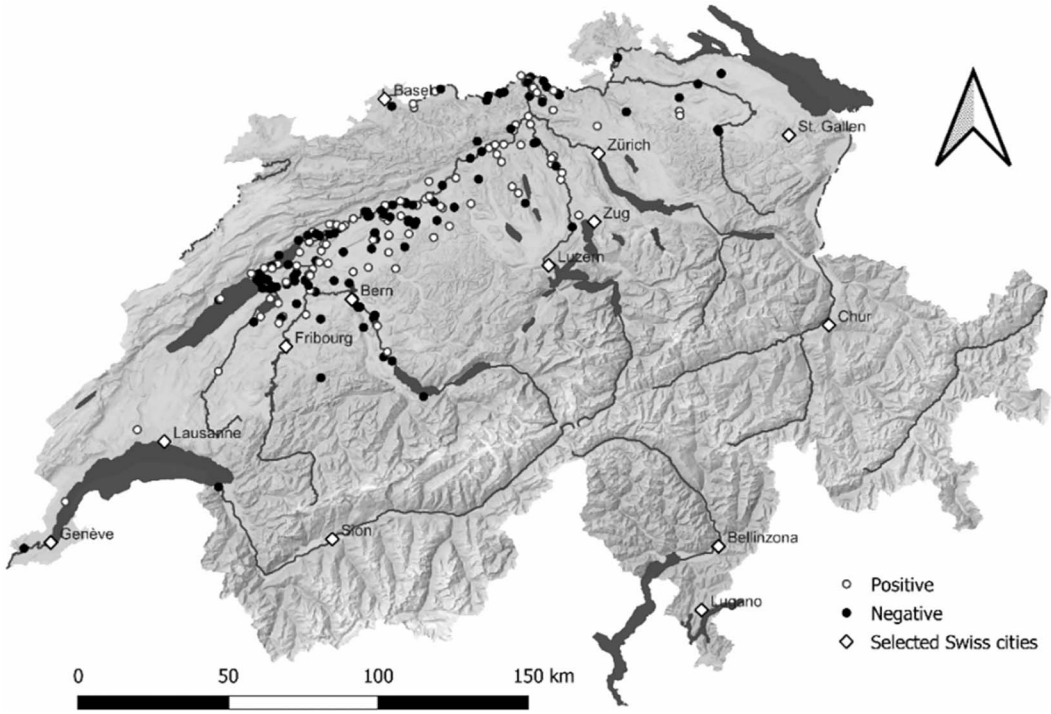

FIGURE 1. Map of Switzerland showing the geographical distribution of Eurasian beavers (*Castor fiber*) tested for antibodies against *Toxoplasma gondii*, and the serologic results.

not include age itself in the model because it was partially determined by the animal weight and because the factor age might act as a confounder rather than a causal factor (Opsteegh et al. 2012). To select the best model, Akaike's information criterion (AIC) was used considering that models with a ΔAIC of 0–2 provide similar support (Anderson et al. 2001). For a subset of samples, we performed only univariate comparison between serology and PCR results (Fisher's exact test) and PCR results and occurrence of encephalitis in histology (Pearson's χ^2 test).

RESULTS

Serology

Overall, 113/247 (45.8%; 95% CI, 39.5–52.0%) sera tested positive for specific anti–*T. gondii* IgG antibodies according to the manufacturer's thresholds of the ELISA kit. None of the samples had an inconclusive result. The Switzerland-wide distribution of seropositive and seronegative individuals along the Swiss water bodies is shown in Figure 1. Apparent prevalences according to the different explanatory variables are shown in Table 1. Univariate comparison showed a highly significant difference ($P<0.001$) with the factor weight and a significant difference regarding factors age ($P=0.005$) and proximity to urban areas ($P=0.007$). Sex did not show a significant effect ($P=0.621$). In the best-fitting generalized linear model, "weight" and "urban area" were retained as significant ($P<0.05$) predictors of seropositivity (Table 2). Heavier animals and animals found within an urban area were more likely to be seropositive. When looking at a subset of animals with PCR results available, univariate comparison revealed a strong association ($P<0.001$) between seropositivity and PCR positive results for *T. gondii* DNA in brain tissue (Table 3). The geographical distribution of beavers for which both serology and PCR results were available is shown in Figure 2.

Real-time PCR on tissue samples and histologic examination

For brain samples, 23/41 (56%; 95% CI, 41–71%) tested positive for *T. gondii* DNA by

TABLE 2. Parameters of the best generalized linear model obtained for *Toxoplasma* gondii serology of Eurasian beaver (*Castor fiber*) from Switzerland, 2002 to 2022, and odds ratios (ORs) with 95% confidence intervals (CIs) and *P* values based on multivariate testing.

Model	Term	OR (95% CI)	*P* value
Mode: serology	Intercept	0.100 (0.037–0.252)	<0.001
Urban area+weight	Urban area (within)	2.467 (1.432–4.324)	0.001**
	Weight	1.112 (1.062–1.181)	<0.001***
Akaike's information criterion: 314.1			

** $P<0.01$.
*** $P<0.001$.

real-time PCR. We found inflammatory changes (i.e., encephalitis, meningitis, choroid plexitis, or a combination of these) in 26/40 histologic specimens and observed parasitic stages (tachyzoite aggregations or tissue cysts) in 6/26 samples (examples in Fig. 3).

Real-time PCR of the corresponding fresh brain samples was positive for all six specimens in which parasites were detected and for an additional 11 samples without visible parasite stages. Univariate comparison showed a mild correlation ($P<0.05$) between positive PCR results and histopathologic findings of encephalitis (Table 3).

Nested PCR multilocus sequence testing and in silico RFLP

We successfully amplified and sequenced all 10 markers (including the three separately amplified SAG2 regions) from 22/23 brain samples that were positive by real-time PCR. Final allele patterns for all the samples are shown in Table 4. Two samples corresponded to the clonal Type II (ToxoDB 1), 19 samples to a lineage II variant strain (ToxoDB 3), and one sample revealed a novel genotype (data submitted to ToxoDB for inclusion) with alleles corresponding to allele Type II for nine markers and allele Type I for two markers (alt. SAG2, Apico). Sequencing revealed single-nucleotide polymorphisms (SNPs) in 21 analyzed sequences (whole amplified sequences including primers were considered to define the SNP positions). Concerning marker SAG3, 17 of 21 sequences showed 100% identity, with isolates having an SNP at position 187 with a change from G to T compared with the clonal Type II reference strain (ME49). Another SAG3 marker sample featured an SNP at location 153 with a change from G to C that did not show 100% homology with any sequence deposited on GenBank. A sequence from the marker alt. SAG2 had an SNP at bp 118. The affected site is a known interface for the enzyme TaqI in the RFLP assay. The reference sequences, both ME49 and VEG, have a TCGA nucleotide sequence at this location, which in our sample had a TCAA sequence. According to the RFLP cut pattern, this sequence is attributed to the Type I allele, but phylogenetically the sequence was more closely related to the Type II reference sequence ME49 (Fig. 4). In two PK1 sequences there was an SNP (bp 816) with a nucleotide change from A to G compared with the reference (ME49). Respective sequences are provided with GenBank accession numbers in Table 4.

TABLE 3. Comparison of *Toxoplasma gondii* PCR results on brain samples collected from Eurasian beavers (*Castor fiber*) in Switzerland, 2002 to 2022, with serologic status and histologic observation of encephalitis. *P* values based on univariate testing are indicated.

	Serology		Encephalitis	
	Positive	Negative	Yes	No
PCR				
Positive	21	2	18	5
Negative	0	18	8	9
P value	<0.0005		<0.05	

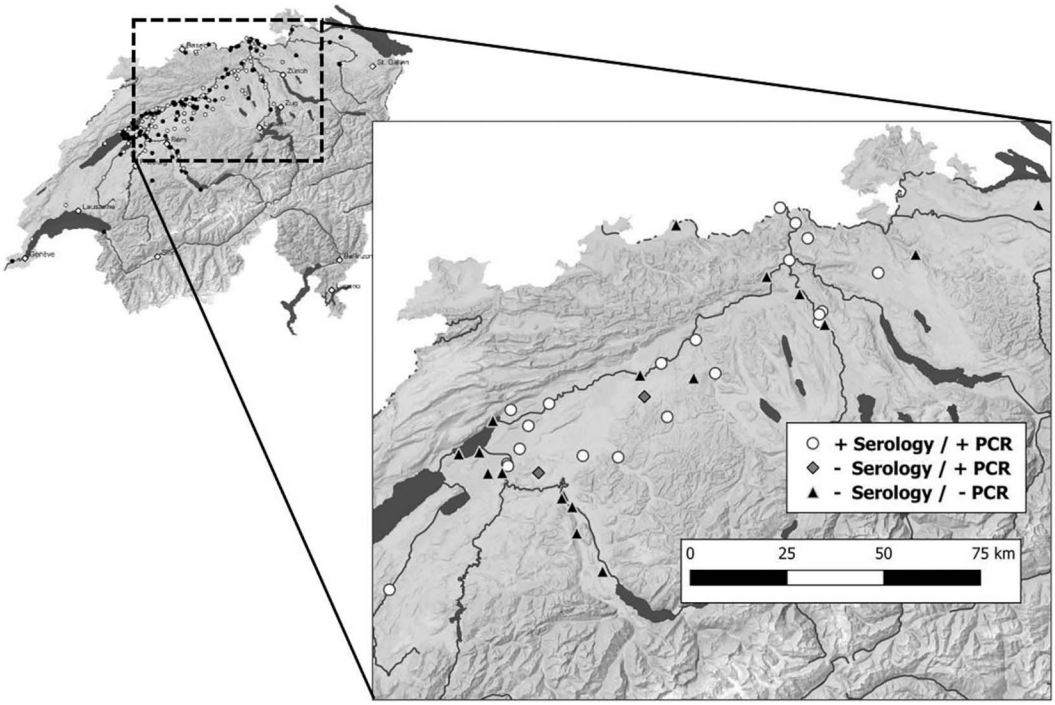

FIGURE 2. Map showing the distribution of Eurasian beaver (*Castor fiber*) samples from Switzerland, 2002–2020, indicating the origins of beavers that were tested for antibodies against *Toxoplasma* gondii and those for which their tissues were tested for *T. gondii* DNA by quantitative PCR. Positive serology and PCR results are indicated by (+) and negative results by (−).

DISCUSSION

With an estimated apparent prevalence of 45.8%, beavers in Switzerland show a higher prevalence than reported, for example, in beavers in the Netherlands (6%; Maas et al. 2022) or in Canadian beavers in Missouri-Kansas and Massachusetts (7% and 10% respectively; Smith and Frenkel 1995; Jordan et al. 2005), suggesting a higher exposure of beavers to *T. gondii* in Switzerland. In some of the mentioned studies, a different detection method was used (modified agglutination test); therefore, the results cannot be directly compared. Although the commercial ELISA used in this study has not been validated for beaver, the clearly distinguishable values of positive and negative results (Fig. 5) are a good indication that the method works for the detection of antibodies in beaver. Furthermore, there was a strong correlation between seropositive animals and positive PCR results from the brain when available. This is interesting because it is not always possible to detect the parasites in the tissues of seropositive animals (Glor et al. 2013).

Our finding of positive association between seropositivity and weight has also been seen in a survey of rats from France, and suggests postnatal transmission (Ayral et al. 2015). This is also reflected by the fact that adult animals were more likely to be seropositive, which is explained by an increasing cumulative likelihood of exposure during an animal's lifetime and long-lasting antibody persistence (Zarnke et al. 2001; Roelke et al. 2008; Opsteegh et al. 2012; Basso et al. 2020, 2022).

A major issue with *T. gondii* in (semi)aquatic ecosystems is the route of transmission. Because beavers are herbivorous, we suspect that they become infected directly through water or plants contaminated with oocysts. Lindsay et al.

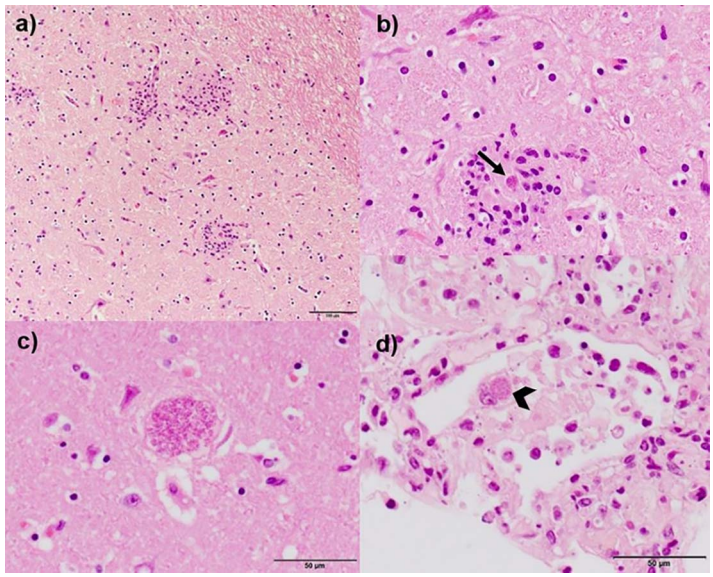

FIGURE 3. Histologic depiction of different stages of *Toxoplasma* infection in Eurasian beavers (*Castor fiber*) from Switzerland. Systemic toxoplasmosis in a single beaver, ID W22_6329 (a, b, and d), and a single tissue cyst in another individual (c). (a) H&E-stained brain tissue with multiple foci of glial cells. (b) Zoomed-in glial nodule with a central cluster of tachyzoite aggregation (arrow). (c) Single tissue cyst in brain tissue without surrounding inflammatory cell infiltration. (d) Tachyzoite aggregation in severely inflamed lung tissue (arrowhead).

(2003) demonstrated that oocysts remained viable for several months in seawater and were able to infect marine mammals (van de Velde et al. 2016; Dubey et al. 2020). In some cases, in both humans and animals, toxoplasmosis outbreaks have been linked to oocyst-contaminated fresh water (e.g., creeks and streams), or water reservoirs, which also confirms viability in freshwater systems (Bowie et al. 1997; Aramini et al. 1999; Keenihan 2002). Often these systems were equipped with inadequate filtration systems, but various methods of water treatment sometimes fail to eliminate oocysts (Dubey 2022). Beavers in our study found within an urban area had a higher probability of being seropositive; such associations between urban areas and higher *T. gondii* seroprevalence have also been found in otters (*E. lutris nereis*), muskrats, and nutrias (Miller et al. 2002; Ahlers et al. 2015; Zanzani et al. 2016). This may be explained by the higher cat density in regions highly populated by humans and thus more oocysts in the environment. Modified drainage systems used in agriculture and urbanized areas have been associated with facilitating transport of *T. gondii* into freshwater ecosystems (Ahlers et al. 2015). In comparison, in a study in Minnesota, far away from most human influences, no antibodies against *T. gondii* were detected in various populations of muskrats (Ahlers et al. 2020). To accurately estimate the risk of oocyst ingestion, additional research on the environmental contamination burden is necessary. It would be interesting to attempt to detect *T. gondii* DNA directly from the water in affected areas. The lack of standardized assays for detection of *T. gondii* oocysts in water bodies may have contributed to an underestimation of the role that oocysts play in the epidemiology of *T. gondii* (Shapiro et al. 2019).

In most of the *T. gondii*–positive brains analyzed in our study, encephalitis was mild and of chronic nature. This suggests that infected beavers tend to develop subclinical and chronic disease courses. The cases with acute encephalitis were usually beavers with systemic toxoplasmosis, that is, with involvement of other organs such as the lungs, heart,

TABLE 4. Genotypes of *Toxoplasma gondii* isolated from brain tissue collected from 2002 to 2022 from Eurasian beavers (*Castor fiber*) in Switzerland. The first column shows the sample ID. Remaining columns show the in silico restriction fragment length polymorphism results for each marker with the assigned ToxoDB number (last column).[a]

Sample ID	SAG1	5′-SAG2	3′-SAG2	alt. SAG2	SAG3	GRA6	BTUB	c22-8	c29-2	L358	PK1	Apico	ToxoDB genotype
W12_3538	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W12_3907	II or III	I or II	II	II	II	II	II	II	II	II	II	I	3
W12_4022	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W12_4326	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W13_6469	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W14_1313	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W14_1982	II or III	I or II	II	II	II	II	II	II	II	II	II	I	3
W16_0760	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W17_4300	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W18_3392	II or III	I or II	II	II	II	II	II	II	II	II	II	I	3
W18_4632	II or III	I or II	II	II	II[b]	II	II	II	II	II	II[c]	I	3
W19_1541	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W19_1678	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W19_1706	II or III	I or II	II	II	II[b]	II	II	II	II	II	II[c]	I	3
W19_2287	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W19_2468	II or III	I or II	II	II	II[d]	II	II	II	II	II	II	I	3
W19_2565	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	II	1
W21_1294	II or III	I or II	II	I[e]	II[b]	II	II	II	II	II	II	I	novel
W21_1498	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W21_2282	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W22_6002	II or III	I or II	II	II	II[b]	II	II	II	II	II	II	I	3
W22_6329	II or III	I or II	II	II	II	II	II	II	II	II	II	II	1

[a] GenBank accession numbers of the marker sequences obtained in this study: SAG1: OQ402826-OQ402847; 3′-SAG2: OQ402870-OQ402891; 5′-SAG2: OQ402848-OQ402869; alt. SAG2: OQ402892-OQ402913; SAG3: OQ402980-OQ403001; BTUB: OQ402958-OQ402979; GRA6: OQ402804-OQ402825; c22-8: OQ402738-OQ402759; c29-2: OQ402760-OQ402781; L358: OQ402782-OQ402803; PK1: OQ402914-OQ402935; Apico: OQ402936-OQ402957.

[b] SNP in position 187 respective to *T. gondii* reference strain ME49, which implies a substitution of guanine in place of thymine.

[c] SNP in position 816 respective to *T. gondii* reference strain ME49, which implies a substitution of adenosine in place of guanine.

[d] SNP in position 153 respective to *T. gondii* reference strain ME49, which implies a substitution of guanine in place of cytosine.

[e] Single-nucleotide polymorphism (SNP) in position 118 respective to *T. gondii* reference strains RH, ME49 and VEG, which implies a substitution of thymine in place of guanine.

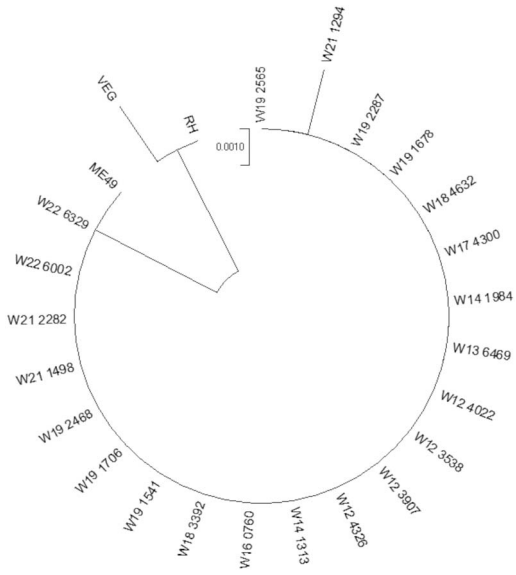

FIGURE 4. Phylogenetic network analysis of *Toxoplasma gondii* isolates from Eurasian beavers (*Castor fiber*) from Switzerland, 2002–2020, regarding marker SAG3. RH=clonal Type I, ME49=clonal Type II, VEG=clonal Type III, WXX XXXX=sample ID beaver.

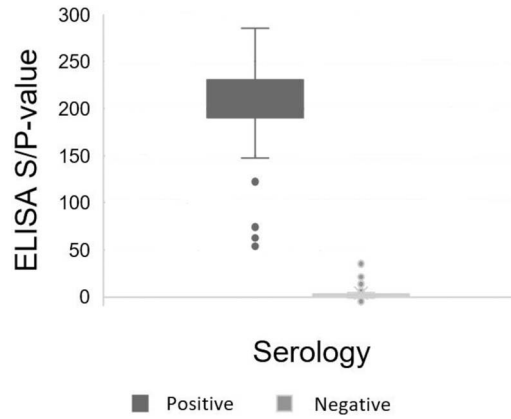

FIGURE 5. ELISA sample to positive (S/P) ratio values for positive and negative serum samples from Eurasian beavers (*Castor fiber*) from Switzerland, 2002–2020.

or liver; with inflammatory and necrotic changes; and with presence of tachyzoites associated to these lesions. Such changes were seen in the histology of a case from 2022 (ID W22_6329; Fig. 3). The pathologic changes are not new to Swiss beavers, as they have been reported associated with *T. gondii* infection in a previous study on beaver health (Zürcher-Giovannini 2018). Documented cases of *T. gondii* infection in beavers exist at the FIWI only from 2012 onwards; this probably does not indicate that toxoplasmosis is an emerging disease in the area, but rather is explained by the fact that previously beaver carcasses, especially skulls, were spared for later preparation and brain tissue was not routinely examined. Deaths due to clinical toxoplasmosis have also been reported in cases from Germany (free-ranging beaver; Herrmann et al. 2013) and the US (captive *C. canadensis*; Forzán and Frasca 2004).

It is difficult to assess to what extent the observed histopathologic changes affected the life of beavers. In our study population the most common cause of death was trauma (Ryser-Degiorgis and Zürcher, pers. comm.). It may be that encephalitis affects the behavior or responsiveness of the animal; for example, sea otters were observed to be significantly more likely to be attacked and killed by sharks if they had concurrent moderate to severe encephalitis caused by *T. gondii* (Kreuder et al. 2003). Loss of aversion towards predators has been described in small rodents such as mice and rats (Vyas et al. 2007) but not yet in beavers. When considering that this change in behavior helps the parasite to be ingested by the final host, the beaver seems likely to be of lesser importance in this role: Domestic cats, wild cats, and Eurasian lynx do not prey on beaver, although in rare cases they might ingest beaver meat through scavenging. Scavenging of dead beavers may play a role in the maintenance of the life cycle among intermediate hosts, (e.g., wild canids, mustelids, scavenging birds). Histopathologic examination is not a sensitive method to detect *T. gondii* because parasitic stages in tissues are generally scarce, especially during the chronic phase of infection, and their distribution is not homogeneous. In some of our cases we were able to clearly distinguish between acute infections (tachyzoite aggregates) and chronic infections (tissue cysts). Conversely, in many other samples we were not able to see the parasites in tissue sections, only the inflammatory reaction to their presence. In such cases the diagnostic value of PCR was shown.

We detected most frequently ToxoDB genotype 3 (in 19/22 positive samples). Within the different marker sequences, we detected SNPs that further distinguish the type. We found most frequently the mutation in marker SAG3. In Switzerland, this mutation has also been detected in a lynx (OQ230332; Scherrer et al., 2023) and different voles (OQ108542–108544; Pardo Gil et al. 2023). ToxoDB 3 is generally considered to be weakly virulent in mice; however, fatalities caused by this genotype have been reported in other species (Spycher et al. 2011; Jokelainen 2012). In our study, 14/19 beavers with this genotype had encephalitis, and in five of them toxoplasmosis was assumed to be the cause of death, showing that clinical toxoplasmosis can be an important disease in beavers.

In Spain, a flock of sheep suffered an abortion storm due to *Toxoplasma* from this genotype exhibiting the same mutation (Fernández-Escobar et al. 2020). In contrast to that Spanish study, we did not see a clear differentiation of ToxoDB 3 from ToxoDB 1 (ME49) due to this mutation.

Two animals infected with *T. gondii* of a ToxoDB 3 genotype exhibiting the mentioned mutation in the SAG3 sequence had an additional mutation in the PK1marker sequence. These two beavers were found in close proximity to each other 6 mo apart, suggesting a local variation of this genotype. The 22 genotyped samples date from 2012 to 2022, showing that genotypes (in our case mainly ToxoDB genotype 3) may persist in a region for several years, sometimes passing on these distinct mutations.

One animal showed a mutation in the alt. SAG2 marker, which resulted in an allele pattern not yet described (ID W21_1294, OQ402909). Although a Type I allele was present, which in principle is considered more virulent (Behnke et al. 2011), pathology did not differ compared with animals infected with other genotypes. However, a more detailed investigation of pathogenicity in this case could not be performed because DNA was isolated from frozen material and a bioassay was not possible.

Our findings confirm that toxoplasmosis is a relevant disease in beavers and that they are able to act as an intermediate host for *T. gondii*, in which role they may contribute to further parasite spread in semiaquatic habitats. The high seroprevalence indicates frequent contact with the parasite, and most beavers appear to develop subclinical to chronic disease courses, though acute and fatal outcomes also occur. Transmission through water contaminated with oocysts seems likely, which opens the floor for further research.

ACKNOWLEDGMENTS

We would like to thank the Swiss cantonal hunting authorities, game wardens and hunters for submitting beaver carcasses for necropsy, all FIWI collaborators who compiled data and collected samples during necropsy and the diagnostic staff of the Institute of Parasitology for their excellent technical assistance in the laboratory, and especially Gaston More for his help with genotyping.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-23-00077.

LITERATURE CITED

Ahlers AA, Mitchell MA, Dubey JP, Schooley RL, Heske EJ. 2015. Risk factors for *Toxoplasma gondii* exposure in semiaquatic mammals in a freshwater ecosystem. *J Wildl Dis* 51:488–492.

Ahlers AA, Wolf TM, Aarrestad O, Windels SK, Olson BT, Matykiewicz BR, Dubey JP. 2020. Survey of *Toxoplasma gondii* exposure in muskrats in a relatively pristine ecosystem. *J Parasitol* 106:346–349.

Anderson DR, Link WA, Johnson DH, Burnham KP. 2001. Suggestions for presenting the results of data analyses. *J Wildl Manag* 65:373–378.

Angst C, Auberson C, Nienhuis C. 2023. *Biberbestandeserhebung 2022 in der Schweiz und Liechtenstein [Beaver population survey 2022 in Switzerland and Liechtenstein]*. info fauna–Biberfachstelle, Neunenberg, Switzerland, and Format AG, Zürich, Switzerland, 140 pp.

Angst C, Fasel M. 2021. Europäischer Biber. In: *Atlas der Säugetiere: Schweiz und Liechtenstein [Atlas of mammals: Switzerland and Lichtenstein*], Graf RF, Fischer C, editors. Haupt Verlag, Bern, Switzerland, pp. 346–351.

Aramini JJ, Stephen C, Dubey JP, Engelstoft C, Schwantje H, Ribble CS. 1999. Potential contamination of drinking water with *Toxoplasma gondii* oocysts. *Epidemiol Infect* 122:305–315.

Ayral F, Artois J, Zilber A-L, Widén F, Pounder KC, Aubert D, Bicout DJ, Artois M. 2015. The relationship between socioeconomic indices and potentially zoonotic pathogens carried by wild Norway rats: A survey in Rhône, France (2010–2012). *Epidemiol Infect* 143:586–599.

Basso W, Grimm F, Ruetten M, Djokic V, Blaga R, Sidler X, Deplazes P. 2017. Experimental *Toxoplasma gondii* infections in pigs: Humoral immune response, estimation of specific igg avidity and the challenges of reproducing vertical transmission in sows. *Vet Parasitol* 236:76–85.

Basso W, Sollberger E, Schares G, Küker S, Ardüser F, Moore-Jones G, Zanolari P. 2020. *Toxoplasma gondii* and *Neospora caninum* infections in South American camelids in Switzerland and assessment of serological tests for diagnosis. *Parasit Vectors* 13:256.

Basso W, Holenweger F, Schares G, Müller N, Campero LM, Ardüser F, Moore-Jones G, Frey CF, Zanolari P. 2022. *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted foetuses. *Food Waterborne Parasitol* 28:e00176.

Behnke MS, Khan A, Wootton JC, Dubey JP, Tang K, Sibley LD. 2011. Virulence differences in *Toxoplasma* mediated by amplification of a family of polymorphic pseudokinases. *Proc Natl Acad Sci U S A* 108:9631–9636.

Biomatters Inc. 2022. *Geneious Prime*, v2022.1.1. https://www.geneious.com/download/previous-versions/. Accessed September 2022.

Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, Marion SA, for the BC *Toxoplasma* investigation team. 1997. Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet* 350:173–177.

Casartelli-Alves L, Pereira SA, Ferreira LC, de Macedo Couto R, Schubach TMP, Amendoeira MRR, da Silva RC, Langoni H, Millar PR, Menezes RC. 2021. Genetic and histopathological characterization of *Toxoplasma gondii* genotypes isolated from free-range chickens reared in the metropolitan region of Rio de Janeiro state, Brazil. *Parasitol Res* 120:665–677.

Castro BBP, Gennari SM, Lorenzi H, Su C. 2020. A simple method to generate PCR-RFLP typing profiles from DNA sequences in *Toxoplasma gondii*. *Infect Genet Evol* 85:104590.

Deiró AGJ, do Prado DP, Sousa IP, Rocha DS, Bezerra RA, Gaiotto FA, Albuquerque GR, Munhoz AD. 2021. Presence of atypical genotypes of *Toxoplasma gondii* isolated from cats in the state of Bahia, northeast of Brazil. *PLoS One* 16:e0253630.

de-la-Torre A, Sauer A, Pfaff AW, Bourcier T, Brunet J, Speeg-Schatz C, Ballonzoli L, Villard O, Ajzenberg D, et al. 2013. Severe South American ocular toxoplasmosis is associated with decreased IFN-γ/IL-17a and increased IL-6/IL-13 intraocular levels. *PLoS Negl Trop Dis* 7:e2541.

Dežkin VV, Safonov VG. 2004. *Die Biber der alten und neuen Welt*. Westarp-Wiss, Hohenwarsleben, Germany. 168 pp.

Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Grigg ME. 2020. Recent epidemiologic and clinical importance of *Toxoplasma gondii* infections in marine mammals: 2009–2020. *Vet Parasitol* 288:109296.

Dubey JP. 2022. *Toxoplasmosis of animals and humans*. CRC Press, Boca Raton, Florida. 547 pp.

Fernández-Escobar M, Calero-Bernal R, Benavides J, Regidor-Cerrillo J, Guerrero-Molina MC, Gutiérrez-Expósito D, Collantes-Fernández E, Ortega-Mora LM. 2020. Isolation and genetic characterization of *Toxoplasma gondii* in Spanish sheep flocks. *Parasit Vectors* 13:396.

Forzán MJ, Frasca S. 2004. Systemic toxoplasmosis in a five-month-old beaver (*Castor canadensis*). *J Zoo Wildl Med* 35:113–115.

Glor SB, Edelhofer R, Grimm F, Deplazes P, Basso W. 2013. Evaluation of a commercial ELISA kit for detection of antibodies against *Toxoplasma gondii* in serum, plasma and meat juice from experimentally and naturally infected sheep. *Parasit Vectors* 6:85.

Grigg ME, Ganatra J, Boothroyd JC, Margolis TP. 2001. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. *J Infect Dis* 184:633–639.

Halonen SK, Weiss LM. 2013. Toxoplasmosis. *Handb Clin Neurol* 114:125–145.

Hamilton CM, Black L, Oliveira S, Burrells A, Bartley PM, Melo RPB, Chianini F, Palarea-Albaladejo J, Innes EA, et al. 2019. Comparative virulence of Caribbean, Brazilian and European isolates of *Toxoplasma gondii*. *Parasit Vectors* 12:104.

Harb OS, Roos DS. 2020. ToxoDB: Functional genomics resource for *Toxoplasma* and related organisms. *Methods Mol Biol* 27–47.

Hassan MA, Olijnik A-A, Frickel E-M, Saeij JP. 2019. Clonal and atypical *Toxoplasma* strain differences in virulence vary with mouse sub-species. *Int J Parasitol* 49:63–70.

Hejlícek K, Literák I, Nezval J. 1997. Toxoplasmosis in wild mammals from the Czech Republic. *J Wildl Dis* 33:480–485.

Herrmann DC, Wibbelt G, Götz M, Conraths FJ, Schares G. 2013. Genetic characterisation of *Toxoplasma gondii* isolates from European beavers (*Castor fiber*) and European wildcats (*Felis silvestris silvestris*). *Vet Parasitol* 191:108–111.

Jokelainen P. 2012. Endemic *Toxoplasma gondii* genotype II causes fatal infections in animal hosts in Europe—lessons learnt. In: *Toxoplasmosis—recent advances*. Djurković Djaković O, editor. IntechOpen. https://www.intechopen.com/books/2816. Accessed December 2022.

Jordan CN, Kaur T, Koenen K, DeStefano S, Zajac AM, Lindsay DS. 2005. Prevalence of agglutinating antibodies to *Toxoplasma gondii* and *Sarcocystis neurona* in beavers (*Castor canadensis*) from Massachusetts. *J Parasitol* 91:1228–1229.

Keenihan SH. 2002. Toxoplasmosis in Brazil. *Trends Parasitol* 18:203–204.

Kohn MA, Senyak J. 2021. *Sample size calculators*. UCSF CTSI. https://www.sample-size.net/. Accessed February 2023.

Kreuder C, Miller MA, Jessup DA, Lowenstine LJ, Harris MD, Ames JA, Carpenter TE, Conrad PA, Mazet JA. 2003. Patterns of mortality in Southern sea otters (*Enhydra lutris nereis*) from 1998–2001. *J Wildl Dis* 39:495–509.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis

across computing platforms. *Mol Biol Evol* 35:1547–1549.

Lindsay DS, Collins MV, Mitchell SM, Cole RA, Flick GJ, Wetch CN, Lindquist A, Dubey JP. 2003. Sporulation and survival of *Toxoplasma gondii* oocysts in seawater. *J Eukaryot Microbiol* 50 Suppl:687–688.

Maas M, Glorie J, Dam-Deisz C, de Vries A, Franssen FFJ, Jaarsma RI, Hengeveld PD, Dierikx CM, van der Giessen JWB, Opsteegh M. 2022. Zoonotic pathogens in Eurasian beavers (*Castor fiber*) in the Netherlands. *J Wildl Dis* 58:404–408.

Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, Dodd E, Harris MD, Ames JA, et al. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of Southern sea otters (*Enhydra lutris nereis*). *Int J Parasitol* 32:997–1006.

Minuzzi CE, Fernandes FD, Portella LP, Bräunig P, Sturza DAF, Giacomini L, Salvagni E, Ribeiro JDS, Silva CR, et al. 2021. Contaminated water confirmed as source of infection by bioassay in an outbreak of toxoplasmosis in South Brazil. *Transbound Emerg* Dis 68:767–772.

Opsteegh M, Haveman R, Swart AN, Mensink-Beerepoot ME, Hofhuis A, Langelaar MFM, van der Giessen JWB. 2012. Seroprevalence and risk factors for *Toxoplasma gondii* infection in domestic cats in the Netherlands. *Prev Vet Med* 104:317–326.

Pardini L, Bernstein M, Carral LA, Kaufer FJ, Dellarupe A, Gos ML, Campero LM, Moré G, Messina MT, et al. 2019. Congenital human toxoplasmosis caused by non-clonal *Toxoplasma gondii* genotypes in Argentina. *Parasitol Int* 68:48–52.

Pardo Gil M, Hegglin D, Briner T, Ruetten M, Müller N, Moré G, Frey CF, Deplazes P, Basso W. 2023. High prevalence rates of *Toxoplasma gondii* in cat-hunted small mammals—evidence for parasite induced behavioural manipulation in the natural environment? *Int J Parasitol Parasites Wildl* 20:108–116.

Pena HFJ, Gennari SM, Dubey JP, Su C. 2008. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. *Int J Parasitol* 38:561–569.

QGIS.org. 2017. *QGIS Geographic Information System*. Open Source Geospatial Foundation Project. http://qgis.org.

R Core Team. 2022. *R: A language and environment for statistical computing*. https://www.R-project.org/. Accessed October 2022.

Roelke ME, Johnson WE, Millán J, Palomares F, Revilla E, Rodríguez A, Calzada J, Ferreras P, León-Vizcaíno L, et al. 2008. Exposure to disease agents in the endangered Iberian lynx (*Lynx pardinus*). *Eur J Wildl Res* 54:171–178.

Ryser-Degiorgis M-P, Segner H. 2015. National competence center for wildlife diseases in Switzerland: Mandate, development and current strategies. *Schweiz Arch Tierheilkd* 157:255–266.

Scherrer P, Ryser-Degiorgis M-P, Marti IA, Borel S, Frey CF, Mueller N, Ruetten M, Basso W. 2023. Exploring the epidemiological role of the Eurasian lynx (*Lynx lynx*) in the life cycle of *Toxoplasma gondii*. *Int J Parasitol Parasites Wildl* 21:1–10

Shapiro K, Bahia-Oliveira L, Dixon B, Dumètre A, de Wit LA, VanWormer E, Villena I. 2019. Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. *Food Waterborne Parasitol* 15:e00049.

Smith DD, Frenkel JK. 1995. Prevalence of antibodies to *Toxoplasma gondii* in wild mammals of Missouri and east central Kansas: Biologic and ecologic considerations of transmission. *J Wildl Dis* 31:15–21.

Spycher A, Geigy C, Howard J, Posthaus H, Gendron K, Gottstein B, Debache K, Herrmann DC, Schares G, et al. 2011. Isolation and genotyping of *Toxoplasma gondii* causing fatal systemic toxoplasmosis in an immunocompetent 10-year-old cat. *J Vet Diagn Invest* 23:104–108.

Stelzer S, Basso W, Benavides Silván J, Ortega-Mora LM, Maksimov P, Gethmann J, Conraths FJ, Schares G. 2019. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. *Food Waterborne Parasitol* 15:e00037.

Su C, Dubey JP. 2020. Isolation and genotyping of *Toxoplasma gondii* strains. *Methods Mol Biol* 2071:49–80.

Swiss Confederation. 2022. *Maps of Switzerland*. map.geo.admin. Accessed November 2022.

van de Velde N, Devleesschauwer B, Leopold M, Begeman L, Ijsseldijk L, Hiemstra S, Ijzer J, Brownlow A, Davison N, et al. 2016. *Toxoplasma gondii* in stranded marine mammals from the North Sea and eastern Atlantic Ocean: Findings and diagnostic difficulties. *Vet Parasitol* 230:25–32.

Vincze T, Posfai J, Roberts RJ. 2003. Nebcutter: A program to cleave DNA with restriction enzymes. *Nucleic Acids Res* 31:3688–3691.

Vyas A, Kim S-K, Sapolsky RM. 2007. The effects of *Toxoplasma* infection on rodent behavior are dependent on dose of the stimulus. *Neuroscience* 148:342–348.

Webster JP. 2001. Rats, cats, people and parasites: The impact of latent toxoplasmosis on behaviour. *Microbes Infect* 3:1037–1045.

Zahner V, Schmidbauer M, Schwab G, Angst C. 2021. *Der Biber: Baumeister mit Biss*. SüdOst Verlag, Regenstauf, Germany. 192 pp.

Zanzani SA, Di Cerbo A, Gazzonis AL, Epis S, Invernizzi A, Tagliabue S, Manfredi MT. 2016. Parasitic and bacterial infections of *Myocastor coypus* in a metropolitan area of Northwestern Italy. *J Wildl Dis* 52:126–130.

Zarnke RL, Dubey JP, Ver Hoef JM, McNay ME, Kwok OC. 2001. Serologic survey for *Toxoplasma gondii* in lynx from interior Alaska. *J Wildl Dis* 37:36–38.

Zürcher-Giovannini S. 2018. Toxoplasmosis in free-ranging Eurasian Beavers (*Castor fiber*) from Switzerland. In: *Proceedings of the 13th European Wildlife Disease Association conference, European Wildlife Disease Association*, Larissa, Greece, 27–31 August. p. 40. http://ewda.org/wp-content/uploads/2022/01/EWDA2018_Abstracts_Book.pdf. Accessed September 2022.

Submitted for publication 26 April 2023.
Accepted 18 August 2023.