Veterinary Parasitology 266 (2019) 27–33

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Seroprevalence, biogeographic distribution and risk factors for *Aelurostrongylus abstrusus* infections in Swiss cats

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ARTICLE INFO

Keywords:
Aelurostrongylus abstrusus
Cat
Antibody detection
Prevalence
Risk factors
Switzerland

ABSTRACT

The metastrongyloid nematode *Aelurostrongylus abstrusus* is a worldwide occurring feline lungworm. The spectrum of clinical signs in infected cats ranges from mild (e.g. nasal discharge or cough) to severe respiratory distress. The aim of this seroepidemiological study was to define prevalence and risk factors for *A. abstrusus* infections in Swiss cats, to assess the biogeographic distribution and to investigate the influence of temperature and altitude on the occurrence of this parasite. Sera of 4067 domestic cats were collected from all over Switzerland, tested for the presence of antibodies against *A. abstrusus* by a novel ELISA and the results correlated with biogeographic aspects. A subsample of 1000 datasets was used for risk factor analyses. Overall, 10.7% (434/4067, 95% confidence intervals [CI]: 9.7–11.7%) of the cats were tested positive, with variations from 0.0% to 20.0% among ten different biogeographic regions. Differences were significant between the Western (13.9%, CI: 11.4–16.7%) and the Eastern (9.2%, CI: 8.0–10.5%) Swiss Plateau, possibly attributable to the suitability of the areas for intermediate hosts. In total 90.3% (392/434) of the seropositive cats originated from regions lower than 700 m above sea level. Correspondingly, 98.9% (429/434) of positive samples were obtained from regions with a mean temperature higher than −2 °C in January, suggesting altitude and temperature being limiting factors for *A. abstrusus* infections in Switzerland. Concerning individual risk factors, prevalence was higher in intact (15.5%, CI: 9.5–23.4%) than in neutered cats (5.8%, CI: 7.9–10.4%). Young adult cats (aged 11–22 months) were significantly more often seropositive (10/76, 13.2%, CI: 6.5–22.9%) than kittens aged 1–10 months (1/34, 2.9%, CI: 0.1–15.3%) or adult and senior cats > 22 months (58/889, 6.5%, CI: 5–8.4%). Outdoor cats and cats presenting respiratory signs tend to be more often positive than indoor cats ($p = 0.077$) and animals without respiratory signs ($p = 0.086$), respectively. We here confirm that the use of a serological test can contribute to improve the identification of infected animals, through evaluation of risk factors on a population level and for a better management on an individual level, overcoming the challenges represented by faecal examinations and the correlated underestimation of the occurrence of *A. abstrusus* in cats.

1. Introduction

Aelurostrongylus abstrusus is a worldwide occurring feline lungworm which belongs to the metastrongyloid nematodes. Domestic cats are considered the main natural hosts (Scott, 1973) and snails and slugs serve as intermediate hosts. Within these, after ingestion of the first larval stage (L1) shed in faeces of infected cats, the infective third stage larva (L3) develops. Cats become infected by either ingesting intermediate hosts or paratenic hosts such as reptiles, amphibians, birds and rodents (Hamilton and McCaw, 1967b; Hobmaier and Hobmaier, 1935; Scott, 1973). The L3s penetrate the intestinal mucosa, moult into L4 and reach the lungs via the lymphatic system. Here, adult worms develop, reproduce and release L1s, both causing inflammatory reactions and parenchymal tissue damages (Gerdin et al., 2011; Hamilton, 1966; Schnyder et al., 2014).

The clinical signs of this disease can be nonspecific (Schnyder et al., 2014) or range from asymptomatic to very severe respiratory distress which can even lead to death (Genchi et al., 2014). The most evident signs are chronic cough, sneezing, nasal discharge and dyspnoea (Crisi et al., 2017; Genchi et al., 2014; Scott, 1973; Traversa et al., 2008b), whereas often the disease is subclinical (Hamilton, 1969). Prevalence rates of *A. abstrusus* infections in cats in European countries are highly variable and difficult to compare due to small sample sizes and/or differing study designs, diverse diagnostic methods or study

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https://doi.org/10.1016/j.vetpar.2018.12.013
Received 21 September 2018; Received in revised form 10 December 2018; Accepted 16 December 2018

populations concerning lifestyle (e.g. domestic versus stray cats) or geographic origin. In a recent and broad study performed in 12 European countries the prevalence of feline lungworms in domestic cats was determined using faecal analysis. The rates ranged from 0.0% in Austria and the United Kingdom to highest (35.8%) in Bulgaria; one of 124 samples (0.8%) from Switzerland was found positive (Giannelli et al., 2017).

The most frequently used diagnostic approaches are coproscopic procedures relying on the detection of L1 in faecal samples. The Baermann method is still the most popular assay (Deplazes et al., 2016). Furthermore, the microscopic and cytologic examination of bronchoalveolar lavages is described, requiring however anesthesia of the animal at each time (Foster et al., 2004). Alternatively, detection of parasite DNA from faecal or pharyngeal swab samples by PCR is possible and occasionally more sensitive than larval detection from faeces (Traversa et al., 2008a) but also relying on the presence of L1 in the tested material and not in use for mass screening.

The Baermann method is performed with fresh faecal samples as it requires alive L1, and good skills of laboratory personnel in discriminating *A. abstrusus* L1 from other species are needed (Traversa et al., 2010). Importantly, the procedure is limited by the fact that larval shedding can be absent (Hamilton, 1968) or intermittent, even in presence of clinical signs (Schnyder et al., 2014). Eventually, the biggest challenge with all coproscopical procedures is the collection of faeces from cats which have outdoor access, because often owners are not aware of the places where their cats defecate and reluctant to segregate them indoors. This represents an issue as especially these animals are at higher risk of infection (Beugnet et al., 2014).

An alternative to diagnose *A. abstrusus* infections in cats is a recently developed serological test which detects antibodies against this parasite in cat sera (Zottler et al., 2017). This highly specific and sensitive ELISA for lungworms represents a valid method allowing reliable results, and is particularly suitable for mass screening in the context of epidemiological surveys. In the study presented here, serological analyses were combined with a spatial analysis investigating the distribution of seropositive cats within Switzerland. The aim was to determine the seroprevalence and risk factors of *A. abstrusus* infections in cats by testing more than 4000 cat serum samples from all over Switzerland by a validated ELISA (Zottler et al., 2017).

2. Material and methods

2.1. Cat sera

Sera of 4067 cats from all over Switzerland submitted by veterinarians for haematological or clinical chemistry analyses for different medical reasons were collected between 2011–2013 and 2015–2017, including corresponding data on the owner's postal code. The sera were provided by 23 private veterinary clinics, by 3 Swiss private veterinary diagnostic laboratories (IDEXX Diavet Labor AG, Bäch; Labor am Zugersee, Hünenberg/Zug; Labor-Zentral AG, Geuensee), by the Clinical Laboratory, Vetsuisse Faculty, University of Bern, and by the Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland.

2.2. ELISA

The serum samples were stored at −20 °C and thawed before being tested for antibodies against *A. abstrusus* by a previously described and validated indirect ELISA (sensitivity 88.2%, specificity 90.0%, Zottler et al., 2017), performed with modifications: following titration experiments, a combination of an Immobilizer Amino Plate (Nunc Roskilde, Denmark) with a goat anti-feline IgG peroxidase labeled conjugate (Southern Biotech, Birmingham, USA) at a dilution of 1:9000 was chosen. The absorbance values were read in a Multiscan RC ELISA reader (Thermo Labsystems, Helsinki, Finland), initially at 450 nm to determine the exact moment for stopping the reaction with sulphuric acid, subsequently the plate was read at 492 nm. Each plate was run with a substrate control, two positive controls (sera from experimentally infected cats), two negative controls (from uninfected laboratory cats) and a conjugate control. A reference serum was added twice on each plate to calculate a correction factor for adjustment between plates (Schnyder et al., 2011). Receiver operating characteristic (ROC) analysis (Zweig and Campbell, 1993) was applied to determine an optimal cut-off value. For this purpose ELISA optical density (OD) values of sera from infected and healthy cats verified by the Baermann method (Deplazes et al., 2016) were used.

Based on previous studies, seropositivity in experimentally infected cats is expected to start two weeks after infection, being 100% after ten weeks and intermediate after six weeks. Antibodies where shown to persist as long as adult worms are present, not necessarily correlating with patency. After anthelmintic treatment, the OD values started to decline within four weeks, and were negative approximately after 6 weeks (Zottler et al., 2017).

2.3. Geoprocessing

In order to represent the geographic distribution of *A. abstrusus* infections in cats in Switzerland and to create appropriate maps, the program Quantum GIS 2.18.6 'Las Palmas' (Quantum GIS Geographic Information System, Open Source Geospatial Foundation Project, http://qgis.osgeo.org) was used. Each sample was depicted on maps based on the Swiss postal code system. To create point data according to postal codes, these were matched with a coordinate in their center by means of the Swiss Cadastral Surveying (www.cadastre.ch). For the biogeographic analysis, Switzerland was divided in ten regions based on data from the Federal Office for the Environment (FOEN, www.bafuadmin.ch): regions were defined relying on the patterns of distribution of flora and fauna, applying a purely statistical approach, which was adapted to communal boundaries (Gonseth et al., 2001). Data on mean temperatures and altitudes in Switzerland were obtained from WorldClim-Global Climate Data (www.worldclim.org) and from the Federal Office of Topography swisstopo (www.toposhop.admin.ch), respectively. The exact binomial 95% confidence intervals (CI) were calculated according to Clopper and Pearson (Clopper and Pearson, 1934) using Microsoft Excel 2010 for Windows (Microsoft Corporation, Redmond, USA).

2.4. Risk factor analysis

A total of 1000 cat sera out of the 4067 were used for risk factor analysis, all originating from the Clinical Laboratory of the Vetsuisse Faculty, University of Zurich: from these cats all requested data were available, and a random selection based on the case history numbers was performed. The following anamnestic data and history of each cat were considered: gender (male/female), neuter status (yes/no), respiratory signs (absent/present; e.g. coughing, nasal discharge, increased respiratory rate), date of sampling and age of the cat were obtained from the OblonData Software belonging to the Small Animal Clinic of the Vetsuisse Faculty, University of Zurich, Switzerland. To determine the lifestyle (indoor/outdoor access) each paper-based dossier was viewed. Cats which only had access to the owner's house were classified as indoor cats. The dates of sampling were categorized in two periods of the year, i.e. into a warm weather period (April-September) and a cold weather period (October-March). Additionally, the cats were ranked in three age groups: 1) 1–10 months (kittens); 2) > 10 to 22 months (juvenile cats); 3) > 22 to 276 months (adult/senior cats).

To evaluate a potential influence of these risk factors on *A. abstrusus* seropositivity, a univariate statistical analysis using crosstabulations and the chi-squared test were performed. In addition, a standard logistic regression including all risk factors was run. The software IBM SPSS statistics for Windows, version 22, was used for analysis and the

Table 1
Seroprevalence for antibodies against *Aelurostrongylus abstrusus* in 4067 cats within defined biogeographic regions of Switzerland (CI: confidence intervals).

Biogeographic regions (number of tested samples)	Antibody positive cats		
	n	%	95% CI
1. Southern Alps (20)	4	20.00	5.7–43.7
2. Western Swiss Plateau (698)	97	13.90	11.4–16.7
3. Northern Alps (224)	31	13.84	9.6–19.1
4. Geneva and High Rhine (371)	45	12.13	9.0–15.9
5. Swiss Prealps (210)	25	11.90	7.9–17.1
6. Jura Mountains and Randen (252)	27	10.71	7.2–15.2
7. Central Western Alps (63)	6	9.52	3.6–19.6
8. Eastern Swiss Plateau (2073)	190	9.17	8.0–10.5
9. Southern Ticino (119)	9	7.56	3.5–13.9
10. Central Eastern Alps (37)	0	0.00	0–7.8
Total (4067)	434	10.7	9.7–11.7

significance level was set at $p < 0.05$.

3. Results

Analysing 4067 collected samples, an average seroprevalence of *A. abstrusus* in cats in Switzerland of 10.7% (434/4067 cats, CI: 9.7–11.7%) was determined.

3.1. Geoprocessing

All 4067 cat serum samples were included in the analysis. The seroprevalences within the different biogeographic regions ranged from 0.0% in the Central Eastern Alps to highest 20.0% (CI: 5.7–43.7%) in the Southern Alps (Table 1, Fig. 1). The number of *A. abstrusus* infections in the Western Swiss Plateau (13.9%, CI: 11.4–16.7%) differed significantly from the Eastern Swiss Plateau (9.2%, CI: 8.0–10.5%). In Fig. 2 all 434 antibody positive cases are depicted in relation to the different altitudes in the country. The highest altitude at which a positive sample was found was 1351 m above sea level (asl), whereas the majority ($n = 392$, 90.3%) of the 434 positive cats originated from areas lower than 700 m asl. Similarly, most ($n = 429$, 98.9%) of the positive samples originated from areas with a mean temperature in January warmer than −2 °C (Fig. 3).

3.2. Risk factor analysis

Overall, six potential risk factors were examined: gender and neuter status, age, lifestyle, presence of respiratory signs and season of sampling (warm vs. cold). Results are summarised in Table 2. *Aelurostrongylus abstrusus* prevalence was significantly higher ($p < 0.001$) in intact cats (18/116, 15.5%, CI: 9.5–23.4%) than in neutered ones (51/884, 5.8%, CI: 4.3–7.5%), while gender itself did not have any influence on seropositivity. In univariate statistics a significant ($p = 0.03$, Chi-squared test) peak of seropositivity was detected for juvenile cats aged between 11–22 months. This group had a seroprevalence of 13.3%; adult/senior cats and kittens in contrast had a prevalence of 6.5% and 2.9%, respectively. The difference was not significant in logistic regression analysis, and also the 95% CI for OR (0.26–1.17) was not significant ($p = 0.119$), which can be explained by the small amount of juvenile cats assessed.

No further significant risk factors using logistic regression analysis were detected, but the following trends were observed: cats with or without respiratory signs showed a seroprevalence of 9.4% and 6.5%, respectively ($p = 0.09$). Of 69 seropositive cats, 14 (20.3%, CI: 11.6–31.7%) had a history of respiratory signs, whereas of 931 seronegative cats, 149 (16%, CI: 13.7–18.5%) presented with respiratory issues ($p = 0.086$).

The prevalence of antibodies against *A. abstrusus* in outdoor cats was 7.9% (51/644) and 5.1% (18/356) in indoor cats, with a *p*-value of 0.08, therefore indicating a trend for a higher infection risk for outdoor cats. The variable season of serum sampling did not have any influence on the probability of cats being seropositive for *A. abstrusus*.

4. Discussion

With a seroprevalence of 10.7%, the rate of *A. abstrusus* antibody

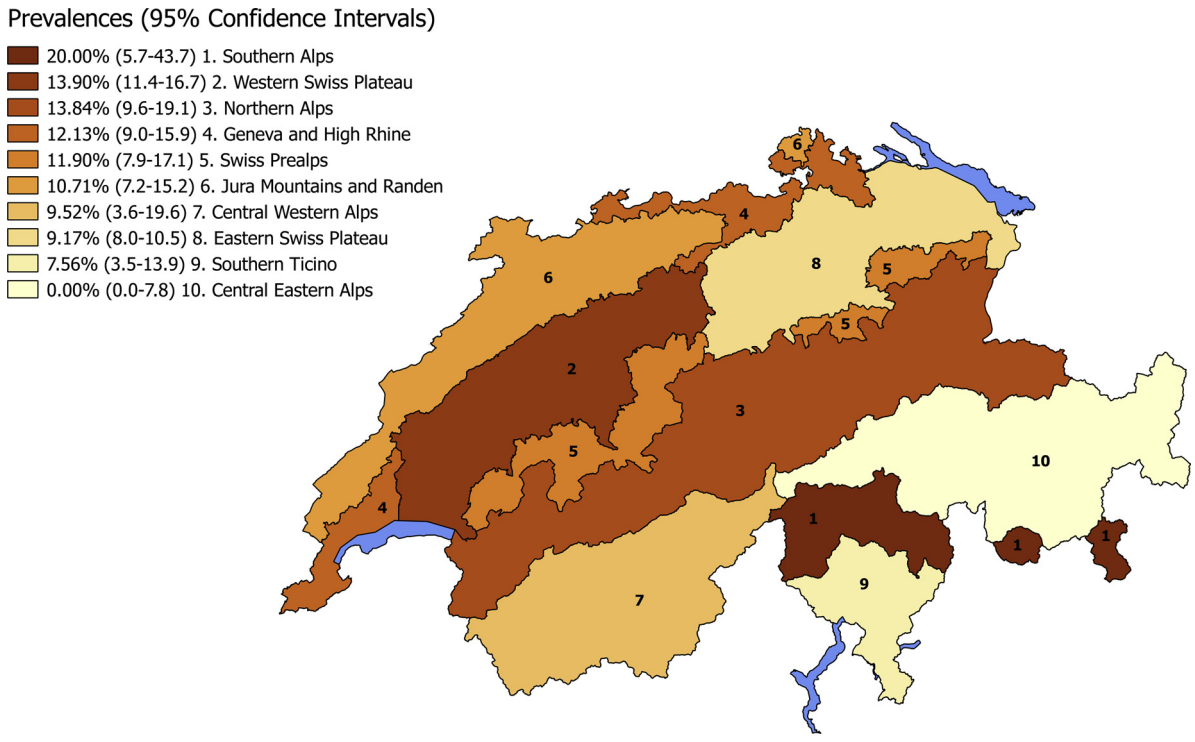

Fig. 1. Antibody seroprevalence against the lungworm *Aelurostrongylus abstrusus* in domestic cats within ten biogeographic regions of Switzerland.

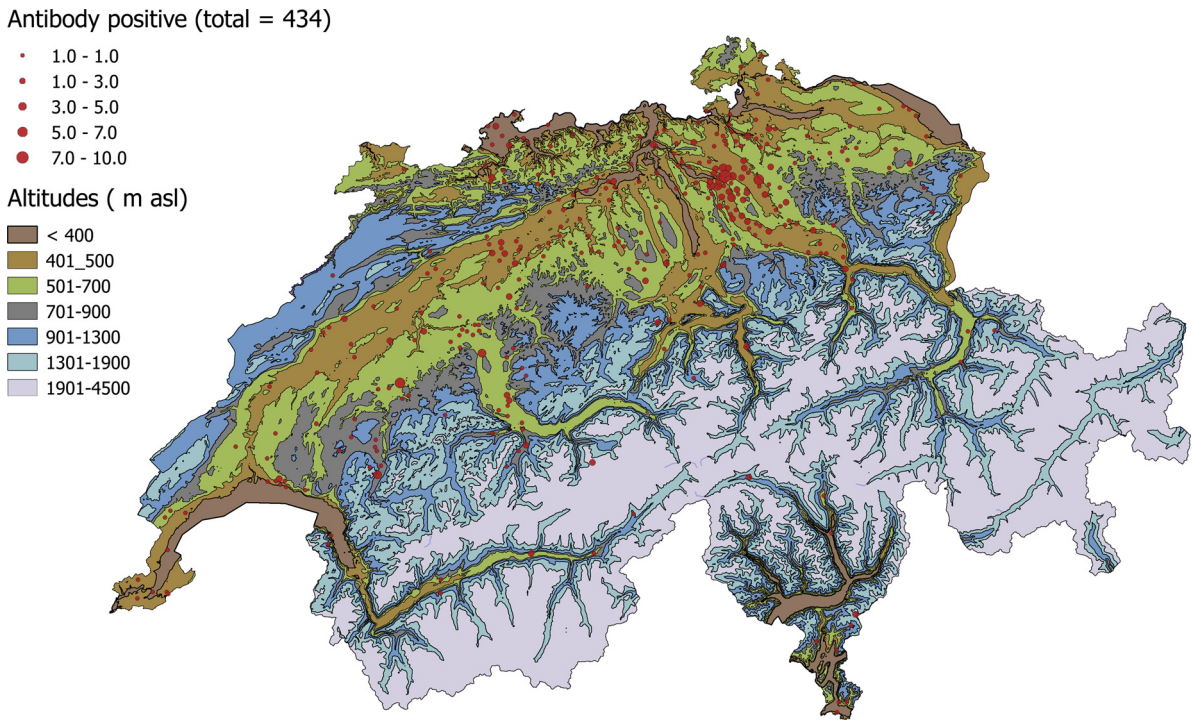

Fig. 2. Geographic location of *Aelurostrongylus abstrusus* antibody positive cat sera (n = 434) in relation to altitude in meters above sea level (m asl) in Switzerland.

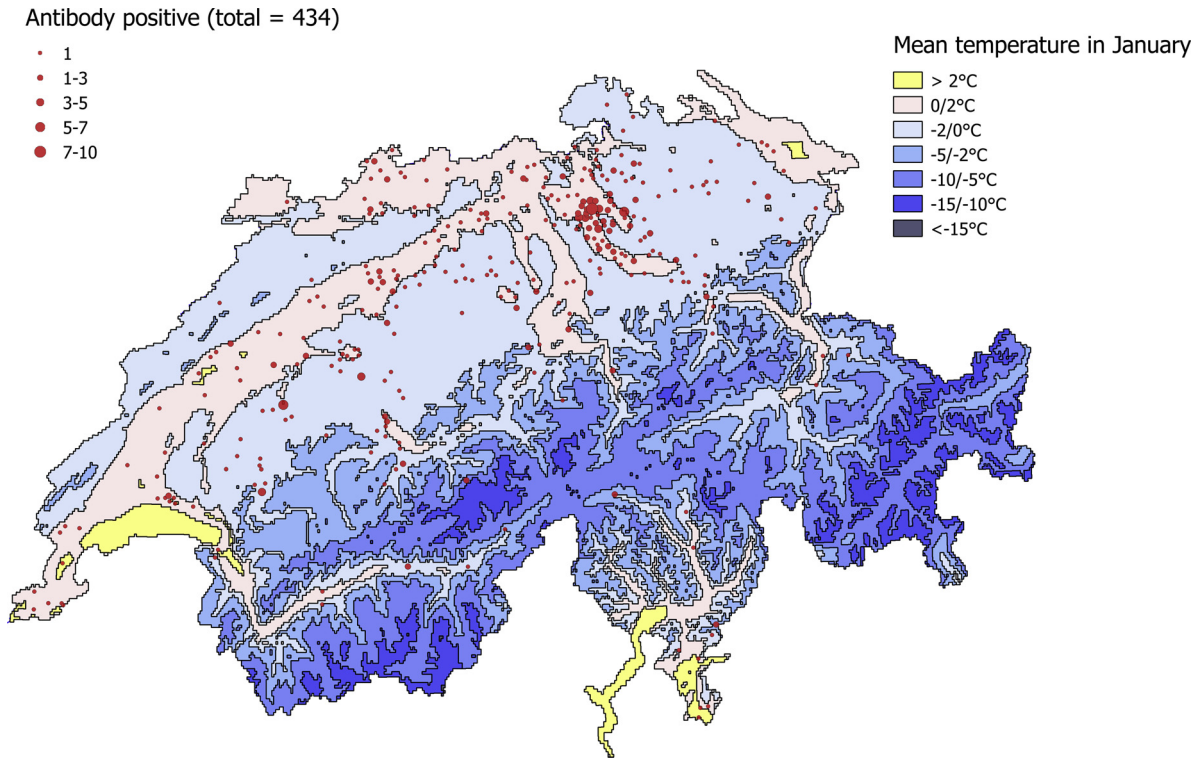

Fig. 3. Geographic location of *Aelurostrongylus abstrusus* antibody positive cat sera (n = 434) in relation to the mean temperature in January in Switzerland.

positive cats in Switzerland is clearly higher than expected. Recent studies in which the Baermann method was used indicated a prevalence of 0.8% (Giannelli et al., 2017) and 2.3% (Zottler et al., 2019) in Swiss cats. Premising that antibodies are detectable already 2–3 weeks before patency and that they may persist for at least 4 weeks after successful anthelmintic treatment (Zottler et al., 2017), a number of reasons could be at the origin of such a discrepancy, among which the adopted diagnostic method plays an important role. In fact, the detection of both, larval excretion and serological antibodies against *A. abstrusus*, presents diagnostic uncertainties with opposite trends.

Comparing six different procedures (serology excluded) for detection of *A. abstrusus* infections in cats on the basis of 80 cadavers of semiferal domestic cats, still the Baermann method was considered the most reliable technique, with a sensitivity of 84.6% and a specificity of 100% (Lacorcia et al., 2009). However, especially in chronic i.e. more than ten weeks old (Hamilton and McCaw, 1968; Schnyder et al., 2014)

Table 2
Risk factor analysis for *Aelurostrongylus abstrusus* infection based on serological antibody detection in 1000 randomly selected cat sera. Significant *p*-values are printed in bold (OR: Odds-Ratios).

	No. of cats (n = 1000)	Cats seropositive for *A. abstrusus* antibodies			Univariate statistical analysis (X^2-Test)		Logistic regression analysis		
	n	*n*	%	95% CI	Crude OR	*p*-value	Adjusted OR	95% CI for OR	*p*-value
Gender									
females	407	28	6.9	4.6–9.8	0.995	0.983	0.89	0.53–1.49	0.657
males	593	41	6.9	5.0–9.3					
Neuter status									
intact	116	18	15.5	9.5–23.4	3.00	**0.000**	3.51	1.89–6.50	**0.000**
neutered	884	51	5.8	4.3–7.5					
Age[a]									
kittens	34	1	2.9	0.1–15.3	0.2[b]	0.099[b]	0.13[b]	0.02–1.09[b]	0.059[b]
juvenile cats	76	10	13.2	6.5–22.9					
adult and senior cats	889	58	6.5	5.0–8.4	0.46[c]	**0.030**[c]	0.55[c]	0.26–1.17[c]	0.119[c]
Lifestyle									
outdoor	644	51	7.9	6–10.3	1.62	0.087	1.67	0.94–2.93	0.077
indoor	356	18	5.1	3–7.9					
Respiratory signs									
present	149	14	9.4	5.2–15.3	1.50	0.193	1.73	0.92–3.25	0.086
absent	851	55	6.5	4.9–8.3					
Season									
cold weather period (Oct.–Mar.) vs.	315	21	6.7	4.2–10	0.948	0.844	1.05	0.61–1.80	0.857
warm weather period (Apr.–Sept.)	685	48	7	5.2–9.2					

[a] Kittens: 1–10 months; juvenile cats: > 10–22 months; adult and senior cats: > 22 months.
[b] Kittens vs. juvenile cats.
[c] Adult and senior cats vs. juvenile cats.

or repeated infections (Ribeiro and Lima, 2001), the interruption of larval excretion precludes the identification of all infected cats, and therefore a notable number of infected animals may be missed by the Baermann method (Zottler et al., 2019), leading to underestimation.

On the other hand, seropositivity indicates that the animal had previous contact with the parasite and/or is currently having an infection that induces an immunological reaction with production of antibodies. At the moment of serodiagnosis, serology is therefore not able to discriminate current and past (some weeks) infections, potentially causing overestimation of current infections. Since clearance of infection by anthelmintic treatment in experimentally infected cats induced declining OD values below cut-off levels within four to eight weeks, in opposition to untreated animals that persisted seropositive (Zottler et al., 2017), the number of seropositive but no longer infected animals under natural conditions is considered low.

In our study for the first time an *A. abstrusus* antibody detection ELISA was adopted for mass-screening detecting *A. abstrusus* exposure in cats. The ELISA is able to identify cats with very low worm burdens and, consequently, with few or even absent clinical signs, and therefore overcomes underestimation due to interrupted/absent larval shedding. In two broad field studies recently performed in 9 (Beugnet et al., 2014) and 12 (Giannelli et al., 2017) European countries, highest prevalences were observed in cats from rural areas of Eastern European countries such as Bulgaria (35.8%), whereas prevalence in Swiss cats was more similar to the ones detected in similar cat populations of i.e. Belgium, lying below 3% (Giannelli et al., 2017). Our data actually support the fact that identifying infected cats by coproscopic methods leads to underestimation of the number of animals that had contact with the parasite, which may be confirmed by future seroepidemiological studies in highly endemic countries. Also cat necropsies for detection of adults and larval stages substantiate that *A. abstrusus* prevalence is probably underestimated, with 50% of cats being positive in Albania (Knaus et al., 2011) and 15.6% in Denmark (Olsen et al., 2015).

Our results obtained by analysing the influence of biogeographic aspects on the distribution of *A. abstrusus* in the country is strongly supported by findings from previous lungworm studies. A temperature limit of −2 °C has also been found in a large scale study on *Angiostrongylus vasorum* in dogs, having a similar life cycle: of more than 6000 assessed Swiss dogs, the majority of the seropositive samples originated from areas with a mean temperature in January warmer than −2 °C and from below 700 m asl (Lurati et al., 2015). These results suggest that colder climatic conditions are limiting factors for the life cycle of these lungworms. According to Hamilton and McCaw (1967a) in fact, *A. abstrusus* L1 can outlive freezing and thawing for several times, but on the other hand experimental infections of *Helix aspersa* with *A. abstrusus* L1 showed that warmer temperature conditions (18.8–29.5 °C) and constant humidity allow a higher rate of larval development in the snails (Di Cesare et al., 2015). Since temperature appears to be a relevant factor for the life cycle, consequently also altitude is, as these two parameters correlate. The higher prevalence of *A. abstrusus* at lower altitudes may be correlated with the presence of suitable compulsory intermediate hosts, and of potentially occurring paratenic hosts. A very common slug in central Europe and competent intermediate host in the field, *Arion lusitanicus* (Lange et al., 2018), mainly lives at altitudes lower than 1000 m asl, as do most of the common gastropods species in Switzerland (Boschi, 2011). Snails and slugs are poorly mobile organisms which cannot evade unfavourable environments over long distances, and they inhabit small scale biotopes forming a close interaction with their local microclimate. Ideal habitats for snails and slugs are represented by wetland biotopes (natural streams, rivers, marshland, alluvial zones), which offer cover (in form of vegetation such as undergrowth and reed beds) from potential predators and from solar radiation, and sustainable food resources (Boschi, 2011). Humidity in the form of precipitations may additionally play a role for survival and propagation of intermediate hosts. Intensively used agricultural areas, instead, are suggested to not be favourable because of the effects of plant protection substances and the lack of retreat zones, and urban areas mostly consist of paved surfaces precluding natural habitats.

The seroprevalence of *A. abstrusus* within the different biogeographic regions extended from the lowest possible prevalence of 0.0% (CI: 0.0–7.8%) in the Central Eastern Alps to a considerably high value

in the Southern Alps (20.0%, CI: 5.7–43.7%). However, the corresponding confidence intervals for these two areas were rather large, due to the small sample sizes. This was not the case for the Western Swiss Plateau (13.9%, CI: 11.4–16.7%), and the Eastern Swiss Plateau (9.2%, CI: 8.0–10.5%). The Western Swiss Plateau is characterized by the presence of more wetlands (federal inventory of the wetlands of national importance, www.bafu.admin.ch/bafu/en/home/suche.html#auengebiete), representing advantageous habitats for gastropods. Furthermore, this area is mainly constituted by cantons (Waadt and Bern) having a higher percentage of wooded areas (10.0 and 13.9%, respectively), while the Eastern Swiss Plateau mainly consists of cantons (Aargau, Zürich, Thurgau and St. Gallen) which have 3.8, 4.0, 1.6 and 4.7% of wooded areas, respectively (www.bfs.admin.ch). In contrast, the proportion of paved surfaces (buildings and streets which result in a loss of natural soil function) are higher in these latter cantons (10.8, 13.4, 8.0 and 6.1%, respectively) compared to the cantons of the Western Swiss Plateau (5.3 and 4.5%, respectively). These factors might also regionally influence the number of available intermediate hosts. Concerning the expected paratenic hosts, the situation is unclear, as information on amphibians, reptiles, rodents or birds infected with larval stages of *A. abstrusus* is very scant (Hobmaier and Hobmaier, 1935). These researchers reported that they had been successful in using mice, frogs, toads, snakes, lizards, ducklings and chickens as experimental "auxiliary hosts" (Hobmaier and Hobmaier, 1935). More recently, a single study confirmed the presence of L3 in rodents (*Apodemus agrarius*) from the field (Jezewski et al., 2013). Extending however the argumentations to correlations between *A. abstrusus* prevalence and the occurrence of paratenic hosts appears speculative.

In contrast to geographic aspects, several individual factors were repeatedly described to influence the risk of a cat to become infected with *A. abstrusus*, in part with contradictory results. The first assessed variable "gender" did not influence the probability of a cat to become seropositive for *A. abstrusus* antibodies. This is in agreement with previous investigations on risk factors for *A. abstrusus* infections (Beugnet et al., 2014; Genchi et al., 2014; Giannelli et al., 2017; Hansen et al., 2017). Interestingly and in opposition to that, the "neuter status" was found to have a significant influence, with intact cats being at higher risk of infection (15.5%) than neutered ones (5.8%). This may be attributed to the fact that intact animals are highly interested in reproduction and are therefore roaming more intensively: especially intact male cats cover long distances and stay away from home for several nights during mating seasons. Therefore, frequent occasions to ingest potentially infected intermediate or paratenic hosts as a cat's prey are guaranteed. To our knowledge, none of the studies evaluating risk factors did consider the neutering status of the animals. It remains therefore to be verified if for instance in areas with higher *A. abstrusus* prevalence the cat populations are generally less well-cared (Capári et al., 2013) and if they correspond to areas where cats are less frequently neutered.

Previous studies report contradictory results concerning the variable "age": in some, cats younger than one year were at higher risk of larval detection (Barutzki and Schaper, 2013; Traversa et al., 2008b), while in others, cats older than one year were more frequently *A. abstrusus* positive (Capári et al., 2013; Lacorcia et al., 2009). We detected a significant peak for seropositivity in 11–22 month old cats, an age at which cats with outside access are very agile, curious and already well trained in hunting, which may explain a higher risk of seropositivity through captured and ingested prey. Genchi et al (2014) categorized the age of cats in comparable categories, but the prevalence in the different age classes did not differ significantly.

Surprisingly, in outdoor cats seroprevalence was not significantly higher than in indoor cats. Some of the indoor cats had access to a balcony or a terrace, thus an infection from there cannot be fully ruled out. Alternatively, these cats may have had outdoor access and ingested intermediate hosts prior to blood sampling or to the moment of anamnesis (e.g. change of owner) and may therefore be uninterruptedly infected and seropositive, and, eventually, false positive results cannot be excluded.

Concerning the presence or absence of respiratory signs no significant difference was assessed, although a trend towards presence was observed. This supports the current opinion of mild infections being frequently unapparent (Genchi et al., 2014; Schnyder et al., 2014). In previous risk factor analyses e.g. performed in Italy and in Denmark, *A. abstrusus* positive cats suffered significantly more often from respiratory signs than negative cats (Di Cesare et al., 2015; Hansen et al., 2017). On the other hand, in a study from Sardinia, 48.1% of *A. abstrusus* positive cats did not manifest respiratory signs though they had radiographic changes in their lungs (Genchi et al., 2014). Importantly, clinically obvious but also asymptomatic infections with *A. abstrusus* should be taken seriously because representing a highly relevant cause for death during anaesthesia (Gerdin et al., 2011).

No significant difference for the frequency of *A. abstrusus* infections during warm or cold weather periods has been found in our study, although this has been observed for other lungworm infections in carnivores such as for *Crenosoma vulpis* or *A. vasorum* in dogs (Maksimov et al., 2017), where gastropods also act as intermediate hosts. In this latter case the life cycle was used to explain how large and possibly infected gastropod populations may accumulate in autumn, leading to patent infections in December/January. In contrast, we can hypothesize that cats may more often get infected by ingestion of paratenic hosts, bypassing seasonality. Additionally, as anticipated, antibodies may persist for at least four weeks after anthelmintic treatment (Zottler et al., 2017), and verisimilar at least as long as the infection persists (for years, Ribeiro and Lima, 2001), and therefore seropositivity does not deliver information about the moment of infection(s).

5. Conclusion

Clinical signs in *A. abstrusus* infected cats are often mild and subtle, so that the disease often stays unobserved and is therefore underestimated. When clinical signs are observed, they are often initially misinterpreted, for example as feline asthma (Traversa and Di Cesare, 2016). The use of a serological test can contribute to enhance correct individual diagnosis and contemporaneously allows mass-screening of cat populations. Considering that an important proportion of cats has outdoor access, serological tests represent a fundamental advantage. This not only because such cats are normally considered at higher risk of infection (although not confirmed in this study), but also because the collection and examination of faecal samples (possibly fresh samples and collected over three days to increase sensitivity) can be challenging and circuitous and needs to be overcome by locking cats indoor or collecting faeces rectally. Serology confirmed that the occurrence of *A. abstrusus* infections in cats is underestimated and that an increase in disease awareness for animal owners and practitioners as well is warranted.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

The authors would like to thank the 5 veterinary diagnostic laboratories for contributing to the collection of cat sera: the Clinical Laboratory, Vetsuisse Faculty, University of Zurich (Prof. Dr. Regina Hofmann-Lehmann, Dr. Barbara Riond and team), the Clinical Laboratory, Vetsuisse Faculty, University of Bern (Dr. Judith Howard), IDEXX Diavet Labor AG, Bäch (Sandra Kägi), Labor am Zugersee, Hünenberg/Zug (Dr. Daniel Schaarschmidt) and the Labor-Zentral AG, Geuensee (Dr. Walter Regli, Sandra Schütze). We would also like to acknowledge all the veterinary clinics and practices for helping with the collection: The Small Animal Clinic of the Vetsuisse Faculty, University

of Zurich, Cabinets Vétérinaire Ecublens-Lausanne (Dr. Jean Pfister and team), Centro veterinario 3 Valli Sagl, Polleggio (Natan Vescovi and team), Dr. Vanna Taminelli, Airolo, Dres. Catherine and Fabio Nägeli, Balerna, Centro veterinario Preluna, Comano (Dr. Federica Leidi and team), Dr. Aaron Balli, Ascona, Dr. Catrina Geret, Davos, Kleintierpraxis Aamatt, Sarnen, Tierklinik Appenzell (Dr. Tobias Fritsche and team), Kleintierpraxis Obersee, Altendorf (Dr. Leslie Wohlgroth), Tierarztpraxis Seeblick, Stansstad (Dr. Josef Rappo), ANIMAL Kleintierpraxis, Visp (Dr. Susanne Spagnoli-Hartmann, Dr. Dominique Sturdy and the whole team), Tierklinik Rhenus, Flurlingen (Dr. Markus Trächsel and team), Clinique vétérinaire Dr. Diego Stornetta et Jean-Bernard Deillon, Delémont, Tierarztpraxis Dres. Martin and Karin Kull, Ernen, Praxis am Wyher, Küblis (Dr. Riccardo Dalla Costa and team), Tierarztpraxis Calanda, Chur (Dr. Urs Iselin and team), Tierarztpraxis Dr. Franco Ardüser, Lenzerheide, Centro veterinario Albanova, Massagno (Dr. Andrea Togni and team), Kleintierpraxis Stadler, Altdorf (Dr. Thomas Stadler), Kleintierpraxis und Petshop Adlergarten, Schattdorf (Dr. Flavio Regli), Tierarztpraxis Farnenbüel, Eschenbach (Felizian Kuster), and the Cabinet vétérinaire du Littoral S.A (Dr. Pierre Sauve), Aubonne.

We also highly acknowledge Katrin Helfer-Hungerbühler for her help in sample collection, Nora Schreiber, Camilla Stella and Lea Rohner for their help in data collection and Sonja Hartnack from the section of Epidemiology of the University of Zurich and Thomas Echtermann for their help in statistical analysis.

This work was partially supported by a grant from Bayer Animal Health GmbH and represents part of the doctoral dissertation of Emily Katharina Gueldner.

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