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# Quantitative detection of *Toxoplasma gondii* in tissues of experimentally infected turkeys and in retail turkey products by magnetic-capture PCR

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ABSTRACT

Magnetic-capture PCR was applied for the quantitative detection of *Toxoplasma gondii* in tissues of experimentally infected turkeys and retail turkey meat products. For experimental infection, three *T. gondii* strains (ME49, CZ-Tiger, NED), varying infectious doses in different matrices (organisms in single mouse brains or $10^3$, $10^5$, or $10^6$ oocysts in buffer) were used. From all animals, breast, thigh, and drumstick muscle tissues and for CZ-Tiger-infected animals additionally brains and hearts were analyzed. Using the magnetic-capture PCR large volumes of up to 100 g were examined. Our results show that most *T. gondii* parasites are present in brain and heart tissue. Of the three skeletal muscle types, drumsticks were affected at the highest and breast at the lowest level. Type III strain (NED) seems to be less efficient in infecting turkeys compared to type II strains, because only few tissues of NED infected animals contained *T. gondii* DNA. Furthermore, the number of detected parasitic stages increased with the level of infectious dose. Infection mode by either oocyst or tissue cyst stage did not have an effect on the amount of *T. gondii* present in tissues. In retail turkey meat products *T. gondii* DNA was not detectable although a contact with the parasite was inferred by serology.



## 1. Introduction

*Toxoplasma gondii* is one of the most common protozoan parasites worldwide. It is assumed to be able to infect all warm-blooded species while felids represent its definitive host. Infection of humans can remain asymptomatic, particularly in immunocompetent hosts, but can also lead to severe toxoplasmosis in immunocompromised patients. Especially at risk are seronegative pregnant women, because an infection with the parasite can severely damage the unborn child (Weiss and Dubey, 2009). Humans mainly get infected by the consumption of raw or undercooked meat from infected animals, in which the parasites persist within tissue cysts in tissues such as muscles and brain. Turkey meat is very popular in some regions of the world. Most turkey meat is consumed in the USA with 7.4 kg/capita in 2012, followed by Austria (6.1 kg), Germany (5.7 kg), France (5.3 kg), and Italy (4.8 kg) (AVEC, 2013). Turkey meat has been identified as a risk factor for *T. gondii* infection (Alvarado-Esquivel et al., 2006, 2008). However, there is only little information available about the presence of *T. gondii* in turkeys. After a fatal toxoplasmosis was reported in a wild turkey (Howerth and Rodenroth, 1985), Dubey et al. (1993a) experimentally infected 14 turkeys with *T. gondii* strain ME49 for the first time and bioassayed brain, breast muscle, leg muscle, liver, and heart. Bioassays were positive for heart and muscles. Sedlák et al. (2000) also performed experimental infection of five turkeys resulting in positive bioassays of pooled brain, liver, spleen, heart, and leg muscles. Several serological studies have been published showing seroprevalences from 10.0 % to 76.6 % in wild or private turkeys in Iran, USA, Egypt, and Iraq (Butty, 2009; El-Massry et al., 2000; Ghorbani et al., 1990; Harfoush and Tahoon, 2010; Lindsay et al., 1994; Quist et al., 1995) while in the Czech Republic no seropositive turkeys were found (Bártová et al., 2009; Literák and Hejlícek, 1993). We have previously published results on fattening turkeys in Germany intended for human consumption.

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Average seroprevalence in these conventionally indoor raised animals was 18.4% but in individual fattening cycles it was as high as 77.1% indicating intensive contact of the turkeys with the parasite (Koethe et al., 2011). Furthermore, we could show by nested PCR that many tissues were parasitized after experimental, oral or parenteral *T. gondii* infection with oocysts or tachyzoites, respectively (Bangoura et al., 2013; Zöller et al., 2013). Previous results are all based on conventional DNA extraction methods, where only as little as about 25 mg of tissue could be used as starting material. Having in mind that *T. gondii* accumulates in tissue cysts that are not equally distributed within tissues it is obvious that with conventional DNA extraction methods *T. gondii* detection and quantification may be flawed. In this context, Opsteegh et al. (2010) described a DNA extraction method by which *T. gondii* DNA sequences specifically are captured using magnetic beads and thereby making it possible to examine large samples of up to 100 g. Accurate and reliable quantification of *T. gondii* equivalents in the samples is possible by subsequent quantitative real-time PCR that has been performed on pig meat and sheep heart samples (Opsteegh et al., 2010). This method was further applied to pig, goat, and mice tissues and organs (Jurànková et al., 2014, 2013a, 2013c) as well as for quantitative detection of *T. gondii* in Serrano ham (Gomez-Samblas et al., 2015) and has been confirmed to be a highly sensitive and specific method.

The aim of our study was to apply the sequence-specific magnetic-capture PCR method to quantify the amount of *T. gondii* equivalents in different tissues and organs of experimentally infected turkeys and to examine whether or not there are differences regarding the parasite strain or infection dose or material. Either brains of infected mice or oocysts shed by infected cats were used for infection to mimic natural infection routes. We mainly focused on different muscle tissues that are relevant for human consumption but particularly also examined brains and hearts for comparison. Furthermore, retail turkey meat and meat products were analyzed to assess a potential risk of infection by the consumption of such products.

## 2. Materials and methods

### 2.1. Parasites

Three different *T. gondii* strains were used in the study: CZ-Tiger (Jurànková et al., 2013c, oocysts kindly provided by Walter Basso, Institute of Parasitology, University of Zurich, Switzerland) and ME49 (Lunde and Jacobs, 1983) as type II strains; NED (Howe and Sibley, 1995) as type III strain. Tachyzoites of ME49 and NED strains were maintained in VERO cell culture in IMDM medium supplemented with 5% fetal bovine serum, 1% penicillin/streptomycin, and 1% amphotericin B at 37 °C and 5% $CO_2$ as previously described (Zintl et al., 2009). Cell culture supernatants were centrifuged at 2000 × *g* for 5 min, the resulting pellet was resolved in 1 ml PBS and tachyzoites were counted in a Neubauer chamber. Six CD1 mice were intraperitoneally infected with either 2000 ME49 tachyzoites (four mice) or 1500 NED tachyzoites (two mice). Two CD1 mice were infected with 100 CZ-Tiger oocysts orally. Cats were fed with infected mouse brains to passage the parasites and yield sufficient amounts of oocysts. In detail, two cats each were fed with one brain of a mouse infected with the CZ-Tiger strain, another two cats each were fed with one NED-infected mouse brain and two other cats each received two ME49-infected mouse brains. To obtain brain tissue cysts for turkey infections, each six CD1 mice were infected with 10–100 oocysts orally or 100–2000 tachyzoites intraperitoneally of the respective strains. Mouse infections were verified by light microscopically examination of brain squashes and by serology. Infections of cats and mice met legal requirements and were granted by the competent authority (Landesdirektion Leipzig, Germany, trial no. V09/12).

### 2.2. Animals

BUT B.I.G. 6 turkeys were stabled as one-day-old chicks and raised at the Institute of Parasitology, University of Leipzig where they were kept on a bedding of wood shavings and underwent daily clinical observations. A total of 48 infected and 6 uninfected turkeys were included in the study. Turkeys were fed with poultry feed for pet birds without anticoccidials (deuka Wild-und Ziergeflügelfutter, Deutsche Tiernahrung Cremer, Germany) which was supplemented with vitamins, minerals (Korvimin ZVT + Reptil, WDT, Germany), and powdered milk to obtain recommended nutritional values for turkeys. Feed and water were available *ad libitum*.

### 2.3. Experimental study design

Turkeys were divided into nine groups of six animals each. Eight groups were infected with different doses ($10^3$–$10^6$ ooysts or tissue cysts within one mouse brain), strains and stages while one group remained uninfected to serve as negative control group as outlined in Table 1. Oocysts or mouse brains were applied orally into the crop of the turkeys. Eight weeks after the day of infection, animals were sacrificed and tissue samples were collected and stored frozen at −20 °C for examination. Infection experiments complied with legal regulations and were approved by the responsible authority (Landesdirektion Leipzig, Germany, trial no. TVV 29/10).

### 2.4. Serology

Sera of experimentally infected turkeys were examined for seroconversion to confirm infection or absence of infection in control group animals, respectively. Blood was drawn from the wing vein (*Vena cutanea ulnaris*) prior to infection and in weekly intervals thereafter. After blood clotting, sera were separated by centrifugation at 2600 × *g* for 15 min at room temperature and collected thereafter. Serum samples were stored frozen at −20 °C until used for testing. Kinetic ELISA (KELA) was performed on turkey sera as described previously (Koethe et al., 2011). All infected animals seroconverted within the first two weeks after infection while all uninfected turkeys remained seronegative throughout the study.

From animals killed at the abattoir, blood was collected at the bleeding station into a sterile 50 ml tube. During transportation to the Institute of Food Hygiene, blood clotted and subsequently serum was obtained and frozen the same day. Afterward, sera were also examined by the above mentioned KELA.

From wholesale breast fillets, meat fluid was used for serologically examination instead of serum. KELA with meat fluid was performed accordingly as described previously (Koethe et al., 2011).

### 2.5. Samples

Focusing mainly on meat, breast muscles, thigh muscles, and lower leg (drumstick) muscles from all experimentally infected turkeys were examined. For comparison, brains and hearts from CZ-Tiger infected turkeys were examined additionally. Of the muscles, 100 g were cut into pieces with edges of approximately 2 cm in length. Complete brains were homogenized with a mortar and pestles and complete hearts were ground with a commercial household blender (La Moulinette, Tefal Groupe SEB, Germany). Fractions of the homogenates (mean weights were 1.8 g for brains and 17.4 g for hearts) were subjected to DNA capture.