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Serological survey and risk factors for *Toxoplasma gondii* in domestic ducks and geese in Lower Saxony, Germany

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ABSTRACT

To obtain estimates for the prevalence of *Toxoplasma gondii* infection in ducks and geese in Germany, enzyme-linked immunosorbent assays (ELISA) were established based on affinity-purified *T. gondii* tachyzoite surface antigen 1 (TgSAG1) and used to examine duck and goose sera for *T. gondii*-specific antibodies. The results of 186 sera from 60 non-infected ducks (*Anas platyrhynchos*) and 101 sera from 36 non-infected geese (*Anser anser*) as well as 72 sera from 11 ducks and 89 sera from 12 geese inoculated experimentally with *T. gondii* tachyzoites (intravenously) or oocysts (orally) and positive in a *T. gondii* immunofluorescent antibody test (IFAT) were used to select a cut-off value for the TgSAG1-ELISA. Sera obtained by serial bleeding of experimentally inoculated ducks and geese were tested to analyze the time course of anti-TgSAG1 antibodies after inoculation and to assess the sensitivity of the assays in comparison with IFAT. In ducks, IFAT titres and ELISA indices peaked 2 and 5 weeks p.i with tachyzoites, respectively. Only three of six geese inoculated with tachyzoites at the same time as the ducks elicited a low and non-permanent antibody response as detected by the IFAT. In the TgSAG1-ELISA, only a slight increase of the ELISA indices was observed in four of six tachyzoite-inoculated geese. By contrast, inoculation of ducks and geese with oocysts led to an increase in anti-TgSAG1 antibodies within 1 or 2 weeks, which were still detectable at the end of the observation period, i.e. 11 weeks p.i. Inoculation of three ducks and three geese with oocysts of *Hammondia hammondi*, a protozoon closely related to *T. gondii*, resulted in a transient seroconversion in ducks and geese as measured by IFAT or TgSAG1-ELISA. Using the newly established TgSAG1-ELISA, sera from naturally exposed ducks and geese sampled in the course of a monitoring program for avian influenza were examined for antibodies to *T. gondii*; 145/2534 (5.7%) of the ducks and 94/373 (25.2%) of the geese had antibodies against TgSAG1. Seropositive animals were detected on 20 of 61 duck and in 11 of 13 goose farms; the seroprevalences within positive submissions of single farms ranged from 2.2% to 78.6%. Farms keeping ducks or geese exclusively indoors had a significantly lower risk (odds ratio 0.05, 95% confidence interval 0.01–0.3) of harboring serologically positive animals as compared with farms where the animals had access to an enclosure outside the barn.

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1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that can infect all warm-blooded animals, i.e. mammals and avian species (Dubey, 2010). *T. gondii* has a clonal population structure with three main clonal lineages designated as types I, II, and III (Howe and Sibley, 1995). These lineages largely predominate in Europe and North America while strains not related to these lineages have been described mainly from other geographic regions (Dardé, 2004, 2008; Sibley et al., 2009). Phylogenetically, *T. gondii* is closely related to *Hammondia hammondi*, a protozoan that – like *T. gondii* – uses felids as definitive hosts (Dubey and Sreekumar, 2003).

T. gondii has been isolated from the tissues of domestic Anseriformes (Literák and Hejlíček, 1993; Dubey et al., 2003, 2007; Bártová et al., 2004; Zia-Ali et al., 2005, 2007). Recent serological studies during the past decade suggest that *T. gondii* infections in domestic Anseriformes may occur worldwide, and are not rare (Literák and Hejlíček, 1993; El Massry et al., 2000; Sroka, 2001; Dubey et al., 2003, 2007; Zia-Ali et al., 2005, 2007; Bártová et al., 2009; Yan et al., 2009; Sroka et al., 2010). Anseriformes may become infected with *T. gondii* by oral ingestion of sporulated *T. gondii* oocysts with feed or water, or of tissue cysts in carcasses of intermediate hosts as demonstrated by experimental studies in domestic ducks (Bártová et al., 2004; Murao et al., 2008). The oocyst is an environmentally resistant stage of the parasite shed by felids such as domestic cats, which are the predominant definitive host of *T. gondii* in Europe. Felids can excrete large numbers of oocysts which become infectious in the environment by sporulation and may remain infectious for several months (Dubey, 2010). Up to 13 million oocysts per gram of feces were reported in naturally infected cats (Schares et al., 2008). Intermediate hosts, such as birds, rodents, domestic mammals or humans can contract the infection by ingesting sporulated *T. gondii* oocysts.

Contamination of drinking water with *T. gondii* oocysts can cause major outbreaks of acute toxoplasmosis in humans (de Moura et al., 2006). However, infection by tissue cysts in raw or undercooked meat is often regarded the predominant route for human infection (Cook et al., 2000). In addition to consumption, handling of raw meat of infected intermediate hosts prior to preparing meals may also pose a risk of infection, as a low standard of kitchen hygiene was demonstrated as a risk factor for primary *T. gondii* infection during pregnancy (Kapperud et al., 1996). To which extent the consumption or handling of poultry meat, including the meat of Anseriformes, contributes to human infections remains to be clarified. The isolation of viable *T. gondii* from the tissue of Anseriforms after acidic pepsin digestions (Zia-Ali et al., 2005, 2007; Dubey et al., 2007) suggests that tissue cysts were present in these tissues.

To assess the potential risk for human infection with *T. gondii* due to exposure to meat from Anseriformes, data on the proportion of infected ducks and geese are needed. Therefore the present study aimed at the development and the validation of serological tests for the detection of *T. gondii*-specific antibodies in ducks and geese. We report for the first time serological data on the *T. gondii* prevalence in ducks and geese in the German federal state of Lower Saxony.

2. Materials and methods

2.1. Parasites

2.1.1. Tachyzoites

The *T. gondii* strains RH (Sabin, 1941), ME49 (Lunde and Jacobs, 1983), and NED (Howe and Sibley, 1995), i.e. representatives of the three main clonal lineages of *T. gondii* (type I, II, and III, respectively), were cultivated at 37 °C, 5% CO_2 in Vero cells for 3–5 days with MEM Dulbecco's medium supplemented with 1% glutamine, 2% fetal calf serum (FCS) and 1% antibiotic solution (10,000 IU penicillin and 10,000 μg streptomycin/ml solution). To harvest *T. gondii* tachyzoites the cells were scraped from the flask and tachyzoites purified by filtration using 5 μm filters (Millipore, Eschborn, Germany), washed five times by centrifugation at $700 \times g$ (4 °C, 8 min) and resuspended in ice-cold phosphate-buffered saline (PBS). For the experimental infection of ducks and geese, the tachyzoites of all three strains were counted using a Neubauer chamber, checked by Trypan Blue exclusion for viability and used immediately after harvest. One day before parasites were harvested for antigen production, the FCS-supplemented medium was removed and infected cells were further cultivated under FCS-free conditions. If used as antigen, RH-strain tachyzoites were centrifuged ($700 \times g$) and dispensed onto IFAT slides or stored as a pellet at −80 °C until used for purification of the *T. gondii* surface antigen 1 (TgSAG1).

2.1.2. Oocysts

T. gondii oocysts were collected from the feces of an experimentally infected cat by flotation using saturated NaCl solution (specific gravidity 1.2). The cat had been orally infected with tissue cysts of the *T. gondii* DX strain (type II; Howe and Sibley, 1995) isolated from a pig (Störmann, 1962). Sporulated oocysts were stored in 2% (v/v) sulfuric acid at 4–8 °C. Prior to use, oocysts were washed with tap water and counted using a Neubauer chamber.

2.2. Experimental infections

All animal experiments reported in this publication were approved by the Ministerium für Landwirtschaft, Umweltschutz und Raumordnung of the German Federal State of Brandenburg or the Lower Saxony State Office for Consumer Protection and Food Safety of the German Federal State of Lower Saxony.

2.2.1. Inoculation of tachyzoites

Ducks (*Anas platyrhynchos*) and geese (*Anser anser*), obtained at the age of 5 weeks from a commercial breeder (Geflügelhof Gaetke, Parchim, Germany) and negative by IFAT, were used to produce positive reference sera. Six geese and six ducks were distributed into three groups of two animals each (Table 1). Two ducks and 2 geese were