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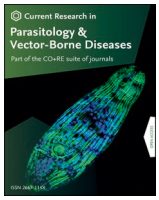

Cats as a sentinel species for human infectious diseases – toxoplasmosis, trichinellosis, and COVID-19

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ABSTRACT

In this study, serological screening for *Toxoplasma gondii*, *Trichinella* spp., and SARS-CoV-2 in domestic cats was conducted, aiming to identify their exposure to the mentioned pathogens and to assess the risk of potential human infection. In total, serum samples from 481 (310 owned and 171 shelter cats) were collected in Bratislava from September 2020 to September 2021, a period that included the initial outbreak wave of the COVID-19 pandemic. The study showed a 37.4% (135/441) seroprevalence of *T. gondii* with a slightly lower seropositivity in shelter cats (35.9%; 61/170) than in owned cats (38.4%; 104/271), but this difference was not statistically significant. Overall, the seroprevalence of *Trichinella* spp. was 2.0% (9/441), with animals from shelters being positive but not significantly more often (2.9%; 5/170) than owned cats (1.5%; 4/271). SARS-CoV-2 antibodies were detected in 2.7% (13/481) of cat sera (2.9% in shelter cats; 2.6% in owned cats). Among ten samples positive by virus neutralisation assay, two were positive for the B.1 variant. The presence of the SARS-CoV-2 virus in buccal and rectal swabs (n = 239) was not detected. The seroprevalence of almost 40% for *T. gondii* in cats suggests a non-negligible risk of human infection. The study confirmed the possibility of *Trichinella* spp. infection in cats, and thus the possibility of infection spreading between the sylvatic and synanthropic cycle *via* this animal species. The presented results also showed that the SARS-CoV-2 virus is likely to circulate in cat populations in Slovakia, not only in cats that may have been in contact with infected persons, but also in shelter cats.

1. Introduction

Over the centuries, the relationship between humans and domestic pets has evolved. Their role has changed from outdoor and working animals to companion animals. Moreover, in modern societies many stray cats and dogs live in close proximity to humans, or people take care of them in shelters. Such close and direct contact, as well as contact with the soil contaminated by their faeces may pose a higher risk of transmission of zoonotic infections to humans (Overgaauw et al., 2020).

Due to the above-mentioned facts, cats can act as a sentinel for a number of parasitic, bacterial, and viral zoonoses, as well as pollutants (Gerhold and Jessup, 2013; Álvarez-Fernández et al., 2018; Aeluro and Kavanagh, 2021). Sentinel species can be defined as animals that share a common environment with humans and can be used to measure the extent of exposure to selected infectious agents or environmental pollutants (Bost et al., 2016).

Toxoplasma gondii is an obligate intracellular protozoan parasite with a worldwide distribution. Its definitive hosts are only members of the family Felidae, while all warm-blooded animals can act as intermediate hosts and may become infected after the ingestion of any of the three infective stages, sporozoites, tachyzoites and bradyzoites. Infection is common in humans and may develop by accidental ingestion of sporulated oocysts from the environment or by consumption of raw or undercooked meat and meat products containing tissue cysts. Toxoplasmosis in healthy adults is often without symptoms or clinical signs and does not cause serious problems. However, if first contracted during

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pregnancy, *T. gondii* infection may cause abortion, neonatal death or foetal abnormalities. In immunocompromised patients, severe disease or even fatal toxoplasmosis may occur (Machala et al., 2015; Dubey et al., 2021). The prevalence of infection varies with the geographical and socioeconomic conditions, hygiene level and eating/cooking habits of the studied population, and globally ranges from less than 10% to more than 60% (Pappas et al., 2009). Cats play an important role in maintaining the life-cycle of *T. gondii*, especially due to shedding millions of environmentally resistant oocysts in faeces. They become infected after preying on small mammals and birds infected with *T. gondii*, being fed raw meat of infected animals or by ingesting oocysts from the environment. Since infected cats excrete oocysts only for a short period of time, detecting infection *via* faecal examination is difficult. Thus, the serological examination can be useful for determining the occurrence of *T. gondii* infection in the feline population (Sroka et al., 2018).

Trichinella spp. are parasitic nematodes with a worldwide distribution on all continents except for Antarctica. The infection is transmitted through the food-borne route in carnivores and in omnivores with scavenging behaviour and can cause serious health problems in infected humans (Pozio and Zarlenga, 2013; Hamed et al., 2022). In Slovakia, *Trichinella* spp. circulate primarily *via* the sylvatic cycle, with several sporadic human outbreaks registered since the 1930s (Antolová et al., 2020). The presence of three species, *T. spiralis*, *T. britovi* and *T. pseudospiralis*, has been documented in the country (Hurníková et al., 2021). Cats may become infected with *Trichinella* spp. after consuming raw meat from infected animals. Since outdoor cats in particular may hunt or scavenge on small mammals living in the vicinity of human dwellings, they can serve as suitable indicators of the presence or circulation of trichinellosis in anthropogenically used areas.

SARS-CoV-2 (severe acute respiratory syndrome coronavirus) caused the global COVID-19 pandemic. The novel virus (family Coronaviridae, genus *Betacoronavirus*) was first reported in Wuhan, China, in 2019, and rapidly spread across the globe. As of April 2024, the pandemic had caused more than 704 million cases and 7 million deaths (WHO, 2024). Circulation of coronaviruses, including SARS-CoV-2, in natural foci is associated with various species of mammals, especially bats, pangolins and civets (Su et al., 2016; Hu et al., 2021). The association of both SARS-CoV and SARS-CoV-2 with animal markets suggests animal trafficking and selling is a key part of this transmission to humans (Lytras et al., 2022). Moreover, coronaviruses have already jumped the interspecies barrier during the SARS and MERS (Middle East respiratory syndrome) epidemics.

Carnivores such as cats, dogs, ferrets and minks are susceptible to SARS-CoV-2 infection. There are reports of infections of dogs and cats from their infected owners (Tiwari et al., 2020). Cases with clinical signs of upper respiratory tract infection were reported in a tiger at the Bronx Zoo, New York, USA (McAloose et al., 2020), and in minks in a farm in Belgium (Larsen et al., 2021), with the death of some animals. All these animals were infected from humans who had COVID-19. In addition, serological screening has confirmed the presence of virus-neutralising antibodies against SARS-CoV-2 in cats in Wuhan, which confirms their susceptibility to the virus. Experimental infections in cats pointed out the possible transmission between cats (Shi et al., 2020; Gerhards et al., 2023), and transmission of the virus from infected cats back to humans has been also reported, though rarely (Sila et al., 2022).

In this study, we conducted serological screening of *T. gondii*, *Trichinella* spp. and SARS-CoV-2 in domestic cats, aiming to identify their exposure to the mentioned pathogens in the environment and to assess the risk of potential human infection in the territory of Bratislava in Slovakia.

2. Materials and methods

2.1. Collection of biological material

The sampling period ran from September 2020 to September 2021, a period that included the initial outbreak wave of the COVID-19 pandemic. In this period, sera of 481 cats (310 owned and 171 shelter cats) were collected in Bratislava, the capital of Slovakia. Shelter cats were sampled in cooperation with shelters "Sloboda zvierat" and "MačkySOS", non-profit organisations in Bratislava, during castration of the animals. Sera from owned domestic cats were obtained during the health-care visits to veterinarians. The exact origin of the majority of the cats (especially the shelter cats) was unknown; therefore, this information was not collected for statistical purposes.

The category "owned cats" encompassed animals kept in households for companionship or other animals kept under the supervision of the owner, with restricted and at least partially controlled movement in the countryside. The group of "shelter cats" comprised stray, lost, abandoned or surrendered animals that had been caught and put into shelters.

Blood samples were collected *via* venipuncture of the cephalic vein; sera were then separated and stored at −80 °C until further processing. For molecular diagnosis of SARS-CoV-2 infection, buccal and rectal swabs were collected from 239 cats (91 owned cats and 148 shelter cats) and stored in a DNA/RNA Shield solution at −80 °C.

2.2. Detection of antibodies to Toxoplasma gondii

Indirect ELISA was used for the detection of antibodies to *T. gondii* using commercially available *Toxoplasma gondii* Antigen (CD Creative Diagnostics, New York, NY, USA) prepared from the RH strain. Microtiter ELISA plates (Nunc; Maxisorp, Denmark) were coated with antigen (100 µl/well) diluted in carbonate buffer (pH 9.6). Optimal antigen dilution (3.4 µg protein/ml) was determined by previous titrations with sera of experimentally infected laboratory mice. Afterwards, 100 µl of sera, negative control serum and positive serum from cat (verified during previous studies) diluted 1:200 in 5% non-fat dry milk in a phosphate buffer (PBS; pH 7.2) were added into the wells. Each serum was loaded into two wells in parallel. Anti-cat IgG immunoglobulin (Anti-Cat IgG (H + L)-Peroxidase antibody produced in goat; Sigma-Aldrich, St. Louis, MO, USA) diluted 1:30,000 was used as the conjugate. After incubation and the washing step, the reactions were visualised by adding 100 µl of substrate (o-phenylenediamine/methanol with 0.05% H_2O_2). The reaction was stopped after 20 min using 50 µl of 4N H_2SO_4, and the optical density (OD) values were read at 490 nm. The cut-off value was calculated according to the OD values of positive controls, and samples with OD higher than 50% of the average OD values of positive controls after subtracting the OD of the "blank" wells were considered positive.

2.3. Detection of antibodies to Trichinella spp.

Indirect ELISA was used also for the detection of antibodies to *Trichinella* spp. *Trichinella spiralis* larval somatic antigen was prepared according to Reiterová et al. (1999). Microtitre plates were coated with antigen containing 1.25 µg/ml of protein diluted in carbonate buffer (pH 9.6). Serum samples (diluted 1:100) were loaded on plates in a volume of 100 µl per well. *Trichinella*-positive and *Trichinella*-negative serum from experimentally infected cats were used as controls. Anti-cat IgG immunoglobulin (Anti-Cat IgG (H + L)-Peroxidase antibody produced in goat; Sigma-Aldrich, St. Louis, MO, USA) diluted 1:30,000 in a volume of 100 µl was used as the conjugate. The cut-off value was calculated according to the OD values of positive controls, and samples with OD values higher than 50% of the average OD of positive controls after subtracting the average OD of the "blank" wells were considered positive.

2.4. Detection of antibodies to SARS-CoV-2 and molecular detection of SARS-CoV-2 virus

The presence of serum antibodies to SARS-CoV-2 infection was tested

using ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA (ID. vet, Grabels, France). The manufacturer declares no cross reactions with other pathogenic coronaviruses (e.g. feline infectious peritonitis virus; hereafter FIP). The diagnostic kit is designed to detect antibodies against the nucleocapsid (N protein) of the SARS-CoV-2 virus. In total, 25 µl of each serum, serum of a cat with confirmed FIP, and negative and positive controls were diluted in 25 µl of dilution buffer and incubated for 45 min at 37 °C. The plates were washed three times with 300 µl of washing buffer. A total of 100 µl of conjugate dilution buffer was added and incubated for 30 min at room temperature. The wash was repeated three times. The reaction was developed by adding of 100 µl of 3,30,5,50-tetramethylbenzidine (TMB), and, after incubation for 20 min at room temperature, the reaction was stopped by adding 100 µl of stop solution. Validation and interpretation of the results was done according to the manufacturer's protocol. The assay was conducted in duplicate of the samples. The OD values reported represent the average of the values obtained from the assays.

RNA from buccal and rectal swabs was extracted using the Quick-RNA Viral Kit (Zymo Research, Irvine, CA). All samples were examined by RT PCR analysis using vDetect COVID-19 RT-qPCR (MultiplexDX, Bratislava, Slovakia) with specific primers provided by the manufacturer. Temperature conditions were as follows: reverse transcription: 50 °C for 30 min; initial denaturation: 95 °C for 3 min; cycling, 45 cycles: denaturation: 95 °C for 5 s, and annealing/extension: 60 °C for 20 s.

2.5. Viral neutralisation

Vero E6 cells were seeded 24 h prior to infection at a density of 100,000 cells/well of a flat-bottom 96-well plate. After 24 h, under BSL-3 conditions, dilutions of sera with a constant amount of virus (30 PFU/well) were incubated. The incubation lasted for 1 h in a CO_2 thermostat (at 37 °C, 5% CO_2). Sera were diluted 2-fold (1:20, 1:40, 1:80, 1:160) in a serum-free culture medium (DMEM). After incubation for 1 h, the media were removed from the Vero E6 cells and replaced with the serum/virus mixture in a volume of 200 µl per well. The plates were incubated for 1 h at 37 °C in a CO_2 thermostat. After incubation, the serum/virus mixture was removed and placed 300 µl of DMEM medium and 500 µl of carboxymethyl cellulose. After 4 days of incubation, the plates were fixed with 4% paraformaldehyde for 30 min and then stained with crystal violet for 10 min. The plates were washed with water and then allowed to dry. After staining, the rate of neutralisation (serum titre) was evaluated by comparing the re-titration of the virus with the individual serum dilutions. The 80% neutralisation ability was evaluated, and at 80% neutralisation, we calculated the serum titre.

The strain Slovakia/SK-BMC5/2020 (available at https://www.european-virus-archive.com/virus/sars-cov-2-strain-slovakiask-bmc52020) represents strains circulating in Europe in the spring of 2020 and carries the Spike D614G mutation (lineage B.1).

2.6. Statistical analyses

The prevalence values of parasitic infection in cats were provided with a 95% confidence interval (95 % CI). The Chi-square (χ^2) test was used to test the differences in seropositivity of owned and shelter cats to tested pathogens, with a value of $P < 0.05$ considered significant. The statistical analyses were performed using the Quantitative Parasitology on the Web software (Reiczigel et al., 2019). The raw data supporting the findings of this study are provided in Supplementary file 1.

3. Results

3.1. Seropositivity to Toxoplasma gondii

For the seroprevalence studies of *T. gondii* and *Trichinella* spp., 441 serum samples were available; 170 sera were collected from shelter cats and 271 owned animals were sampled during their visits to veterinarians.

A total of 165 (37.4%) cats were seropositive to *T. gondii*. The seropositivity of cats derived from shelters (27.6%) was lower than the seropositivity of owned animals (38.4%), but this difference was not statistically significant ($\chi^2 = 0.28$, $P = 0.60$) (Table 1).

3.2. Seropositivity to Trichinella spp.

The overall seropositivity to *Trichinella* spp. was 2.0% (9/441). Animals coming from shelters were positive slightly more often (2.9%) than those with owners (1.5%), but the observed difference was not statistically significant ($\chi^2 = 1.13$, $P = 0.27$) (Table 1).

3.3. Seropositivity to SARS-CoV-2 and molecular PCR detection of SARS-CoV-2 virus

For testing seropositivity to SARS-CoV-2, 481 samples were tested. In total, 2.7% cat sera were positive for the purified N protein recombinant antigen of the SARS-CoV-2 virus. The positivity of shelter cats (2.9%; 5/171) was similar to the positivity of owned animals (2.6%; 8/310) (Table 1), with no significant difference between the positivity rates ($\chi^2 = 0.02$, $P = 0.90$). Among the ten samples testing positive for virus neutralisation assay, two (one owned and one shelter cat) had SARS-CoV-2 neutralising antibodies to the variant B.1. No serological cross-reactivity was detected between the test and serum of a cat with FIP.

RNA of the SARS-CoV-2 virus in buccal and rectal swabs ($n = 239$) was not detected.

3.4. Co-seropositivity of cats to Toxoplasma gondii, Trichinella spp. and SARS-CoV-2

Antibodies to *T. gondii* and *Trichinella* spp. were recorded in seven animals (1.6%); three of the four owned cats seropositive to *Trichinella* spp. were also positive to *T. gondii*, and four out of five shelter cats with antibodies to *Trichinella* also appeared to have antibodies to *T. gondii* (Table 2). Overall, the seropositivity of cats to *T. gondii* was higher with statistical significance than positivity to *Trichinella* spp. ($\chi^2 = 131.8$, $P < 0.001$).

Antibodies to *T. gondii* and SARS-CoV-2 were recorded in 0.9% of the cats (two owned cats and two shelter cats) and one shelter cat (0.2%) was positive to all three pathogens (Table 2). Overall, the difference among the seropositivity of tested cats to *T. gondii* was statistically significantly higher than the positivity to SARS-CoV-2 ($\chi^2 = 133.0$, $P < 0.001$).

The hypothesis that *T. gondii* seropositivity may represent a risk factor for *Trichinella* spp. or SARS-CoV-2 in terms of shared exposure to pathogens was also tested. Seven (4.2%) of 165 cats seropositive to *T. gondii* were also positive to *Trichinella* spp., while only two (0.7%) of *T. gondii* negative animals had anti-*Trichinella* spp. antibodies (Table 3) and this difference was statistically significant ($\chi^2 = 6.7$, $P = 0.009$). Seropositivity to SARS-CoV-2 and *T. gondii* did not correlate significantly

Table 1
Seropositivity of cats to *Toxoplasma gondii*, *Trichinella* spp. and SASR-CoV-2.

Cat category	*Toxoplasma gondii*		*Trichinella* spp.		SARS-CoV-2	
	n/N	% (95% CI)	*n/N*	% (95% CI)	*n/N*	% (95% CI)
Owned cats	104/271	38.4 (32.6–44.5)	4/271	1.5 (0.4–3.7)	8/310	2.6 (1.3–5.0)
Shelter cats	61/170	35.9 (28.7–43.6)	5/170	2.9 (1.0–6.7)	5/171	2.9 (1.3–6.7)
Total	165/441	37.4 (32.9–42.1)	9/441	2.0 (0.9–3.8)	13/481	2.7 (1.6–4.6)

Abbreviations: *n*, number of positive cats; *N*, number of examined cats; %, prevalence in %; CI, confidence interval.

Table 2
Co-seropositivity of cats to *Toxoplasma gondii*, *Trichinella* spp. and SARS-CoV-2.

Cat category	*T. gondii* + *Trichinella* spp.		*T. gondii* + SARS-Cov-2		*Trichinella* spp. + SARS-CoV-2		*T. gondii*, *Trichinella* spp. + SARS-CoV-2	
	n/N	% (95% CI)	*n/N*	% (95% CI)	*n/N*	% (95% CI)	*n/N*	% (95% CI)
Owned cats	3/271	1.1 (0.2–3.2)	2/271	0.7 (0.2–2.7)	0/271	0	0/271	0
Shelter cats	4/170	2.4 (0.6–5.9)	2/170	1.2 (0.3–4.2)	1/170	0.6 (0.1–3.3)	1/170	0.6 (0.1–3.3)
Total	7/441	1.6 (0.6–3.2)	4/441	0.9 (0.4–2.3)	1/441	0.2 (0.04–1.3)	1/441	0.2 (0.04–1.3)

Abbreviations: *n*, number of positive cats; *N*, number of examined cats; %, prevalence in %; CI, confidence interval.

Table 3
Seropositivity to *Trichinella* spp. and SARS-CoV-2 in *Toxoplasma gondii*-seropositive and seronegative cats.

Pathogen		*Trichinella* spp.		SARS-CoV-2	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
T. gondii	Positive ($n = 165$)	7 (4.2)	158 (95.8)	4 (2.5)	161 (97.6)
	Negative ($n = 276$)	2 (0.7)	274 (99.3)	8 (2.9)[a]	267[a] (97.1)

[a] One cat tested to *T. gondii* was not tested to SARS-CoV-2.

($\chi^2 = 0.07$, $P = 0.79$), four (2.5%) of 161 *T. gondii*-seropositive cats were positive to SARS-CoV-2 and eight (2.9%) of *T. gondii*-negative animals had also antibodies to the virus (Table 3).

4. Discussion

The presented study showed an overall seroprevalence for *T. gondii* of 37.4% among owned and shelter cats living in Bratislava and its surroundings, suggesting a non-negligible risk of human infection. All seropositive cats, even only for a short period of their life, shed thousands of oocysts and contaminate the environment where they live. Thus, information on the seroprevalence of infection in cats can be useful for evaluating *T. gondii* environmental contamination and the risk to public health. In Europe, seropositivity values range, for example, between 22.7% in Portugal, 40.7% in Italy, 47.6% in Hungary, 68.8–75.0% in Poland, and 62.8% in Romania (Papini et al., 2006; Hornok et al., 2008; Sroka et al., 2010, 2018; Darabus et al., 2011; Fernandes et al., 2019). In general, seropositivity to *T. gondii* in cats varies among countries, within different areas of a country and even within the same city which may be attributable to the lifestyle of cats (Dubey et al., 2020) and depends on several factors, e.g. geographical conditions, hygiene level, the feeding of animals, etc. The seroprevalence of infection is generally higher in stray and outdoor cats that can hunt for prey than in indoor pet cats. Cats fed with raw meat are at increased risk of being infected with *T. gondii* than cats fed only with dry or canned food. Differences in seropositivity can be also connected to the serological tests used, and according to Dubey et al. (2020), little information is available comparing different tests in sera from naturally exposed cats. The lower seropositivity recorded in our study, when compared to data from other European countries, can be related to lifestyle and the feeding of cats. Based on the responses of 3673 cat owners from Australia, Canada, New Zealand, the UK and the USA, 32% of cats were fed exclusively on conventional (heat-processed) foods, and almost 90% of cats were fed such products regularly (Dodd et al., 2020). This is an easy and quick way of feeding animals and similar tendencies have also been observed in Slovakia, where the majority of owned cats, both indoor and outdoor, are fed with commercial food. However, many owners also offer their animals raw meat and meat-derived products, or their cats are partially or completely outdoors and thus have the opportunity to catch rodents. In such a case, the possibility of being infected with *T. gondii* increases.

In the same way, by eating raw meat and meat products or catching rodents, cats can become infected with *Trichinella* spp. In the present study, antibodies to *Trichinella* were detected in nine (2.0%) animals, with shelter cats testing seropositive slightly more often (2.9%) than pet animals (1.5%). In total seven of the nine cats positive for *Trichinella* were also positive for *T. gondii*. In statistical analysis, seropositivity to *Trichinella* spp. was significantly higher in *T. gondii*-seropositive than in *T. gondii*-seronegative cats. It indicates the shared exposure risks to both pathogens, when especially the same feeding practices increase the risk of infection with both parasites.

In Slovakia, *Trichinella* spp. circulates mainly *via* the sylvatic cycle, with an average prevalence in 2007–2018 of 0.04% ($n = 204{,}516$) in wild boars, 9.6% ($n = 3100$) in red foxes and 1.7% ($n = 232$) in brown bears. Molecular analyses of 236 isolates from the mentioned free-living animals revealed *T. britovi* to be the predominant species (69.4%), followed by *T. spiralis* (1.4%) and *T. pseudospiralis* (0.4%). On the other hand, an examination of more than 1.8 million domestic pigs in the same period did not reveal any positive animals (Hurníková et al., 2021). In humans, trichinellosis occurs sporadically; six infection outbreaks were recorded in Slovakia between 1980 and 2008, and since then a few individual cases have been reported every year (Dubinský et al., 2016; Antolová, unpublished data). The consumption of meat and meat-derived products from wild boars is the most frequent source of human infection in Slovakia (Dubinský et al., 2016), indicating a spillover between the sylvatic and domestic cycles and the risk of infection for humans and domestic animals, including dogs and cats. The seropositivity in cats recorded in the presented study signals the contact of animals with the pathogen and subsequent production of antibodies. Correspondingly, antibodies to *Trichinella* spp. were also recorded in dogs in Slovakia. In total, 12.8% of 439 tested animals were seropositive in the study of Miterpáková et al. (2017). Similar research by Thi et al. (2013) in Vietnam revealed seropositivity to *Trichinella* in 4% of 125 dogs and none of 98 cats. In Finland, where the prevalence of sylvatic trichinellosis is generally high, 7.3% of examined dogs were positive using the ELISA method (Oivanen et al., 2005). Even though epidemiological studies in feline and canine populations are scarce, they confirm the possibility of infection of domestic carnivores and the spread of infection between the sylvatic and synanthropic cycles.

In the present study, the overall seroprevalence of SARS-CoV-2 virus in cats living in Bratislava and its surroundings was 2.7% (2.6% in owned cats and 2.9% in feral cats). Although there was no significant difference between shelter and owned cats, shelter cats can be assumed to be more exposed to infections than owned cats, as they move freely outdoors, being in contact with different people and environments (Chalkowski et al., 2019).

Several other studies tested seropositivity to the virus in cat populations. The seroprevalence of SARS-CoV-2 in domestic cats during the first pandemic wave in Europe in April-August 2020 was 4.2% in Germany, 3.3% in the UK, 4.2% in Italy and 6.4% in Spain (Schulz et al., 2021). In 2021–2022, the prevalence of antibodies in cats from Europe varied from 1.3% in Italy (Bianco et al., 2023), 2.7% in the Netherlands

(Duijvestijn et al., 2023), 3.2% in the UK (Tyson et al., 2023) to 4.6% in Spain (Sánchez-Morales et al., 2023). In Portugal, Oliveira et al. (2022) found a correlation between contact of cats with infected people and the occurrence of antibodies to the virus, as the seropositivity of cats from COVID-19-positive and -negative households was 5.0% and 0.7%, respectively.

Out of ten ELISA-positive samples evaluated for the presence of SARS-CoV-2 neutralising antibodies, two were positive (one owned and one shelter cat) to the original Wuhan variant. This variant was used for the test because the majority of the selected positive sera were from the period when this variant dominated in our country. However, the occurrence of another variant in Slovakia during the sampling period is not excluded either.

In the present study, the SARS-CoV-2 virus was not detected in buccal and rectal swabs. At the time of the first wave of the pandemic, SARS-CoV-2 RNA was not detected in swab specimens from cats in Italy (Patterson et al., 2020) or in Rio de Janeiro (Dias et al., 2021), while in Hong Kong and Texas (USA) viral RNA was detected in 12% (6/50) and 17.6% (3/17) of domestic cats, respectively (Barrs et al., 2020; Hamer et al., 2021). In Italy, 0.4% of owned cats (1/260) were positive for the virus (Klaus et al., 2021). However, direct detection of the virus by RT-PCR in cats was logistically complicated, since owners with acute COVID-19 disease were quarantined.

Both humans and cats are susceptible to the SARS-CoV-2 virus and the course of the disease may be asymptomatic/subclinical, which increases the risk of disease transmission. The households of patients with COVID-19 are contaminated with SARS-CoV-2; thus, domestic animals living with infected people are directly exposed to the virus. Since the possible circulation and persistence of the SARS-CoV-2 virus in the stray cat population was confirmed (Spada et al., 2021), cats with a free outdoor range may represent a risk factor for transmission of infection to other cats (owned or stray). Fear among the public that pets might play a role in spreading COVID-19 had a negative impact on the welfare of animals during the pandemic (Parry, 2020); therefore, knowing the circulation of the virus between humans and animals and domestic and wild animals can be crucial from the point of view of a possible pandemic outbreak.

5. Conclusions

The seroprevalence of almost 40% for *T. gondii* recorded in this study suggests a non-negligible risk of human infection. The study also confirmed the possibility of *Trichinella* spp. infection in cats and thus the possibility of infection spreading between sylvatic and synanthropic cycles *via* this animal species. The present results showed that SARS-CoV-2 virus is likely to circulate in cat populations in Slovakia, not only in cats that may have been in contact with infected persons but also in stray cats. Whether shelter cats have become infected after contact with a contaminated environment or other infected cats or animals should be the subject of further research. Human-to-pet transmission of the virus can be minimised by hygiene and by minimising contact between infected people and animals.

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Ethical approval

This study was conducted according to the guidelines of the Declaration of Helsinki, as revised in 2013, and approved by the Ethics Committee of the Institute of Parasitology, SAS, under the Statement EK/01/2020 issued on 30 July 2020. All cat owners agreed with the participation in the study and signed informed consent forms.

CRediT authorship contribution statement

Diana Selyemová: Validation, Methodology, Resources, Writing – original draft, Writing – review & editing. **Daniela Antolová:** Validation, Funding acquisition, Methodology, Resources, Writing – original draft, Writing – review & editing. **Barbara Mangová:** Formal analysis, Writing – review & editing. **Júlia Jarošová:** Methodology, Writing – review & editing. **Martina Ličková:** Methodology, Validation, Writing – review & editing. **Sabína Fumačová Havlíková:** Methodology, Writing – review & editing. **Monika Sláviková:** Methodology, Writing – review & editing. **Veronika Rusňáková Tarageľová:** Methodology, Writing – review & editing. **Markéta Derdáková:** Conceptualization, Funding acquisition, Writing – review & editing, All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article. The raw data generated in the study are provided in Supplementary file 1.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2024.100196.

References

Aeluro, S., Kavanagh, T.J., 2021. Domestic cats as environmental lead sentinels in low-income populations: A One Health pilot study sampling the fur of animals presented to a high-volume spay/neuter clinic. Environ. Sci. Pollut. Res. Int. 28, 57925–57938. https://doi.org/10.1007/s11356-021-14769-7.

Álvarez-Fernández, A., Breitschwerdt, E.B., Solano-Gallego, L., 2018. *Bartonella* infections in cats and dogs including zoonotic aspects. Parasites Vectors 11, 624. https://doi.org/10.1186/s13071-018-3152-6.

Antolová, D., Fecková, M., Valentová, D., Hurníková, Z., Miklisová, D., Avdičová, M., et al., 2020. Trichinellosis in Slovakia - epidemiological situation in humans and animals (2009–2018). Ann. Agric. Environ. Med. 27, 361–367. https://doi.org/10.26444/aaem/125194.

Barrs, V.R., Peiris, M., Tam, K.W.S., Law, P.Y.T., Brackman, C.J., To, E.M.W., et al., 2020. SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. Emerg. Infect. Dis. 26, 3071–3074. https://doi.org/10.3201/eid2612.202786.

Bianco, A., Bortolami, A., Miccolupo, A., Sottili, R., Ghergo, P., Castellana, S., et al., 2023. SARS-CoV-2 in animal companions: A serosurvey in three regions of southern Italy. Life 13, 2354. https://doi.org/10.3390/life13122354.

Bost, P.C., Strynar, M.J., Reiner, J.L., Zweigenbaum, J.A., Secoura, P.L., Lindstrom, A.B., et al., 2016. U.S. domestic cats as sentinels for perfluoroalkyl substances: Possible linkages with housing, obesity, and disease. Environ. Res. 151, 145–153. https://doi.org/10.1016/j.envres.2016.07.027.

Chalkowski, K., Wilson, A.E., Lepczyk, C.A., Zohdy, S., 2019. Who let the cats out? A global meta-analysis on risk of parasitic infection in indoor *versus* outdoor domestic cats (*Felis catus*). Biol. Lett. 15, 20180840 https://doi.org/10.1098/rsbl.2018.0840.

Darabus, G., Hotea, I., Oprescu, I., Morariu, S., Brudiu, I., 2011. Toxoplasmosis seroprevalence in cats and sheep from western Romania. Revue Méd. Vét 162, 316–320. https://doi.org/10.1186/s13071-022-05634-8.

Dias, H.G., Resck, M.E.B., Caldas, G.C., Resck, A.F., da Silva, N.V., Dos Santos, A.M.V., et al., 2021. Neutralizing antibodies for SARS-CoV-2 in stray animals from Rio de Janeiro, Brazil. PLoS One 16, e0248578. https://doi.org/10.1371/journal.pone.0248578.

Dodd, S., Cave, N., Abood, S., Shoveller, A.K., Adolphe, J., 2020. An observational study of pet feeding practices and how these have changed between 2008 and 2018. Vet. Rec. 186, 643.. https://doi.org/10.1136/vr.105828.

Dubey, J.P., Cerqueira-Cézar, C.K., Murata, F.H.A., Kwok, O.C.H., Yang, Y.R., Su, C., 2020. All about toxoplasmosis in cats: the last decade. Vet. Parasitol. 283, 109145 https://doi.org/10.1016/j.vetpar.2020.109145.

Dubey, J.P., Murata, F.H.A., Cerqueira-Cézar, C.K., Kwok, O.C.H., Villena, I., 2021. Congenital toxoplasmosis in humans: An update of worldwide rate of congenital infections. Parasitology 148, 1406–1416. https://doi.org/10.1017/S0031182021001013.

Dubinský, P., Antolová, D., Reiterová, K., 2016. Human *Trichinella* infection outbreaks in Slovakia, 1980–2008. Acta Parasitol. 61, 205–211. https://doi.org/10.1515/ap-2016-0029.

Duijvestijn, M.B.H.M., Schuurman, N.N.M.P., Vernooij, J.C.M., van Leeuwen, M.A.J.M., Bosch, B.J., van den Brand, J.M.A., et al., 2023. Serological survey of retrovirus and coronavirus infections, including SARS-CoV-2, in rural stray cats in The Netherlands, 2020–2022. Viruses 15, 1531. https://doi.org/10.3390/v15071531.

Fernandes, S., Brilhante-Simões, P., Coutinho, T., Cardoso, L., Dubey, J.P., Lopes, A.P., 2019. Comparison of indirect and modified agglutination tests for detection of antibodies to *Toxoplasma gondii* in domestic cats. J. Vet. Diagn. Invest. 31, 774–777. https://doi.org/10.1177/1040638719868753.

Gerhards, N.M., Gonzales, J.L., Vreman, S., Ravesloot, L., van den Brand, J.M.A., Doekes, H.P., et al., 2023. Efficient direct and limited environmental transmission of SARS-CoV-2 lineage B.1.22 in domestic cats. Microbiol. Spectr. 11, e0255322 https://doi.org/10.1128/spectrum.02553-22.

Gerhold, R.W., Jessup, D.A., 2013. Zoonotic diseases associated with free-roaming cats. Zoonoses Public Health 60, 189–195. https://doi.org/10.1111/j.1863-2378.2012.01522.x.

Hamed, A.M.R., Abdel-Shafi, I.R., Elsayed, M.D.A., Mahfoz, A.M., Tawfeek, S.E., Abdeltawab, M.S.A., 2022. Investigation of the effect of curcumin on oxidative stress, local inflammatory response, COX-2 expression, and microvessel density in *Trichinella spiralis* induced enteritis, myositis and myocarditis in mice. Helminthologia 59, 18–36. https://doi.org/10.2478/helm-2022-0002.

Hamer, S.A., Pauvolid-Corrêa, A., Zecca, I.B., Davila, E., Auckland, L.D., Roundy, C.M., et al., 2021. SARS-CoV-2 infections and viral isolations among serially tested cats and dogs in households with infected owners in Texas, USA. Viruses 13, 938. https://doi.org/10.3390/v13050938.

Hornok, S., Edelhofer, R., Joachim, A., Farkas, R., Berta, K., Répási, A., et al., 2008. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infection of cats in Hungary. Acta Vet. Hung. 56, 81–88. https://doi.org/10.1556/AVet.56.2008.1.8.

Hu, B., Guo, H., Zhou, P., Shi, Z.L., 2021. Characteristics of SARS-CoV-2 and COVID-19. Nat. Rev. Microbiol. 19, 141–154. https://doi.org/10.1038/s41579-020-00459-7.

Hurníková, Z., Miterpáková, M., Zaleśny, G., Komorová, P., Chovancová, G., 2021. Fifteen years since the first record of *Trichinella pseudospiralis* in Slovakia: What's new? Vet. Parasitol. 297, 109129 https://doi.org/10.1016/j.vetpar.2020.109129.

Klaus, J., Zini, E., Hartmann, K., Egberink, H., Kipar, A., Bergmann, M., et al., 2021. SARS-CoV-2 infection in dogs and cats from southern Germany and northern Italy during the first wave of the COVID-19 pandemic. Viruses 13, 1453. https://doi.org/10.3390/v13081453.

Larsen, H.D., Fonager, J., Lomholt, F.K., Dalby, T., Benedetti, G., Kristensen, B., et al., 2021. Preliminary report of an outbreak of SARS-CoV-2 in mink and mink farmers associated with community spread, Denmark, June to November 2020. Euro Surveill. 26, 2100009 https://doi.org/10.2807/1560-7917.ES.2021.26.5.210009.

Lytras, S., Hughes, J., Martin, D., Swanepoel, P., de Klerk, A., Lourens, R., et al., 2022. Exploring the natural origins of SARS-CoV-2 in the light of recombination. Genome Biol. Evol. 14 https://doi.org/10.1093/gbe/evac018.

Machala, L., Kodym, P., Malý, M., Geleneky, M., Beran, O., Jilich, D., 2015. [Toxoplasmosis in immunocompromised patients]. Epidemiol. Mikrobiol. Imunol. 64, 59–65 (In Czech).

McAloose, D., Laverack, M., Wang, L., Killian, M.L., Caserta, L.C., Yuan, F., et al., 2020. From people to *Panthera*: Natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. mBio 11, e02220. https://doi.org/10.1128/mBio.02220-20, 20.

Miterpáková, M., Antolová, D., Hurníková, Z., Březinová, N., Čabanová, V., Reiterová, K., 2017. Seroprevalence of *Trichinella* infections in domestic dogs from Slovakia. J. Helminthol. 91 (5), 549–554. https://doi.org/10.1017/S0022149X16000602.

Oivanen, L., Nareaho, A., Jokela, S., Rikula, U., Gamble, R., Sukura, A., 2005. The prevalence of *Trichinella* infection in domestic dogs in Finland. Vet. Parasitol. 132, 125–129. https://doi.org/10.1016/j.vetpar.2005.05.040.

Oliveira, A., Pereira, M.A., Mateus, T.L., Mesquita, J.R., Vala, H., 2022. Seroprevalence of SARS-CoV-2 in client-owned cats from Portugal. Vet. Sci. 9, 363. https://doi.org/10.3390/vetsci9070363.

Overgaauw, P.A.M., Vinke, C.M., Hagen, M.A.E.V., Lipman, L.J.A., 2020. A One Health perspective on the human-companion animal relationship with emphasis on zoonotic aspects. Int. J. Environ. Res. Publ. Health 17, 3789. https://doi.org/10.3390/ijerph17113789.

Papini, R., Sbrana, C., Rosa, B., Saturni, A.M., Sorrentino, A.M., Cerretani, M., et al., 2006. Serological survey of *Toxoplasma gondii* infections in stray cats from Italy. Rev. Med. Vet. 157, 193–196.

Pappas, G., Roussos, N., Falagas, M.E., 2009. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int. J. Parasitol. 39, 1385–1394. https://doi.org/10.1016/j.ijpara.2009.04.003.

Parry, N.M.A., 2020. COVID-19 and pets: When pandemic meets panic. Forensic Sci. Int. Rep. 2, 100090 https://doi.org/10.1016/j.fsir.2020.100090.

Patterson, E.I., Elia, G., Grassi, A., Giordano, A., Desario, C., Medardo, M., et al., 2020. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat. Commun. 11, 6231. https://doi.org/10.1038/s41467-020-20097-0.

Pozio, E., Zarlenga, D.S., 2013. New pieces of the *Trichinella* puzzle. Int. J. Parasitol. 43, 983–997. https://doi.org/10.1016/j.ijpara.2013.05.010.

Reiczigel, J., Marozzi, M., Fabian, I., Rozsa, L., 2019. Biostatistics for parasitologists – a primer to quantitative parasitology. Trends Parasitol. 35, 277–281. https://doi.org/10.1016/j.pt.2019.01.003.

Reiterová, K., Dubinský, P., Klimenko, V.V., Tomašovičová, O., Dvorožňáková, E., 1999. Comparison of *Trichinella spiralis* larva antigens for the detection of specific antibodies in pigs. Vet. Med. Czech. 44, 1–5.

Sánchez-Morales, L., Sánchez-Vizcaíno, J.M., Domínguez, L., Barroso-Arévalo, S., 2023. A retrospective study of SARS-CoV-2 seroprevalence in dogs and cats in the community of Madrid, Spain. Front. Microbiol. 14, 1264172 https://doi.org/10.3389/fmicb.2023.1264172.

Schulz, C., Martina, B., Mirolo, M., Müller, E., Klein, R., Volk, H., et al., 2021. SARS-CoV-2-specific antibodies in domestic cats during first COVID-19 wave, Europe. Emerg. Infect. Dis. 27, 3115–3118. https://doi.org/10.3201/eid2712.211252.

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., et al., 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science 368, 1016–1020. https://doi.org/10.1126/science.abb7015.

Sila, T., Sunghan, J., Laochareonsuk, W., Surasombatpattana, S., Kongkamol, C., Ingviya, T., et al., 2022. Suspected cat-to-human transmission of SARS-CoV-2, Thailand, July-September 2021. Emerg. Infect. Dis. 28, 1485–1488. https://doi.org/10.3201/eid2807.212605.

Spada, E., Vitale, F., Bruno, F., Castelli, G., Reale, S., Perego, R., et al., 2021. A pre- and during pandemic survey of Sars-Cov-2 infection in stray colony and shelter cats from a high endemic area of northern Italy. Viruses 13, 618. https://doi.org/10.3390/v13040618.

Sroka, J., Karamon, J., Dutkiewicz, J., Wójcik Fatla, A., Zajac, V., Cencek, T., 2018. Prevalence of *Toxoplasma gondii* infection in cats in southwestern Poland. Ann. Agric. Environ. Med. 25 (3), 1–5. https://doi.org/10.26444/aaem/94675.

Sroka, J., Wójcik-Fatla, A., Szymańska, J., Dutkiewicz, J., Zając, V., Zwoliński, J., 2010. The occurrence of *Toxoplasma gondii* infection in people and animals from rural environment of Lublin region – estimate of potential role of water as a source of infection. Ann. Agric. Environ. Med. 17, 125–132.

Su, S., Wong, G., Shi, W., Liu, J., Lai, A.C.K., Zhou, J., et al., 2016. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 24, 490–502. https://doi.org/10.1016/j.tim.2016.03.003.

Thi, N.V., De, N.V., Prate, N., Claes, L., Gabriël, S., Dorny, P., 2013. Seroprevalence of trichinellosis in domestic animals in northwestern Vietnam. Vet. Parasitol. 193, 200–205. https://doi.org/10.1016/j.vetpar.2012.11.023.

Tiwari, R., Dhama, K., Sharun, K., Mm, Yatoo I., Malik, Y.S., Singh, R., et al., 2020. COVID-19: animals, veterinary and zoonotic links. Vet. Q. 40, 169–182. https://doi.org/10.1080/01652176.2020.1766725.

Tyson, G.B., Jones, S., Logan, N., McDonald, M., Marshall, L., Murcia, P.R., et al., 2023. SARS-CoV-2 seroprevalence and cross-variant antibody neutralization in cats, United Kingdom. Emerg. Infect. Dis. 29, 1223–1227. https://doi.org/10.3201/eid2906.221755.

WHO, 2024. WHO COVID-19 dashboard. https://data.who.int/dashboards/covid19. (Accessed 4 April 2024).