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PROTOZOOLOGY - ORIGINAL PAPER

Comparison of a commercial modified direct agglutination test and a commercial enzyme-linked immunosorbent assay for screening for antibodies against *Toxoplasma gondii* in naturally exposed domestic cats

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Abstract

Domestic cats and other felids are definitive hosts for the zoonotic protozoan parasite *Toxoplasma gondii*. Serology is widely used in epidemiological studies conducted to estimate the proportion of domestic cats that have encountered the parasite. However, a limited number of such studies are available from some regions, including eastern parts of Europe and Russia. Various serological tests have been applied for *T. gondii* serology for feline samples. Seropositivity indicates previous exposure, and seropositive cats are presumed to have shed oocysts of the parasite earlier and to be chronically infected. In this study, we included a random sample of 200 sera and plasma samples from a larger sampling frame comprising samples from domestic cats from Estonia, where *T. gondii* is common. The samples, which had been previously screened for anti-*T. gondii* immunoglobulin G antibodies using a commercial modified direct agglutination test (DAT: Toxo-Screen DA; bioMérieux SA, Marcy-l'Étoile, France), were screened using a commercial enzyme-linked immunosorbent assay (ELISA: VectoToxo-antibodies [VektoToksoantytila], VectorBest, Novosibirsk, Russian Federation). The cut-off for seropositivity with DAT was titer of 40. Of the 200 samples, 120 (60.0%) tested positive with DAT and 114 (57.0%) tested positive with ELISA; 112 samples (56.0%) tested positive with both tests. Percent agreement of 95.0% and Kappa 0.8971 indicated an almost perfect agreement between the screening results using the two methods. The results of this study can be useful for comparison, evaluation, and interpretation of results obtained with these two tests in seroepidemiological studies and may encourage more studies on the topic from eastern parts of Europe and Russia.

Keywords Antibodies · Cat · DAT · ELISA · Feline · Toxoplasmosis

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Introduction

Toxoplasma gondii is a protozoan parasite that can infect warm-blooded animals, including domestic cats and humans (Dubey 2010). The parasite has been shown to reproduce sexually only in felids, which can result in shedding of high numbers of oocysts into the environment (Dabritz and Conrad 2010). After sporulation, the oocysts are highly resistant to environmental conditions and can remain infective for long periods of time (Dubey 1998, 2010).

Domestic cats are epidemiologically important hosts for *T. gondii*. Serology is widely used in epidemiological studies conducted to estimate the proportion of domestic cats that have encountered *T. gondii*. Seropositive cats are presumed to have shed oocysts of the parasite after the infection and to be chronically infected (Dubey 2010; Lappin 2010). Various

serological tests have been applied for feline samples. An in-house modified agglutination test (MAT) and a commercial modified direct agglutination test (DAT; Toxo-Screen DA; bioMérieux SA, Marcy-l'Étoile, France) are widely used for several host species, including cats (Dubey 2010; Jokelainen et al. 2012; Must et al. 2015). Based on literature searches, MAT and DAT do not appear to be used in Russian-speaking regions. In Ukraine, a commercial enzyme-linked immunosorbent assay (ELISA; VectoToxo-antibodies [VektoToksoantytila], VectorBest, Novosibirsk, Russian Federation) has been used for testing different host species (Rissanen et al. 2019), but literature searches did not identify any publications applying it for feline samples.

The aim of this study was to compare the two commercial serological tests, the DAT and the ELISA, for screening for antibodies against *T. gondii* in serum and plasma samples from cats.

Materials and methods

Ethical statement

The samples were surplus from sera and plasma samples that had been collected for unrelated diagnostic purposes from pet cats and shelter cats in Estonia, and used for a seroepidemiological *T. gondii* study (Must et al. 2015). Participation in the seroepidemiological study was voluntary. The owners of the pet cats signed an informed consent form, and the animal shelter provided an oral consent. Data were treated confidentially, and only the research group of the original seroepidemiological study handled the background information of the cats. In this study, the samples were stored and tested coded.

Samples

The sampling frame was 490 sera and plasmas used in the previous seroepidemiological study, comprising samples from domestic cats of different ages and both sexes from Estonia, northeastern Europe, where *T. gondii* is common: 60.8% of them tested *T. gondii* seropositive with DAT (Must et al. 2015). That is, the samples had been already tested with DAT.

The random selection of 200 samples for this study was done using random number generator of Stata 13.1 (Stata Corporation, TX, USA). If there was insufficient amount of serum or plasma left for the ELISA, that sample was excluded and the sample indicated by the next random number was included in the study.

The samples had been collected in 2013 and tested with DAT in 2014, and they had been stored frozen and thawed altogether up to five times before testing with ELISA in 2018.

Modified direct agglutination test

The samples had been screened for presence of specific immunoglobulin G antibodies against *T. gondii* using the commercial modified direct agglutination test (Toxo-Screen DA; bioMérieux SA, Marcy-l'Étoile, France), using only a single dilution but otherwise following the instructions of the manufacturer, in our earlier seroepidemiological study (Must et al. 2015). In this test, possible immunoglobulin M antibodies are denatured by 2-mercaptoethanol; the test only detects specific anti-*T. gondii* immunoglobulin G antibodies. The kit is intended for human samples, but the method does not include any host-species-specific reagents and it has been used for numerous different host species, including domestic cats (Dubey 2010; Jokelainen et al. 2012; Must et al. 2015, 2017).

This testing was performed in Estonia. The samples were tested at a single dilution, 1:40. Each plate included the negative and positive controls of the kit, as well as control for autoagglutination (no sample, all reagents). The results were read after 18 h using a 4-point scale: "0" for a button, "1" for a ring or a mat covering less than half of the bottom of the well, "2" for a large mat covering at least half of the bottom of the well, and "3" for an unshrunken mat covering the bottom of the well (Jokelainen 2013). Samples that tested clearly positive (an agglutination mat covering at least half of the bottom of the well, "2" or "3") were considered positive. The cut-off for seropositivity was thus a titer of 40.

Enzyme-linked immunosorbent assay

The sera were tested for presence of total antibodies against *T. gondii* using the commercial ELISA (VectoToxo-antibodies [VektoTokso-antytila], VectorBest, Novosibirsk, Russian Federation), following the instructions of the manufacturer. In this test, the sera are pipetted on the plate without a preceding dilution step. This kit is intended for the detection of total antibodies against *T. gondii* in human or animal serum or plasma; however, it should be noted that it does not mention cats specifically and that at present, it is marketed in Ukraine for use for human samples only. The kit does not provide details about the test (e.g., type of antigen).

This testing was carried out in Ukraine. Each plate included the negative and positive controls of the kit. Optical density was measured at 450 nm using an iMark Microplate Absorbance Reader (BIO-RAD). The samples were tested blinded: coded so that the performer of the test did not know whether the sample was negative or positive with DAT. The cut-off optical density was obtained by adding 0.3 to the arithmetic mean optical density of the negative controls on the same plate. The samples with optical density equal to or higher than the cut-off optical density were considered positive, and those with lower optical density were considered negative. We also calculated an ELISA proportion index for

each sample: optical density of the sample/mean optical density of the negative controls on the plate *100.

Comparison of the tests

Proportions were compared using two-by-two tables (2-tailed *P* value, Mid-*P* exact) of OpenEpi (Dean et al. 2018). The level of agreement between the two independent categorical results, seropositivity with DAT and seropositivity with ELISA, was assessed by calculating percent agreement and Kappa with Stata 13.1 (Stata Corporation, TX, USA).

Data availability All data generated or analyzed during this study are included in this published article.

Results

The sample of 200 cats included 12 (6.0%) young cats (< 12 months old) and 181 (90.5%) adults (≥ 12 months old); for seven (3.5%) cats, the age group was unknown. Of the cats included in the sample, 90 (45.0%) were females and 101 (50.5%) were males; for nine (4.5%) of the cats, the sex was unknown.

Of the 200 cats, 120 (60.0%, 95% CI 53.09–66.62) had tested seropositive with DAT and 114 (57.0%, 95% CI 50.06–63.74) tested seropositive with ELISA (Table 1). These estimates of proportion *T. gondii* seropositive did not differ statistically significantly from each other (*P* value 0.5447), and both confidence intervals included the *T. gondii* seroprevalence estimate of the sampling frame (Must et al. 2015).

Altogether, 112 (56.0%, 95% CI 49.06–62.77) of the cats tested seropositive with both DAT and ELISA (the two tests used in series), and 122 (61.0%, 95% CI 54.10–67.58) of the cats tested seropositive with at least one of the two tests (the two tests used in parallel). Altogether, 78 of the cats tested seronegative with both tests.

Eight samples tested positive only with DAT (Table 1). Of these eight samples, seven had a DAT result of "2" and one had a DAT result of "3" on the 4-point scale; their ELISA proportion indexes were 28–701 (mean, 176) and 58, respectively. Two samples tested positive only with ELISA (Table 1). One of these had a DAT result "0" and the other "1"; their ELISA proportion indexes were 547 and 316, respectively.

The percent agreement between the two tests was 95.00%, and Kappa was 0.8971 (standard error 0.0706), indicating almost perfect agreement. Figure 1 shows the ELISA proportion indexes by the DAT results.

Discussion

The zoonotic parasite *T. gondii* has a global distribution, and different tests for detecting the infection are available and used in different parts of the world. Comparison of *T. gondii* seroprevalence estimates that have been obtained using different tests is challenging. Comparing the performance of different tests in different host species is therefore useful, as it allows evaluation and comparison of results obtained with them. For domestic cats, e.g., MAT and a commercial ELISA kit have been previously compared (Zhu et al. 2012), and in this study, we compared a commercial DAT and another commercial ELISA. Other studies have compared the performance of other serological tests (e.g., Lappin and Powell 1991; Ljungström et al. 1994; Dabritz et al. 2007; Macrì et al. 2009; Al-Adhami and Gajadhar 2014). Studies like these have the potential of widening the usability of results obtained with tests that are available and used in different parts of the world.

The agreement between the results from the DAT and the ELISA was almost perfect, and the apparent estimates of proportion *T. gondii* seropositive that were yielded with the two tests did not differ statistically significantly from each other. There are several possible explanations for the 10 disagreeing results (Table 1), which comprised 5% of all results. Serology tests are indirect methods, i.e., they detect the reaction of the host to the parasite; here, it should be emphasized that the DAT detects only immunoglobulin G antibodies whereas the ELISA detects total antibodies. In the initial phase of infection, serology tests, especially the DAT that detects only one antibody class, might yield false negative results. The cut-off we used for seropositivity with DAT is the one recommended for human samples by the manufacturer, and we have used this cut-off in other studies for feline samples (Jokelainen et al. 2012, Must et al. 2015, 2017). However, this cut-off may be

Table 1 Agreement between *Toxoplasma gondii* serology screening results obtained using a commercial modified direct agglutination test (DAT) and a commercial enzyme-linked immunosorbent assay (ELISA): the number of domestic cats that tested seropositive and seronegative with the tests are shown

	Seronegative with DAT	Seropositive with DAT	Total
Seronegative with ELISA	78	8	86
Seropositive with ELISA	2	112	114
Total	80	120	200

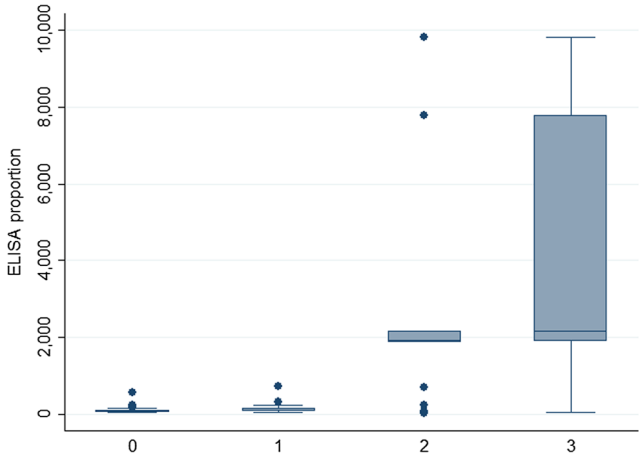

Fig. 1 A box plot of *Toxoplasma gondii* serology results obtained using a commercial modified direct agglutination test (DAT) and a commercial enzyme-linked immunosorbent assay (ELISA). DAT results using a 4-point scale are shown on the *X*-axis, and ELISA proportion indexes of the samples (optical density value of each sample/the mean optical density of negative controls *100) are shown on the *Y*-axis. DAT results "0" and "1" are negative and "2" and "3" are positive

considered relatively high. Relatively high cut-off could explain the two samples testing negative with DAT and positive with ELISA, as their ELISA proportion indexes were relatively low: it remains possible that the two cats were seropositive but had a low titer. Moreover, there may be some false negative results with DAT due to the prozone phenomenon, which occurs in the presence of high titers: too many antibodies in the sample for an agglutination mat to be formed. The two samples that tested negative with DAT and positive with ELISA might have been false negative with DAT because of this phenomenon; however, of the different explanations, this is perhaps unlikely as their ELISA proportion indexes were relatively low. False negative results due to prozone phenomenon can be avoided by using several dilutions for DAT, as the samples will test positive when sufficiently diluted. This was not done for these samples (Must et al. 2015) due to the limited benefit expected from using several dilutions (Jokelainen et al. 2012), the cost of the test, and the limited amount of sample available from some of the cats. Furthermore, for the eight samples that were positive with DAT and negative with ELISA, the long storage period and the repeated freezing-thawing before the ELISA was performed may be potential explanations for the disagreeing results, as these may negatively affect the antibodies. On the other hand, our results indicate that feline sera and plasma can be used for estimating *T. gondii* seroprevalence even after long storage and freeze-thaw cycles without major effect. This is useful information for planning studies, and generally in line with what has been reported, e.g., for rabbit meat juice samples and human sera previously (Mecca et al. 2011; Dard et al. 2017). Furthermore, it should be emphasized that the reading of results is more subjective for the DAT than for the ELISA, because the DAT plates are read by a person and the ELISA plates are read by a machine. Despite the DAT results were read by researchers who are experienced in using the method, interpretation error is possible. Several of the samples with disagreeing results had been scored one of the two middle options of the 4-point scale for DAT. As the true serostatus of the cats remains unknown, false positive results remain possible, and potential cross-reactions were not directly evaluated. Finally, the DAT could simply be more sensitive: MAT has been evaluated to be a sensitive test for the detection of *T. gondii* infection in experimentally infected cats (Dubey et al. 1995). In summary, there are several potential explanations for the disagreeing results, which were altogether relatively few in number and did not significantly affect the overall estimate of proportion of seropositives.

It should be emphasized that our focus was to compare the two methods for their applicability for screening the extent of exposure to *T. gondii* in feline populations in the context of observational seroepidemiological studies, not for detecting *T. gondii* infections of individual cats in the context of clinical diagnostic work. It should also be emphasized that the sample material was not optimal for the tests: using samples after long storage and freeze-thaw cycles is inconsistent with the recommendations of the test manufacturers; however, this is often a reality for samples available for seroepidemiological studies. The opportunity to optimize the performance of either of the test, e.g., by testing several dilutions, was not explored in this study due to the limited amount of sample material available as well as limited resources. For example, testing the samples in several dilutions with the DAT could have resulted in even better agreement between the tests. However, the agreement appeared so good using single dilution that further increase would be of limited value for screening purposes.

The *T. gondii* infections in the sampled population were naturally acquired, which is both a strength and a limitation of this study. The true serostatus and infection status of the cats included in this study remain unknown. Moreover, details that remain unknown for the infected cats include the strain of the parasite, the infection dose, the infection route, when the infection was acquired, and whether the cats had been infected several times. For all the cats, it remains unknown whether they had other infections that could potentially cause cross-reactions in serology and whether they had concomitant other diseases that could have affected their immunological responses. However, in the real-life situation in which serology is applied for screening cats for exposure to *T. gondii* in seroepidemiological studies, these details are also often unknown.

Conclusions

The two serological tests evaluated in this study for using in screening the extent of exposure to *T. gondii* in feline populations

in the context of observational seroepidemiological studies showed an almost perfect agreement. The results of this study, as well as the discussion about the performance of the two tests, can be useful when comparing, evaluating, and interpreting *T. gondii* serology results obtained with these tests in seroepidemiological studies. This might encourage more studies on the topic from eastern parts of Europe and Russia.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed Consent The owners of the pet cats signed an informed consent form, and the animal shelter provided an oral consent. The samples were stored and tested coded.

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