



Toxoplasma gondii prevalence in Israeli crows and Griffon vultures

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ARTICLE INFO

Article history:

Received 17 June 2012

Received in revised form 12 July 2012

Accepted 27 July 2012

Keywords:

Toxoplasma gondii

MAT

Crows

Griffon vultures

Israel

ABSTRACT

A cross-sectional *Toxoplasma gondii* seroprevalence study was performed on free ranging crows (*Corvus cornis*, *Corvus monedula*, *Corvus splendens*) and Griffon vultures (*Gyps fulvus*) from Israel in order to assess exposure to this pathogen in scavenger birds that feed on animal carcasses and their possible role in the epidemiology of toxoplasmosis. Using the modified agglutination test (MAT) with a cutoff titer of 1:25, 52 of 122 crows (42.6%) and 40 of 101 Griffon vultures (39.6%) were found to be *T. gondii* seropositive. Crow *T. gondii* seroprevalence was significantly higher in northern areas of Israel ($p = 0.007$) where annual precipitation is higher and annual summer maximum temperatures are lower than in the drier and warmer south. Seroprevalence in crows was positively associated with higher human population densities possibly related to the increased cat population in these areas. PCR analysis of brain extracts from crows resulted in the detection of *T. gondii* DNA in 1 seropositive crow from northern Israel. Genetic analysis of DNA from the positive crow brain confirmed infection with *T. gondii* type 2 using a multiplex multilocus nested PCR-RFLP (Mn-PCR-RFLP) of the SAG1, 5–3' SAG2, alt.SAG2, SAG3, BTUB, GRA6, C22–8, c29–2, L358, PK1 and Apico loci. The high *T. gondii* seroprevalence in these bird species suggests that infected carrion may be responsible for widespread infection of carcass scavenger birds which may further transmit infection to other carnivorous intermediate hosts or feline definitive hosts when consumed post-mortally.

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1. Background

Toxoplasmosis is one of the most common zoonoses worldwide (Tenter et al., 2000). The causative protozoal agent, *Toxoplasma gondii*, can infect all warm-blooded animals, including birds which become infected by ingesting tissue cysts from infective meat or by consuming food or drink contaminated with oocysts excreted by cats, the definitive host for the parasite (Dubey and Beattie, 1988). The disease is of veterinary and medical importance, because it may cause severe clinical disease, abortion or congenital disease in its intermediate hosts.

In Israel, the rate of infection of various populations of ground feeding animals that likely become infected by ingesting oocysts contaminating the environment has been recently examined. Amongst ground-feeding animals, 41/458 (8.9%) 4–6 months old slaughtered sheep from the south of the country and 48/128 (37.5%) sheep of ages 5 months to 2 years and raised in the central region were *T. gondii* seropositive. Amongst poultry, infection rates were 12–44% of 248 free-range chickens from various regions (Dubey et al., 2004b; Salant, 2009). Lastly, in a study of wild pigeons (*Columba livia*) captured from various locations in Israel, *T. gondii* seropositivity was found in 20/495 (4%) of the birds (Salant et al., 2009a).

No local study on intermediate hosts which consume the other infective form of *T. gondii*, namely tissue cysts in infected meat has been done. Crows are commonly found in all parts of Israel. They are extremely successful in their

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Table 1
Capture location, species and serology of crows sampled for *Toxoplasma gondii*.

Crow sample location	Sampling point	Crow species	Number sampled	Seroprevalence results	
				Proportion positive (%)	Cumulative percentage seroprevalence
North	Haifa	<i>Corvus cornix</i>	28	17/28 (60.7)	42.6%
	Gaash	<i>Corvus cornix</i>	14	11/14 (78.6)	
	Raanana	<i>Corvus cornix</i>	2	2/2 (100)	
Center	Jerusalem	<i>Corvus cornix</i>	25	5/25 (20)	
	Ganei Yavne	<i>Corvus monedula</i>	5	2/5 (40)	
	Ashkelon	<i>Corvus cornix</i>	11	3/11 (27)	
South	Kibbutz Beeri	<i>Corvus cornix</i>	21	10/21 (46.6)	
	Eilat	<i>Corvus splendens</i>	16	2/16 (12.5)	

ability to establish large populations in urban and rural areas and feed on almost any source of nutrition including carrion, insects, grains, berries, fruit, small birds and other animals. Crows tend to remain in the same territory continually. Griffon vultures, found in various parts of Israel, feed entirely on carrion, including sheep and goat, cow, donkey and camel carcasses. The daily flight distance of a Griffon vulture is about 35–100 km. Exceptional long range forays covered more rarely, reach a distance of 400–1700 km from their home range (Nathan et al., 2012). Griffon vultures that were tagged in Bulgaria and Croatia have been observed in Israel (Society for the protection of nature in Israel, <http://www.birds.org.il/872-en/Birding-Israel.aspx>).

Fourteen percent of the wild crows tested in a study from California were found to have antibodies to the parasite by the indirect hemagglutination (Franti et al., 1976). Dubey (2002) has reviewed toxoplasmosis in wild birds extensively, including studies of infections in crows and vultures. However, many of the studies reviewed in crows were performed more than 3 decades ago (Finlay and Manwell, 1956; Franti et al., 1975; Pak, 1976). Generalized signs of toxoplasmosis as well as fatal encephalitis in 5 Hawaii crows including their serological results were described by Work et al. (2000). A survey of 240 common African black vultures in eastern Nigeria by modified agglutination test (MAT) resulted in a positive *T. gondii* seroprevalence of 64.8% with a base line titer of 1:25 (Arene, 1999). In a recent study performed in Spain, the prevalence of *T. gondii* antibodies in ravens was found to be as high as 80.5% using MAT in 115 wild caught ravens (Molina-López et al., 2012).

The objective of this study was to perform a *T. gondii* seroprevalence study in crows and Griffon vultures in Israel in order to assess exposure to this pathogen in scavenger birds that feed on animal carcasses and their possible role in the epidemiology of toxoplasmosis.

2. Materials and methods

2.1. Birds

One hundred and twenty two crows of the species, *Corvus cornix* (Hooded crow), *Corvus monedula* (Jackdaw) and *Corvus splendens* (House crow, Table 1) were included in the survey. Crows were captured using Australian traps and sacrificed humanely as permitted by the Israeli Nature

and Parks Authority during January–November 2011. Sera and brain material were collected from each individual crow and immediately frozen until further examination.

One hundred and one sera from wild Griffon vultures (*Gyps fulvus*) locally captured for monitoring purposes, ringed and wing-tagged prior to their release, were included in the study.

2.2. Sampling locations

Crows were captured mainly in the coastal plains of Israel in areas that ranged from Haifa (32°49'49.30"N) in the north, to Eilat (29°33'27.60"N) in the south (Fig. 1). Crow sampling regions were divided into 3 groups: (1) Haifa (32°49'49.30"N) in the north, (2) a central region comprised of the area between Beerli (31°40'0"N) to Gaash (32°13'40"N) and (3) Eilat (29°33'27.60"N) in the south.

Griffon vulture capture was performed in four areas ranging from Rash Mankushiya in the Judea Desert close to the Dead Sea (31°35'28.30"N) to Yotvata (29°52'60"N) in the south (Fig. 1).

2.3. Environmental data

Human population density data for the years, 2010–2011, was obtained from the Israel Bureau of Statistics website, http://www1.cbs.gov.il/reader/cw_usr_view_Folder?ID=141. Average annual summer temperatures (°C) for the months, April–September, maximum temperatures (°C), and average yearly precipitation figures for the years 2010–2011, were obtained from the Israel Meteorological Service website, <http://www.ims.gov.il>.

2.4. Serological examination

The modified agglutination test (MAT) for *T. gondii* antibodies was carried out as previously described by Dubey and Desmonts (1987). MAT antigen used in this study consisted of commercially supplied formalinized tachyzoites obtained from Dr. Villena Isabelle (Laboratoire de Parasitologie-Mycologie, Reims Cédex, France). Sera from birds were diluted serially in PBS at 1:25, 1:50, 1:100, 1:200 and 1:400. Each plate contained positive and negative controls for comparison. Random positive and negative reactions were repeated in order to confirm their reliability. Titers of 1:25 or higher were considered positive. This

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