Exposure to *Toxoplasma gondii* and *Neospora caninum* in free-ranging and captive wild animals

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ABSTRACT

Toxoplasma gondii (T. gondii) and *Neospora caninum (N. caninum)* are apicomplexan parasites with definitive hosts from the order Carnivora and with a wide range of intermediate hosts. Information concerning the prevalence of antibodies to these parasites in wild animals is limited. In the current study, sera samples from four Israeli zoological gardens (n=110) and from free-ranging wild animals in Israel (n=149) were tested. All sera were analyzed by Modified Agglutination Test (MAT) for initial screening, followed by confirmation of all positive samples and a portion of negative samples by in-house Immunofluorescence Antibody Test (IFAT). Antibodies to *T. gondii* were detected in 67 of 259 animals (25.87%). Antibodies to *N. caninum* were detected in 18 of 259 animals (6.95%). *T. gondii* exposure tended to be higher in zoo animals as compared to free-ranging wild animals (30.9%) and (22.15%), respectively; (p=0.056), showing almost significantly higher risk of infection for zoo animals (OR=1.57 (CI (p (95%)): 0.9-2.75)). *N. caninum* exposure was found to be significantly higher in free-ranging animals (10.07%) as compared to zoo animals (2.73%, p=0.021), showing a higher risk of infection for free-ranging animals (OR=3.99 (CI (p (95%)): 1.13-14.15)). In addition, seropositivity to *T. gondii* was found to differ significantly between zones of different climates, with the lowest exposure measured in desert climates and the highest in Mediterranean climates with an average of up to 600 ml precipitation per year (p=0.008). The results of this study indicate that wild animals in Israel are more exposed to *T. gondii* than to *N. caninum*. In addition, it appears that zoo animals are at higher risk for *T. gondii* infection and at lower risk for *N. caninum* infection when compared to free-ranging wildlife. Moreover, our results suggest that MAT can be used as an efficient screening technique for *T. gondii* exposure in wildlife.

Keywords: IFAT; MAT; *Neospora caninum*; Serological survey; *Toxoplasma gondii*; Wildlife.

INTRODUCTION

Toxoplasma gondii (T. gondii) and *Neospora caninum (N. caninum)* are protozoan parasites with worldwide distribution, both parasites are known to infect and have a significant economic effect on farm and wild animals as well as on in-house pets (1, 2, 3, 4, 5). Information regarding the impact and prevalence of these parasites in wildlife is scarce and has not been fully clarified to date.

Serological analysis of *T. gondii* and *N. caninum* in wildlife is complicated. Species-specific secondary antibodies are often unavailable and the specificity, sensitivity and cutoff values for many of these tests have not been evaluated in

many wild species (6, 7). Furthermore, the use of at least two different serological methods has been suggested for confirmation of positive results in wild animals (7, 8).

Exposure to *T. gondii* has been reported in captive and free-ranging asymptomatic wild animals worldwide (9, 10, 11). Antibodies to *T. gondii* were detected in 34.7% of tested zoo animals in the Czech Republic, with 63.1% tested species being seropositive (12). Certain species of zoo animals, including new world primates, wallabies and kangaroos, are considered to be highly susceptible to toxoplasmosis (13, 14, 15). Previous studies examining the prevalence of *T. gondii* in free-ranging wild mammals reveal percentages of seropositivity ranging from 24% in the USA (16) to 90% in Portugal (17).

Information regarding toxoplasmosis in wild animals, both free-ranging and in captivity, in Israel is limited. Toxoplasmosis outbreaks were documented in captive wildlife (18, 19, 20). Apart of a study showing high seroprevalence of *T. gondii* using Modified Agglutination Test (MAT) in free-ranging crows and griffon vultures *(Spenicus demersus)*, (21), the seroprevalence of toxoplasmosis in free-ranging wild animals in Israel is unknown.

The route of transmission and infection of *T. gondii* is not always clear. Currently, there is no consensus on the diagnostic test to be implemented for the detection of oocyst contamination of soil and water (22, 23, 24). However, risk factors such as cat presence, contaminated food and water sources and/or soil contamination (affected by moisture, rainfall etc.) are often suspected as major routes of transmission in cases of *T. gondii* (19, 24, 25, 26, 27, 28, 29, 30, 31).

Several studies have been conducted worldwide regarding *N. caninum* infection in wildlife, both in captivity and free-ranging (4, 7, 12, 32). Seroprevalence of *N. caninum* in asymptomatic wild animals worldwide ranged from zero to 78% per species (in studies in which n>100) (1). Seroprevalence of *N. caninum* in free-ranging wild canids in Israel was estimated to be 2.7% in one study (33). A more recent survey conducted on free-ranging wild animal species in Israel, documented seropositivity in 6 out of 11 species tested (n=871), ranging from 3.2% (n=189) in *golden jackals* to 66.7% (n=18) in water buffaloes *(Bubalus bubalis)* (34). To the best of our knowledge, no previous information is available on the prevalence of *N. caninum* in zoo animals in Israel.

Information regarding the survival of *N. caninum* oocysts in the environment is scarce. Nevertheless, several risk factors for *N. caninum* infection have been identified including presence of dogs, population density and high environmental temperatures (1, 35, 36).

In the current study, we evaluated the exposure to *T. gondii* and *N. caninum* in both free-ranging and captive wild animals in Israel, using two serological methods. Risk factors for seropositivity in these two populations of wild animals as well as the agreement between MAT and IFAT (Immunofluorescence Antibody Test) were assessed.

MATERIALS AND METHODS

Study Design

A total of 259 serum samples from animals of 42 different species were available for our study. The samples were obtained from free-ranging wildlife throughout the country (n=149) and from four different zoos in Israel: the Jerusalem Biblical Zoo (28 samples), the Israeli Primate Sanctuary (64 samples); the Safari-Zoological Center (8 samples) and Gan Garoo (10 samples). Samples were collected for clinical and husbandry indications between 2001 and 2019 with the majority collected during the time of the study (2016-2019). Samples used which were stored as leftovers were not especially collected for this study.

Five additional available samples of the species meerkats (*Suricata suricatta*) presenting clinically with toxoplasmosis and previously confirmed as positive for *T. gondii* (20), were used as a positive control.

Serological Methods

In the current research, MAT was used to test all samples for both *N. caninum* and *T. gondii*. The test was carried out as previously described (6, 34, 36, 37). The cutoff value for positive results was set at a dilution of 1:50. Briefly, *N. caninum* and *T. gondii* tachyzoites were propagated in Vero cell cultures as previously described (38). Free tachyzoites from heavily infected cells were passed through a 25-gauge needle and subjected to centrifugation at 70×g for 5 min. The supernatant containing the parasites was then centrifuged at 900×g for 20 min and the pelleted parasites resuspended in PBS. The parasites were washed at least three times in PBS. The final pellet was resuspended in 2-3 ml of PBS to 6% formaldehyde (37% pure). Parasites were fixed at 4◦C, for at least 16h until use. Before the performance of the test, the tachyzoites were resuspended in alkaline buffer adjusted

to pH 8.7, containing 7.02 g NaCl, 3.09 g H3BO3, 24 ml 1N NaOH, 4 mg/ml of bovine albumin (fraction V), 50 mg eosin Y and 1.0 g sodium azide per liter, counted in Neubauer chamber to obtain a final dilution of 10^7 tachyzoites/ml and stored at 4◦C. Next, 50 µl of the antigen suspension in alkaline buffer were distributed in each well to U-shaped well microtiter plates and dilutions of test sera were then applied. Positive samples produced agglutination that could be graded, while negative samples produced a 'button' of precipitated tachyzoites at the bottom of the well.

The cutoff value for positive results was set at a dilution of 1:50 (39). To serve as controls, each plate contained three positive samples and four negative controls (three negative samples and one PBS without serum) previously verified by IFAT. Positive and negative controls had different combinations of *T. gondii* and *N. caninum* positive/negative in order to monitor cross-reactivity between the parasites. After testing all samples by MAT, all positive samples and part of the negative samples by MAT were tested by IFAT to confirm seropositivity, to validate the MAT results and to examine its validity as a screening test in wild animals.

Samples were considered positive when both MAT and IFAT were positive. Since previous studies in wild animals (34) showed that the sensitivity of the MAT was high but its specificity was low (compared to IFAT and/or WesternBlot), samples that were positive by MAT and negative by IFAT were considered negative.

The IFAT was carried out as previously described (20, 34, 40, 41). The cutoff value for seropositivity was set at a dilution of 1:50. The final titer were not evaluated and samples were examined at titers of 1:50 and 1:100.

The choice of secondary antibodies for the IFAT in the current study was based on selecting the closest relatives to a given species for which secondary antibodies were available. Species with no close relatives for which secondary antibodies were available were tested with protein-G (abcam, native protein G (FITC) ab7459) as a multispecies secondary antibody (42) (Table 1S).

Statistical methods

The required sample size was determined using the software 'winpepi', version 11.63 (Abramson JH, Peritz E: Calculator Programs for the Health Sciences. New York: Oxford University Press; 1983), based on 12.

Comparison of continuous variables was done using either independent samples t-test or Mann-Whitney test. Discrete variables were examined using either the chi-squared test or the likelihood ratio test. Finally, logistic regression models with multiple variables were conducted in order to identify risk factors for *T. gondii* and *N. caninum* seropositivity in different climates. The significance level of all tests was set at $p<0.05$.

The agreement between MAT and IFAT was evaluated by calculating Kappa values.

RESULTS

Exposure to *Neospora caninum* and *Toxoplasma gondii*

A total of 259 serum samples were available for this study (free-ranging animals n=149, zoo animals n=110), including 42 species representing 23 families and 6 orders. The prevalence of *T. gondii* and *N. caninum* antibodies by species is presented in Table 1.

The overall exposure to *T. gondii* in this study was 25.87% (CI (p (95%)) 20.54%-31.2%). Seropositive samples were identified in 20 of 42 species tested (Table 1).

The overall exposure to *N. caninum* in this study and was 6.95% (CI (p (95%)) 3.85%-10.05%) Seropositive samples were identified in 6 of 42 species tested (Table 1).

Co-infection with *T. gondii* and *N. caninum* was identified in 12 of 259 samples (4.6% (CI (p (95%)) 2.4%-7.6%)), including two zoo animals and ten free-ranging animals (Table 1).

Considering the different orders examined, seropositivity to both parasites were observed in the following orders: carnivora (only in *golden jackals*), in ungulate and in primates (only in one *grivet* that was co-infected with *T. gondii* and *N. caninum*). Seropositivity for *T. gondii* was also observed in several ungulate and primate species (Table 1). In the aves order *T. gondii* seropositivity was observed only in two *Griffon vultures* and in the order diprotodontia in *Eastern grey kangaroo*. In the order rodentia only one *Indian crested porcupine* was examined and found seronegative to both parasites.

Risk factors

T. gondii exposure tended to be higher in zoo animals when compared to free-ranging wildlife (34/110 (30.9%), 33/149 (22.15%), respectively. P-value=0.056). *N. caninum* exposure rate was significantly higher in free-ranging wildlife compared with zoo animals ((15/149(10.07%), 3/110 (2.73%),

Table 1: Exposure to *T. gondii* and *N. caninum* in wild animals in Israel.

Order	Species	No. Tested	*T. gondii* Seropositive Samples	*N. caninum* Seropositive Samples	Origin of samples
Carnivora	*South American coati (Nasua nasua)*	1	0	0	Z
	Jungle cat (Felis chaus)	1	0	0	FR
	Cheetah (Acinonyx jubatus)	1	1	0	Z
	Golden jackal (Canis aureus)	75	24	8	FR
Ungulate	*Plains zebra (Equus quagga)*	4	2	0	Z
	African wild donkey (Equus africanus)	2	0	0	B
	Fallow deer (Dama dama)	2	0	0	Z
	Persian fallow deer (Dama dama mesopotamica)	7	0	0	FR
	Wild goat (Capra aegagrus)	3	0	1	B
	Arabian oryx (Oryx leucoryx)	19	1	0	B
	Nubian ibex (Capra nubiana)	16	3	4	FR
	addax (Addax nasomaculatus)	1	0	0	FR
	Dorcas gazelle (Gazella dorcas)	1	0	0	FR
	Mountain gazelle (Gazella gazella)	13	4	3	FR
	White rhinoceros (Ceratotherium simum)	1	0	0	Z
	Wild boar (Sus scrofa)	14	1	0	FR
	vicuna (Vicugna vicugna)	1	1	1	Z
	Asian elephant (Elephas maximus)	5	3	0	Z
Primates	*Ring-tailed lemur (Lemur catta)*	1	0	0	Z
	common brown lemur (Eulemur fulvus)	1	0	0	Z
	Barbary macaque (Macaca sylvanus)	6	0	0	Z
	Sooty mangabey (Cercocebus atys)	1	1	0	Z
	Hamadryas baboon (Papio hamadryas)	1	0	0	Z
	Olive baboon (Papio anubis)	3	2	0	Z
	Crab-eating macaque (Macaca fascicularis)	18	3	0	Z
	Diana monkey (Cercopithecus diana)	2	1	0	Z
	Grivet (Chlorocebus aethiops)	11	6	1	Z
	Mandrill (Mandrillus sphinx)	4	0	0	Z
	Angola colobus (Colobus angolensis)	3	3	0	Z
	Rhesus macaque (Macaca mulatta)	2	0	0	Z
	Collins' squirrel monkey (Saimiri collinsii)	1	0	0	Z
	Tufted capuchin (Sapajus apella)	11	4	0	Z
	White-faced saki (Pithecia pithecia)	1	0	0	Z
	Black-headed night monkey (Aotus nigriceps)	3	1	0	Z
	Red-handed tamarin (Saguinus midas)	1	1	0	Z
Aves	*African penguin (Spheniscus demersus)*	4	0	0	Z
	Griffon vulture (Gyps fulvus)	2	2	0	Z
	Red-crowned crane (Grus japonensis)	1	0	0	Z
	White stork (Ciconia ciconia)	1	0	0	Z
	Black swan (Cygnus atratus)	1	0	0	Z
Diprotodontia	*Eastern grey kangaroo (Macropus giganteus)*	12	3	0	Z
Rodentia	*Indian crested porcupine (Hystrix indica)*	1	0	0	Z
Total Animals		259	67	18	

Origin of samples: Z=zoo animals, FR= free-ranging animals, B= both

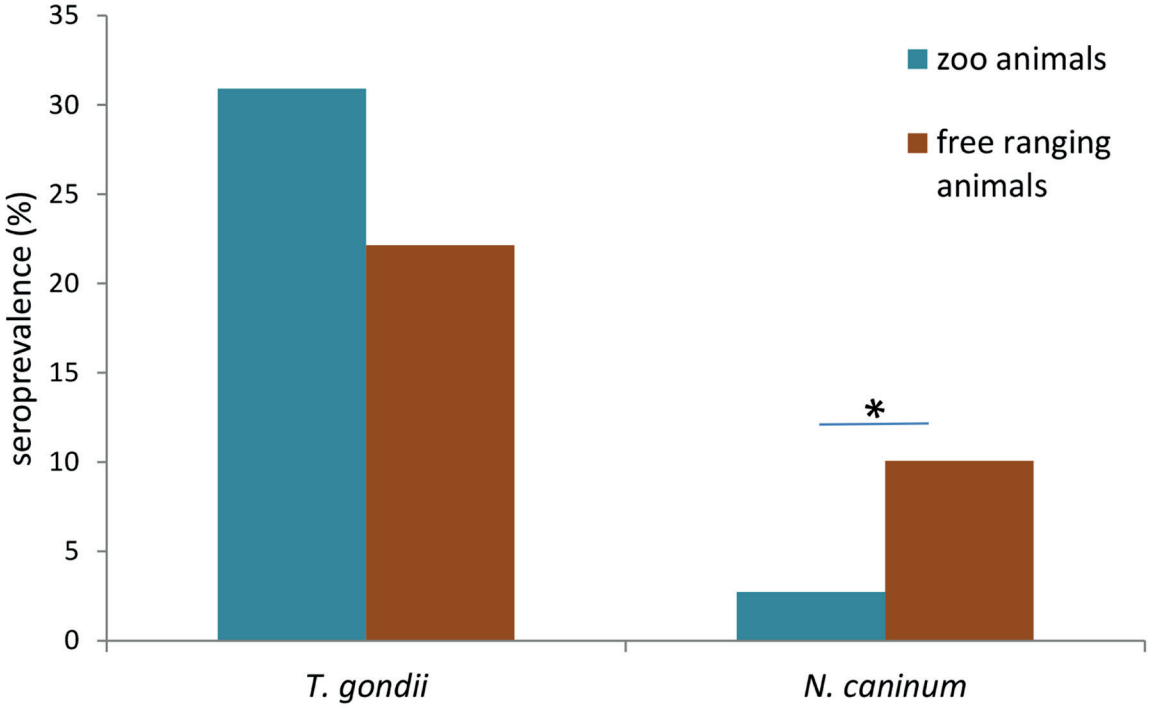

Figure 1: Comparison of exposure to *T. gondii* and *N. caninum* in zoo animals vs. free-ranging animals.
*Statically significant

respectively. P-value=0.0214) (Figure 1). Calculated OR shows higher risk of exposure with *T. gondii* for zoo animals compared with free-ranging animals, (OR=1.57 (CI (p (95%)): 0.9-2.75)) (Figure 1). Higher risk of exposure to *N. caninum* was found in free-ranging animals when compared to zoo animals, (OR=3.99 (CI (p (95%)): 1.13-14.15) (Figure 1).

The lowest exposure to *T. gondii* was found in the desert climate followed by Mediterranean 2 (>600 mm precipitation per year), semi-arid and finally, the Mediterranean 1 climate (<600 mm precipitation per year) presented as the highest. These differences were found to be significant (Pearson Chi-Square test p-value=0.008), (Figure 2). No significant differences where observed in seroprevelance of *N. caninum* in different geographical areas.

3.3. Agreement between MAT and IFAT

A total of 74 samples positive and 14 samples negative for *T. gondii* antibodies by MAT were tested by IFAT. The precentage of observed agreement in detecting *T. gondii* antibodies by both MAT and IFAT was 88.64% of the observations (n=78), when the number of agreements expected by chance is 60.4 (68.60% of the observations). Kappa=0.638 (SE=0.103, CI (p(95%)): 0.436-0.840). The strength of agreement between the two tests was therefore considered to be 'substantial'. Sensitivity and specificity values of MAT for detection of *T.gondii* antibodies as compared to IFAT as gold standard were 66/68 (97.06%) and 12/20 (60.0%), respectively.

A total of 24 *N.caninum* positive and 8 negative samples detected by MAT were also tested by IFAT. The percentage of observed agreements for detection of *N. caninum* antibodies for both MAT and IFAT was 78.13% (n=25), Number of agreements expected by chance: 16.5 (51.56% of the observations). Kappa=0.548 (SE=0.135, CI (p(95%)): 0.257-0.772). The strength of agreement between the two tests is considered to be 'moderate'. Sensitivity and specificity values of MAT for detection of *N. caninum* antibodies as compared to IFAT as gold standart were 17/17 (100%) and 8/15 (53.3%), respectively.

DISCUSSION

This is the first serological survey of *T. gondii* and *N. caninum* in captive wild animals and the first sero-survey of *T. gondii* in free-ranging animals in Israel. The results of this study indicate that wild animals were exposed to both parasites.

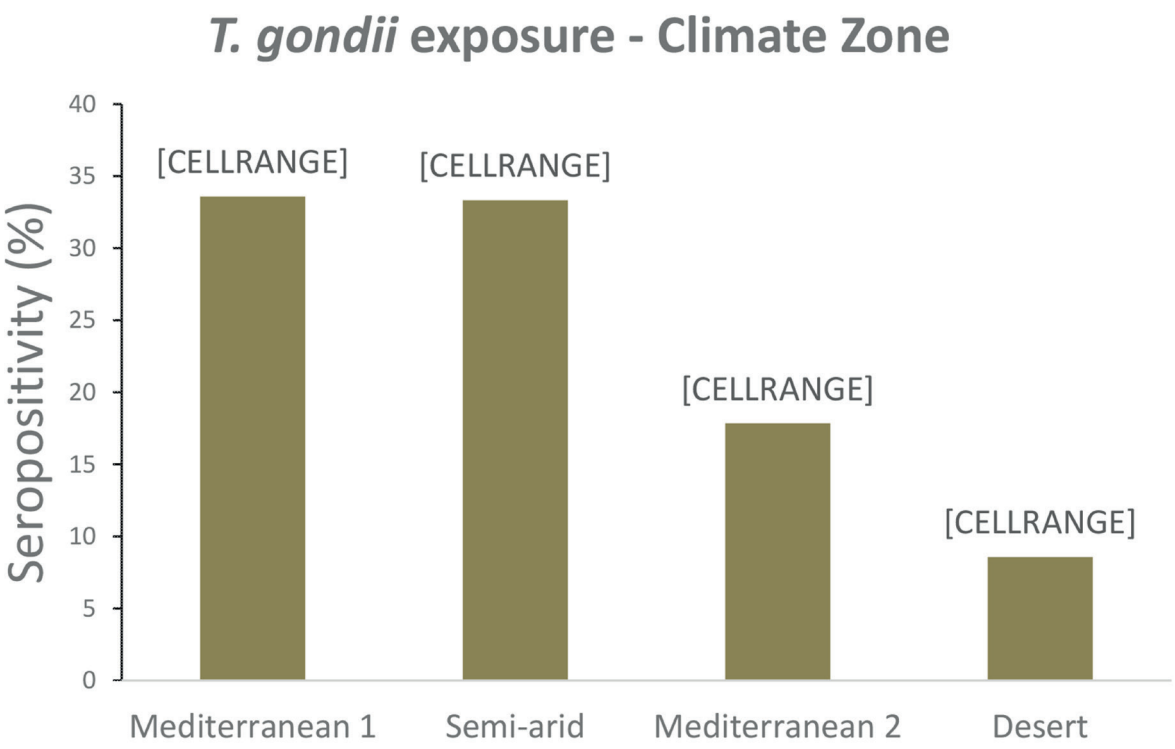

Figure 2: Comparison of exposure rate of *T. gondii* in four different climate zones in Israel.

Seropositive rate to *T. gondii* (25.87%) in wild animals was higher than to *N. caninum* (6.95%). The seropositivity for both parasites observed in this study resemble those of similar studies performed worldwide (1, 7, 12, 16, 34), giving further validation to the estimated rates of *T. gondii* and *N. caninum* in wild animals in Israel and in the world.

Seropositivity to *N. caninum* was found in 6 of 42 species in the study: *Nubian ibex, mountain gazelle, golden jackal, vicuna, grivet, wild goat*. Presence of antibodies in all species, except for *grivet*, have been previously described (4, 34, 43). The *grivet* positive for *N. caninum* arrived at the Israeli Primate Sanctuary from Khan Younes in the Gaza strip on September 2016. The sample we tested was taken upon his arrival. The *grivet* was positive for *N. caninum* at a dilution of 1:50 and was positive for *T. gondii* as well, at a dilution of 1:100 (higher dilutions were not tested). The possibility of cross-reaction between the two parasites must be considered. Further inquiry must be done into the possibility of natural infection with *N. caninum* in primates. Exposure rate to *N. caninum* in *golden jackals* in the current study was higher than previously reported and reached 10.7%. A survey conducted in Israel in 2006 (which tested samples from 1999 to 2004), showed seroprevalence of 1.7% (33). Another survey, conducted in 2018 showed a 3.2% exposure rate of *N. caninum* in *golden jackals* (samples from 2006 to 2013) (34). All *golden jackal* samples used in the current study were collected between 2016 and 2018; therefore, it appears that the exposure of jackals to *N. caninum* parasites has increased in the last years.

The significant rise in exposure rates could be due to a combination of factors. *Golden Jackal* population size and complexity of controlling population growth (43), could be affecting exposure rates. Urbanization and increasing loss of natural open spaces in Israel, contribute to rising population densities which consequently could lead to a closer contact with domesticated dogs, thus exposing wild animals to various pathogens including *N. caninum* (44, 45). If this hypothesis is correct, this finding emphasizes the possible negative effects of urbanization and loss of natural open spaces, on the epidemiology of disease transmission between free-ranging wild animals and domesticated animals. The high exposure to *T. gondii* observed in jackals (32%) can suggest a possibility of close relation between this species and domestic animals. Rates of co-infection with *T. gondii*

and *N. caninum* (4.6%) resemble those evaluated in previous studies (44; 46).

An increased, although not significant, rate of exposure to *T. gondii* was detected in zoos when compared to free-ranging animals (30.9% versus 22.15%, OR=1.57). This fact could be attributed to the large concentrations of cats roaming the zoos, contaminating the soil as well as food and water sources (2, 9, 10, 19, 31). Additionally, population density in zoos is a possible important risk factor. In a study performed in Namibia, free-ranging cheetahs and leopards from different sites were tested for *T. gondii* seroprevalence and no difference concerning location was observed (32). Thus, although higher seroprevalence of *T. gondii* in zoo animals was observed, it is possible that the difference is not solely attributed to environmental conditions. Additionally, it is important to note that the species tested in zoos differ from those of free-ranging animals. Thus, the difference in seropositivity observed can be a result of different susceptibility of the examined animals to *T. gondii* parasites. Further studies comparing the same animal species in different environmental conditions should be performed to obtain a better understanding of this finding.

When comparing exposure rates of *N. caninum* in zoo animals with those of free-ranging wildlife, significantly higher rates were detected in free-ranging wildlife (10.07% versus 2.73%, OR=3.99). This difference was found to be statistically significant even when jackals were removed from the analysis ((7/74 (9.46%) in zoo animals versus 3/110 (2.73%) in free-ranging animals, P-value = 0.047). The fact that the final hosts of *N. caninum*, canids, do not roam zoos freely, could provide a feasible explanation as to why exposure rates are significantly lower in zoos. Additionally, it must be taken into consideration that the difference in exposure between captive and free-ranging animals can be attributed to the difference in animal species tested in each population as discussed above concerning toxoplasmosis.

The high exposure rate in free-ranging animals could be intensified by the fact that *golden jackals* are carnivorous, have close contact with and are natural predators of domestic animals such as sheep and cattle which are known to be endemic for neosporosis in Israel (3, 47, 48). The role of *jackals* in the sylvatic life cycle and epidemiology of *N. caninum* is still not fully understood. The proven definitive hosts of *N. caninum* are canids and dogs (49), coyotes (50), gray wolves (51) and dingoes (52). As *jackals* are also from the canids family and closely related to the definitive hosts of *N. caninum*, they are suspected to be definitive hosts of *N. caninum* thus potentially having an important role in the epidemiology of neosporosis in Israel. However, *jackals* are yet to be proven definitive hosts of N. *caninum* and further studies demonstrating shedding of oocysts should be conducted in order to verify their full role.

Our study demonstrated a possible effect of climate on the prevalence of *T. gondii* with lowest exposure rates detected in desert climates (Figure 2). This can be explained by the fact that *T. gondii* oocysts are able to survive longer in humid climates (26). Similar trends were found in a study that examined the prevalence of *T. gondii* in crows and *griffon vultures* in Israel, in which seroprevalence in crows was significantly higher in areas with increased precipitation and humidity (21). Notwithstanding, it must be taken into consideration that the difference in exposure rates between climate zones could also be a result of other characteristics not evaluated in our study such as population density, urbanization, contact between domesticated and wild species, all with the potential of affecting possibility of exposure.

According to our study, MAT can be used as a first stage screening technique for exposure to *T. gondii* in large scale surveys. This test was found to be accurate in 88.64% of the cases and is an efficient, time and money-saving solution as previously established (6, 36, 53). This was not the case when evaluating MAT as a screening test for *N. caninum*, where agreement between the two tests was found to be moderate (78.13%). A previous study showed similar results with MAT presenting very high sensitivity and low specificity, thus being efficient only for a first screening though not for assessing true prevalence values of *N. caninum* (34).

Validation of the use of protein-G as a secondary antibody when a specific secondary antibody is not available for IFAT was analyzed. Protein-G combined with protein-A at a dilution of 1:1000 had previously been used for serological analysis of bats (54). The combination of these two proteins had also been tested in our work (data not shown). However, no comprehensive results were obtained using various dilutions. Thus, both proteins were tested separately in animals with available secondary antibodies (data not shown) and the best results were obtained with the use of protein-G at a dilution of 1:250. Therefore, following validation, all samples from species with no close relatives for which specific secondary antibodies are available were tested using Protein G.

One limitation we faced in this study was the amount of serum obtained. As this study was performed with samples

taken for clinical and husbandry purposes and not with samples collected specifically for this study, the amount of the serum was limited. Therefore, we chose to perform MAT and IFAT for detection of antibodies against both *Toxoplasma* and *Neospora* parasites as these are commonly accepted tests for diagnostic of seroprevalence of wild animals. Unfortunately, the evaluation of cross reactivity and validation of the tests with a third method such as western blot could not be performed. Yet, we observed that a portion of animals was seropositive for *Neospora caninum* only or *Toxoplasma gondii* only while others were co-infected with both parasites.

Another limitation is the fact that there is no gold standard for diagnose seroprevalence for *T. gondii* and *N. caninum* in wild animals thus we chose to use first a more sensitive but less specific method (MAT) and then to confirm the results with IFAT (55). Thus, it appears that the use of MAT followed by IFAT could be a good choice for screening of both parasites with specific and sustainably results.

In this study we report exposure to *T. gondii* and *N. caninum* in wild animals in Israel, showing high rates of *T. gondii* exposure. Higher rate of seropositivity to *T. gondii* is documented amongst zoo animals compared with free-ranging wildlife, whereas *N. caninum* exposure rate is higher in free-ranging wildlife. In addition, we provide evidence that MAT can be used as an efficient screening technique for *T. gondii* exposure in wild animals and protein-G can be an alternative option when no specific secondary antibodies are available.

BIBLIOGRAPHY

1. Dubey, J.P., Schares, G. and Ortega-Mora, L.M.: Epidemiology and control of neosporosis and *Neospora caninum*. Clin. Microbiol. Rev. 20(2), 323-367, 2007.
2. Dubey, J.P.: The history of *Toxoplasma gondii* – the first 100 years. J. Eukaryot, Microbiol. 55(6), 467-475, 2008.
3. Mazuz, M.L., Fish, L., Reznikov, D., Wolkomirsky, R., Leibovitz, B., Savitzky, I., Golenser, J. and Shkap, V.: Neosporosis in naturally infected pregnant dairy cattle. Vet. Parasitol. 205(1-2), 85-91, 2014.
4. Donahoe, S.L., Lindsay, S.A., Krockenberger, M., Phalen, D. and Šlapeta, J.: A review of neosporosis and pathologic findings of Neospora caninum infection in wildlife. International Int. J. Parasitol. Parasites Wildl. 4(2), 216-238, 2015.
5. Aguirre, A.A., Longcore, T., Barbieri, M., Dabritz, H., Hill, D., Klein, P.N., Lepczyk, C., Lilly, E.L., McLeod, R., Milcarsky, J., Murphy, C.E., Su, C., VanWormer, E., Yolken, R. and Sizemore, G.C.: Correction to: The One Health Approach to Toxoplasmosis: Epidemiology, Control, and Prevention Strategies. *EcoHealth* 16, 391, 2019.
6. Dubey, J.P.: Toxoplasmosis of animals and humans. CRC press, 2010.
7. Almería, S.: *Neospora caninum* and wildlife. ISRN Parasitology, 2013.
8. Sobrino, R., Dubey, J.P., Pabón, M., Linarez, N., Kwok, O.C., Millán, J., Arnal, M.C., Luco, D.F., López-Gatius, F., Thulliez, P. and Gortázar, C.: *Neospora caninum* antibodies in wild carnivores from Spain. Vet. Parasitol. 155(3-4), 190-197, 2008.
9. Dubey, J.P.: Toxoplasmosis – a waterborne zoonosis. Vet. Parasitol. 126(1-2), 57-72, 2004.
10. De Campos, S., Dubey, J.P. and Saville, W.A.: Seroepidemiology of *Toxoplasma gondii* in zoo animals in selected zoos in the mid-western United States. J. Parasitol. 94(3), 648-653, 2008.
11. Cano-Terriza D, Almería S, Caballero-Gómez, J., Díaz-Cao, J. M., Jiménez-Ruiz, S., Dubey J.P. and García-Bocanegra, I.: Serological survey of *Toxoplasma gondii* in captive nonhuman primates in zoos in Spain. Comp. Immunol. Microbiol. Infect. Dis. 65, 54-57. 10.1016/j.cimid.2019.04.002, 2019.
12. Sedlák, K., and Bártová, E.: Seroprevalences of antibodies to *Neospora caninum* and *Toxoplasma gondii* in zoo animals. Vet. Parasitol. 136(3-4), 223-231, 2006.
13. Shamir, A.: Toxoplasmosis in a monkey. Isr. J. Vet. Med. 10(3), 104-109, 1953.
14. Ratcliffe, H.L. and Worth, C.B.: Toxoplasmosis of captive wild birds and mammals. Amer. J. Pathol. 27(4), 65, 1951.
15. Dubey, J.P., Ott-Joslin, J., Torgerson, R.W., Topper, M.J. and Sundberg, J.P.: Toxoplasmosis in black-faced kangaroos *Macropus fuliginosus melanops*. Vet. Parasitol. 30(2), 97-105, 1988.
16. Smith, D.D. and Frenkel, J.K.: Prevalence of antibodies to *Toxoplasma gondii* in wild mammals of Missouri and east central Kansas: biologic and ecologic considerations of transmission. J. Wildl. Dis. 31(1), 15-21, 1995.
17. Lopes, A.P., Sargo, R., Rodrigues, M. and Cardoso, L.: High seroprevalence of antibodies to *Toxoplasma gondii* in wild animals from Portugal. Parasitol. Res. 108(5), 1163-1169, 2011.
18. Nobel, T.A., Klopfer, U. and Neumann, F.: Toxoplasmosis in wild animals in Israel. J. Compar. Pathol. 79(1), 127-129, 1969.
19. Salant, H., Weingram, T., Spira, D.T. and Eizenberg, T.: An outbreak of Toxoplasmosis amongst squirrel monkeys in an Israeli monkey colony. Vet. Parasitol. 159(1), 24-29, 2009.
20. Margalit, L., Bueller-Rosenzweig, A., Horowitz, I., Bouznach, A., Edery, N., Savitsky, I., Fleiderovitz, L., Baneth, G. and Mazuz, M.L.: Clinical toxoplasmosis in two meerkats (*Suricata suricatta*) in Israel. Isr. J. Vet. Med. 72(1), 49-54, 2017.
21. Salant, H., Hamburger, J., King, R. and Baneth, G.: *Toxoplasma gondii* prevalence in Israeli crows and Griffon vultures. Vet. Parasitol. 191(1-2), 23-28, 2013.
22. Afonso, E., Lemoine, M., Poulle, M.L., Ravat, M.C., Romand, S., Thulliez, P., Villena, I., Aubert, D., Rabilloud, M., Riche, B. and Gilot-Fromont, E.: Spatial distribution of soil contamination by *Toxoplasma gondii* in relation to cat defecation behavior in an urban area. Intern. J. Parasitol. 38(8-9), 1017-1023, 2008.
23. Lélu, M., Gilot-Fromont, E., Aubert, D., Richaume, A., Afonso,

E., Dupuis, E., Gotteland, C., Marnef, F., Poulle, M.L., Dumètre, A. and Thulliez, P.: Development of a sensitive method for *Toxoplasma gondii* oocyst extraction in soil. Vet. Parasitol. 183(1-2), 59-67, 2011.
24. Gotteland, C., McFerrin, B.M., Zhao, X., Gilot-Fromont, E. and Lélu, M.: Agricultural landscape and spatial distribution of *Toxoplasma gondii* in rural environment: an agent-based model. International journal of health geographics, 13(1), 45, 2014.
25. Borst, G.H.A. and Van Knapen, F.: Acute acquired toxoplasmosis in primates in a zoo. The J. Zoo Anim. Med, 15(2), 60-62, 1984.
26. Dubey, Jitender Prakask, and C. P. Beattie.: Toxoplasmosis of animals and man. CRC Press, Inc., 1988.
27. Burns, R., Williams, E.S., O'Toole, D. and Dubey, J.P.: Toxoplasmosis in black-footed ferrets (*Mustela nigripes*) at the Louisville Zoo. In: Proceedings of the American Association of Zoo Veterinarians (p. 48), 1993.
28. Juan-Sallés, C., Prats, N., Lopez, S., Domingo, M., Marco, A.J. and Moran, J.F.: Epizootic disseminated toxoplasmosis in captive slender-tailed meerkats (*Suricata suricatta*). Vet. Pathol. 34(1), 1-7, 1997.
29. Afonso, E., Thulliez, P. and Gilot-Fromont, E.: Local meteorological conditions, dynamics of seroconversion to Toxoplasma gondii in cats (Felis catus) and oocyst burden in a rural environment. Epidemiol. Infect. 138(8), 1105-1113, 2010.
30. VanWormer, E., Fritz, H., Shapiro, K., Mazet, J.A. and Conrad, P.A.: Molecules to modeling: *Toxoplasma gondii* oocysts at the human–animal–environment interface. Comp. Immunol. Microbiol. Infect. Dis. 36(3), 217-231, 2013.
31. Burger, M., Du Plessis, E.C., Suleman, E. and Gardner, B.R.: Fatal disseminated toxoplasmosis in a zoological collection of meerkats (*Suricata suricatta*). J. S. African Vet. Assoc. 88(1), 1-5, 2017.
32. Seltmann A, Schares, G., Aschenborn, O.H.K., Heinrich, S.K., Thalwitzer, S., Wachter, B. and Czirják, G.Á.: Species-specific differences in *Toxoplasma gondii*, *Neospora caninum* and *Besnoitia besnoiti* seroprevalence in Namibian wildlife. Parasitol. Vectors. 2020 Jan 8;13(1):7. doi: 10.1186/s13071-019-3871-3. PMID: 31915056; PMCID: PMC6950991, 2020.
33. Steinman, A., Shpigel, N.Y., Mazar, S., King, R., Baneth, G., Savitsky, I. and Shkap, V.: Low seroprevalence of antibodies to Neospora caninum in wild canids in Israel. Vet. Parasitol. 137(1-2), 155-158, 2006.
34. Mazuz, M.L., Alvarez-García, G., King, R., Savisky, I., Shkap, V., Ortega-Mora, L.M. and Gutiérrez-Expósito, D.: Exposure to Neospora spp. and Besnoitia spp. in wildlife from Israel. Int. J. Parasitol. Parasites, Wildl: 7(3), 317-321, 2018.
35. Rinaldi, L., Fusco, G., Musella, V., Veneziano, V., Guarino, A., Taddei, R. and Cringoli, G.: *Neospora caninum* in pastured cattle: determination of climatic, environmental, farm management and individual animal risk factors using remote sensing and geographical information systems. Vet. Parasitol. 128(3-4), 219-230, 2005.
36. Desmonts, G. and Remington, J.S.: Direct agglutination test for diagnosis of Toxoplasma infection: method for increasing sensitivity and specificity. J. Clin. Microbiol. 11(6), 562-568, 1980.
37. Packham, A.E., Sverlow, K.W., Conrad, P.A., Loomis, E.F., Rowe, J.D., Anderson, M.L., Marsh, A.E., Cray, C. and Barr, B.C.: A modified agglutination test for *Neospora caninum*: development, optimization, and comparison to the indirect fluorescent-antibody test and enzyme-linked immunosorbent assay. Clin. Diagn. Lab. Immunol., 5(4), 467-473, 1998.
38. Mazuz, M.L., Haynes, R., Shkap, V., Fish, L., Wollkomirsky, R., Leibovich, B., Molad, T., Savitsky, I. and Golenser, J.: Neospora caninum: in vivo and in vitro treatment with artemisone. Vet. Parasitol. 187, 99-104, 2012.
39. Hove T. and Mukaratirwa, S.: Seroprevalence of *Toxoplasma gondii* in farm-reared ostriches and wild game species from Zimbabwe. Acta Trop. 2005; 94: 49-53. doi: 10.1016/j.actatropica.2004.10.010.
40. Conrad, P.A., Sverlow, K., Anderson, M., Rowe, J., BonDurant, R., Tuter, G., Breitmeyer, R., Palmer, C., Thurmond, M., Ardans, A. and Dubey, J.P.: Detection of serum antibody responses in cattle with natural or experimental Neospora infections. J. Vet. Diagn. Invest. 5(4), 572-578, 1993.
41. Shkap, V., Reske, A., Pipano, E., Fish, L. and Baszler, T.: Immunological relationship between Neospora caninum and Besnoitia besnoiti. Veterinary Parasitology, 106(1), pp. 35-43, 2002.
42. Akerström, B., Brodin, T.H., Reis, K. and Björck, L.: Protein G: a powerful tool for binding and detection of monoclonal and polyclonal antibodies. J. Immunology, 135(4), 2589-2592, 1985.
43. Bártová, E., Kobédová, K., Lamka, J., Kotrba, R., Vodička, R. and Sedlák, K.: Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in exotic ruminants and camelids in the Czech Republic. Parasitol. Res. 116(7), 1925-1929, 2017.
44. Lapid, R., Motro, Y., Juravel, K., King, R., Moran-Gilad, J. and Gila Kahila Bar-Gal, G.: The golden jackal (*Canis aureus*): interaction of fur and fecal microbiota, host genetics and animal traits, Animal Microbiome, 10.1186/s42523-025-00470-4, 7, 1, 2025.
45. Woodroffe, R., Prager, K. C., Munson, L., Conrad, P. A., Dubovi, E. J. and Mazet, J. A.: Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (*Lycaon pictus*). *PloS one*, 7(1), e30099, 2012.
46. Hughes, J.M., Williams, R.H., Morley, E.K., Cook, D.A.N., Terry, R.S., Murphy, R.G., Smith, J.E. and Hide, G.: The prevalence of Neospora caninum and co-infection with Toxoplasma gondii by PCR analysis in naturally occurring mammal populations. Parasitology, 132(1), pp. 29-36, 2006.
47. Mazuz, M.L., Leibovitz, B., Savitsky, I., Blinder, E., Yasur-Landau, D., Lavon, Y., Sharir, B. and Tirosh-Levy, S.: The Effect of Vaccination with *Neospora caninum* Live-Frozen Tachyzoites on Abortion Rates of Naturally Infected Pregnant Cows. Vaccines (Basel)., 2021 Apr 19;9(4):401. doi: 10.3390/vaccines9040401. PMID: 33921584; PMCID: PMC8074125.
48. Tirosh-Levy, S., Savitsky, I., Blinder, E. and Mazuz, M.L.: The involvement of protozoan parasites in sheep abortions – A ten-year review of diagnostic results. Vet. Parasitol. 2022 Mar; 303:109664. doi: 10.1016/j.vetpar.2022.109664. Epub 2022 Jan 25. PMID: 35131575.
49. McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R.A. and McGuire, A.M.: Dogs are definitive hosts of Neospora caninum. Int. J. Parasitol. 1998 Sep; 28(9):1473-8. PMID: 9770635.
50. Gondim, L.F., McAllister, M.M., Pitt, W.C. and Zemlicka, D.E.: Coyotes (*Canis latrans*) are definitive hosts of *Neospora*

caninum. Int. J. Parasitol. 2004 Feb; 34(2):159-61. doi: 10.1016/j.ijpara.2004.01.001. PMID: 15037103.

51. Dubey, J. P., Jenkins, M. C., Rajendran, C., Miska, K., Ferreira, L. R., Martins, J., Kwok, O. C. H. and Choudhary, S.: Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. Vet. Parasitol. 181, 382-387, 2011.
52. King, J.S., Slapeta, J., Jenkins, D.J., Al-Qassab, S.E., Ellis, J.T. and Windsor, P.A.: Australian dingoes are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 2010 Jul; 40(8):945-50. doi: 10.1016/j.ijpara.2010.01.008. Epub 2010 Feb 10. PMID: 20149793.
53. Dubey, J.P.: Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. Vet. Parasitol. 71(4), 307-310, 1997.
54. He, B., Huang, X., Zhang, F., Tan, W., Matthijnssens, J., Qin, S., Xu, L., Zhao, Z., Wang, Q., Hu, T. and Bao, X., 2017: Group A rotaviruses in Chinese bats: genetic composition, serology, and evidence for bat-to-human transmission and reassortment. J. Virol. 91(12), e02493-16, 2027.
55. Carvalho NOR, Gaspar RC, Lima SG, da Silva Zanini D, Rodrigues NJL, Guimarães VY, da Silva AV, Rahal SC, Henrique ALP, Fornazari F, Langoni H.: Comparison Between Indirect Fluorescent Antibody Test and Modified Agglutination Test for Detecting Anti-Toxoplasma gondii IgG Antibodies in Neotropical Primates. J Med Primatol.; 54(1), 2025.