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Seasonality in the proportions of domestic cats shedding *Toxoplasma gondii* or *Hammondia hammondi* oocysts is associated with climatic factors

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ABSTRACT

A previous study on domestic cats in Germany and neighbouring countries suggested seasonality in shedding *Toxoplasma gondii* oocysts. The aim of the present study was to elucidate whether this seasonality in shedding could be explained by climatic effects and whether differences between years in the proportions of cats shedding oocysts could also be explained by climatic factors. To this end, a long-term study over a period of 55 months on domestic cats for *T. gondii* and *Hammondia hammondi* oocysts was performed and the results compared with climatic data. Using species-specific PCR, *T. gondii* oocysts were identified in 0.14% (84/61,224) and *H. hammondi* in 0.10% (61/61,224) of the samples. *Toxoplasma gondii* oocysts were predominantly observed from summer to autumn, while *H. hammondi* oocysts were mainly found during autumn and winter. In statistical analyses using climatic data, even differences in parasitological findings between years could be partially modelled using monthly temperature, North Atlantic Oscillation indices and precipitation. Of the three climatic variables analysed, precipitation as an explanatory variable had the lowest impact in the statistical models while those taking only temperature and North Atlantic Oscillation indices into account were sufficiently predictive. Interestingly, time lags between the climatic event and the parasitological findings had to be implemented in all models. For *T. gondii*, North Atlantic Oscillation indices with a time lag of 7 months and temperature with a time lag of 2 months had the best predictive value. In contrast, temperature (with a time lag of 6 months) and the interaction of precipitation (with a time lag of 5 months) and North Atlantic Oscillation indices (with a time lag of 11 months) were optimal for predicting the seasonality of *H. hammondi*. These results suggest prominent differences in the life cycles of the two closely related parasites. Previous findings showed that *H. hammondi* lack avian hosts, in contrast to *T. gondii*, and the coincidence in the periods of high abundance of birds and high proportions of cats shedding *T. gondii* suggest that birds may play an important role in the epidemiology of this infection. The result that North Atlantic Oscillation index is an important variable in modelling variations in the proportion of cats shedding *T. gondii* and *H. hammondi* over the year is an indication that global warming may also influence the infection risk of animals and humans with *T. gondii* and *H. hammondi*. The findings have important implications for planning epidemiological studies and for estimating the risk of human infection.

1. Introduction

Toxoplasma gondii is an intracellularly multiplying parasite able to infect a large variety of warm blooded intermediate host species (Dubey, 2010). It is estimated that approximately one-third of the human population is infected with this parasite worldwide (Montoya and Liesenfeld, 2004). Most human *T. gondii* infections are asymptomatic. However, infection during pregnancy can cause severe congenital toxoplasmosis of the foetus due to transplacental transmission of the parasite. Infection in adults may be severe and even life threatening, especially in immunocompromised individuals, if not treated appropriately (Luft et al., 1993; Montoya and Liesenfeld, 2004; Carme et al., 2009; Schlüter et al., 2014). In immunocompetent adults, *T. gondii* may cause ocular toxoplasmosis (Maenz et al., 2014). In veterinary medicine, *T. gondii* is known

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as an important abortifacient, especially in small ruminants (Buxton, 1998). The parasite may cause severe disease in a variety of domestic and zoo animals (Dubey, 2010; Schlüter et al., 2014). Infections with *T. gondii* can be caused by several stages parasitizing in tissues of infected intermediate hosts, i.e. the fast-growing tachyzoites or the slow-growing and intracellularly encysted bradyzoites (Dubey, 2010). Pigs, small ruminants and chickens are known as efficient intermediate host species of *T. gondii* and the ingestion of raw or under-cooked meat with encysted *T. gondii* bradyzoites is regarded as an important cause of human infection (Kijlstra and Jongert, 2008; Dubey, 2010; Schlüter et al., 2014). Felines are definitive hosts for *T. gondii* (Dubey, 2010). *Toxoplasma gondii* completes the sexual part of its life cycle in the intestine of cats, resulting in the excretion of millions of environmentally-resistant oocysts (Schares et al., 2008b; Dabritz and Conrad, 2010; Dubey, 2010). These oocysts become infectious after a few days of sporulation, i.e. after development of sporozoites, which are protected from environmental stress by oocyst and sporocyst walls (Dubey, 2010). Rodents and birds are regarded as natural intermediate hosts (Literak et al., 1992; Hejlicek et al., 1997; Dubey, 2002; Lehmann et al., 2003; Kijlstra and Jongert, 2008; Reperant et al., 2009) and transmission from these animal species to cats via predation can expand the parasite population several million-fold (Dabritz and Conrad, 2010; Dubey, 2010).

The closest existing relative of *T. gondii* is *Hammondia hammondi* (Walzer et al., 2013, 2014), which also uses cats as definitive hosts, i.e. it also completes its life cycle in the intestine of cats (Dubey and Sreekumar, 2003). Compared with *T. gondii*, much less is known on the life cycle of this parasites including its spectra of definitive and intermediate hosts (Dubey and Sreekumar, 2003). *Hammondia hammondi* oocysts are able to infect laboratory mice (Eydelloth, M., 1977. Experimentelle Untersuchungen über das Wirtspektrum von *Hammondia hammondi*. Dissertation, Veterinary Faculty Munich, Germany; Mason, 1978; Dubey and Sreekumar, 2003; Schares et al., 2008a), rats (Frenkel and Dubey, 1973, 1975), wild rodents (Eydelloth, M., 1977. Dissertation, cited earlier), hamsters (Frenkel and Dubey, 1975; Christie and Dubey, 1977), guinea pigs (Frenkel and Dubey, 1975; Wallace, 1975), rabbits Eydelloth, M., 1977. Dissertation, cited earlier), goats (Dubey, 1981), dogs (Wallace, 1975) and monkeys (Dubey and Wong, 1978) as intermediate hosts but the host range appears to be more limited compared with *T. gondii*. To the best of our knowledge, there are no reports on disease caused by *H. hammondi* in humans and infection in humans has not yet been demonstrated. In contrast to *T. gondii*, chickens (Dubey and Streitel, 1976), quails (Wallace, 1975) and pigeons (Frenkel and Dubey, 1975; Wallace, 1975) could not be infected with *H. hammondi*, suggesting that the intermediate host spectrum of *H. hammondi* lacks avian species. *Toxoplasma gondii* oocysts can induce oocyst shedding in cats (Dubey, 1996), while oocyst shedding has not been observed after oral infection of cats with *H. hammondi* oocysts (Frenkel and Dubey, 1975).

In a previous study conducted between June 2007 and December 2008, we investigated the proportion of cats shedding *T. gondii* among samples submitted to a commercial German veterinary laboratory. The results showed a statistically significant higher proportion of *T. gondii*-positive cats between July and December than between January and June (Herrmann et al., 2010). These seasonal differences in the proportion of cats shedding *T. gondii* might be of importance in explaining differences in the number of human cases of toxoplasmosis during different periods of the year. Such seasonal differences in the incidence of human toxoplasmosis have been reported from Germany (Braveny et al., 1973), the Netherlands (Meenken et al., 1991) and Slovenia (Logar et al., 2005). These studies showed increased proportions of acute toxoplasmosis from January to June, increased birth rates of children with ocular toxoplasmosis in May, and increased incidences of human toxoplasmosis during winter and spring. A more recent study from Serbia reported that in symptomatic patients, acute infections occurred more frequently between October and March and asymptomatic acute infections were diagnosed significantly more often between February and July. Based on the low IgG avidity in patient sera, it was assumed that the infections had occurred between November and April (Bobic et al., 2010). Another study on various human infectious diseases including toxoplasmosis showed a correlation of the number of yearly cases with a climatic index called North Atlantic Oscillation (NAO) winter index (Hubalek, 2005). This suggested that climatic effects may have an impact on the exposure of humans to *T. gondii*. The NAO winter index is based on the difference in normalised sea level pressure between Gibraltar and southwestern Iceland. Positive NAO winter indices are associated with more intense and more frequent winter storms crossing the Atlantic Ocean towards Europe, usually causing milder winters in northern Europe associated with above-average precipitation. In contrast, negative phases of the NAO winter index are typically characterised by cold winters (Osborn, 2011).

Sporulation of *T. gondii* oocysts and those of *H. hammondi* is influenced by temperature (Frenkel and Dubey, 1975; Dubey, 2010) and the survival of oocysts is also moisture-dependent (Bergler et al., 1980; Dubey, 2010). In addition, the expansion and survival of natural intermediate host species such as rodents and birds rely on favourable climatic conditions (Walther et al., 2002). It is therefore conceivable that climatic factors may influence the life cycle of both parasites.

The aim of the present study was to analyse whether seasonality in the proportion of cats shedding *T. gondii* or *H. hammondi* is associated with climatic factors. To provide sufficient data for analysis, we included our previously published data (Herrmann et al., 2010) and further extended this study. In addition, we examined not only the shedding of *T. gondii* but also of *H. hammondi*. The results show that seasonality in the proportions of domestic cats shedding *T. gondii* or *H. hammondi* oocysts was associated with climatic factors. However, statistical models established for *T. gondii* and *H. hammondi* differed markedly, which suggest clear differences in the impact of these variables on the life cycles of the two parasites.

2. Materials and methods

2.1. Sample acquisition and analysis

Faecal samples from owned domestic cats, which had been submitted to IDEXX Laboratories (previous name 'Vet Med Labor GmbH'), Ludwigsburg, Germany, were examined as previously reported (Herrmann et al., 2010). The analysis comprised a conventional flotation method using 262 mg/ml of $ZnCl_2$ and 275 mg/ml of NaCl (specific gravity of 1.3) followed by microscopic examination (Schares et al., 2005). When oocysts with a diameter of about 9–14 μm were observed, the remaining sample was posted to the Friedrich-Loeffler-Institut (FLI), Wusterhausen, Germany, where they were further examined by a combined sedimentation and flotation procedure (Schares et al., 2005). Isolated oocysts were used for DNA extraction employing a previously published protocol (Herrmann et al., 2010). For the detection of *T. gondii* and *H. hammondi*, the primer pairs Tox4/Tox5 (Homan et al., 2000), Tox5/Tox-8 (Reischl et al., 2003) and Hham34F/Hham3R (Schares et al., 2008a) were used as previously described (Herrmann et al., 2010).

2.2. Source of climatic data

Monthly data on average air temperature (later referred to as temperature; regional mean for Germany) and monthly