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Research paper

Development of an *in vivo* model for *Toxoplasma gondii* infections in chickens and turkeys simulating natural routes of infection

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ABSTRACT

Turkeys and chickens were orally infected with tissue cysts (one mouse brain) or oocysts (10^3, 10^5 or 10^6 oocysts) of three *T. gondii* strains of the clonal types II and III (ME49, CZ-Tiger, NED) to investigate the influence of the applied *T. gondii* strain and infective doses on the distribution of *T. gondii* in several organs and tissues and the serologic response of chickens and turkeys. Organ samples from 16 different tissues, including heart, brain, muscles and gizzard were analyzed by PCR. Brain and heart were found most frequently positive for *T. gondii* DNA in both species, followed by gizzard. Serological analysis with kinetic ELISA for turkey samples and IFAT for chicken samples were performed once a week. In both species a dose-depending serological response was found. Turkeys seroconverted one week after infection with CZ-Tiger strain and medium and high doses of ME49 oocysts. In chickens, infection with medium and high doses of CZ-Tiger led to seroconversion one week p.i. Frequency of *T. gondii* positive organs showed a trend of a dose-effect in both species after infection with the type II strains. The NED strain showed low virulence in chickens and turkeys, demonstrated by clearly less *T. gondii* positive organs. Infection with tissue cysts of all three strains revealed *T. gondii* stages in tissues of turkeys and chickens. In conclusion, our data show a risk for human infection with *T. gondii* due to consumption of chicken and turkey meat.

1. Introduction

Toxoplasma (T.) gondii is a widespread protozoan parasite infecting warm-blooded animals including humans and birds (Tenter et al., 2000). Felids are the definitive hosts of *T. gondii* and shed oocysts after infection with one of the three infectious stages (Frenkel et al., 1970). Almost all warm-blooded animals and humans can act as intermediate hosts. The life cycle is facultative heteroxenous, i.e. intermediate hosts develop tissue cysts in several organs when infected by ingesting oocysts from fecal contamination by definitive hosts, or consuming cyst-containing tissues from another intermediate host (Dubey et al., 1998). Tissue cysts are formed especially in neural and muscular tissue (Dubey et al., 1998; Tenter et al., 2000).

Infected cats may shed up to 360 million of oocysts (Dubey, 2001, 2002, 2005) and contaminate wide areas of soil with oocysts (Gotteland et al., 2014). Thus, free-range poultry often is infected with *T. gondii* oocysts when feeding on contaminated soil. For that reason, free-range chickens are frequently used as sentinel animals to show environmental contamination with *T. gondii*, as described in numerous studies (Dubey et al., 2005, 2006, 2008, 2015). In addition, an infection of poultry via tissue cysts by feeding on infected rodents is possible (Koethe et al., 2015) enhancing infection prevalence. As *T. gondii* causes zoonotic infections, humans most often acquire *T. gondii* infections by ingestion of tissue cysts through consumption of raw or undercooked meat (Cook et al., 2000; Wilking et al., 2016).

Consumption of poultry meat has expanded over the last 20 years all over the world (OECD, 2019). Chickens and turkeys are the most important source of meat worldwide and in North America and the second important source in Europe following pork (OECD, 2019). Usually, poultry meat is consumed well-cooked, rendering tissue cysts no longer

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infective (Dubey et al., 1990). Nonetheless, in many countries, an increasing number of raw poultry meat products is placed onto the market, for example, dry-cured ham and short-matured raw sausages. The processing of these products is not always sufficient to inactivate *T. gondii* tissue cysts (Dubey, 1997; Pott et al., 2013). Hence, such products and undercooked poultry meat can pose an infection risk for consumers.

Worldwide, there are different *T. gondii* strains causing infections in animals and humans. In Europe and North America, the species *T. gondii* is currently assumed to contain four major clonal lineages (Howe and Sibley, 1995; Khan et al., 2011) and atypical strains. Across Europe, type II strains are clearly dominating in humans and animals (Aubert et al., 2010; Herrmann et al., 2012; Nowakowska et al., 2006; Schares et al., 2008; Shwab et al., 2014). However, additionally type III strains were found in Europe (De Sousa et al., 2006; Dubey et al., 2006; Messaritakis et al., 2008). In contrast, evidence of type I strains in animals in Europe is rare in the literature (Ajzenberg, 2010).

For mammalian host species, many data are available on *T. gondii* strain- and dose-dependent cyst formation patterns (Dubey, 1988; Dubey et al., 2016; Esteban-Redondo et al., 1999; Jungersen et al., 1999; Opsteegh et al., 2010). Regarding poultry, previous experimental infection studies use a variety of *T. gondii* strains in different gallinaceous bird species (Bangoura et al., 2013; Dubey et al., 1993a; Kaneto et al., 1997; Koethe et al., 2015; Schares et al., 2018; Sedlak and Franti, 2000) leading to inconsistent results. In addition, many field studies refer to the *T. gondii* positivity of the presumed predilection organs heart and brain though they do not play a significant role in human consumption (Dubey, 2010; Schares et al., 2017).

Evidence of T. gondii cyst organ distribution differences between turkeys and chickens was already shown by our group (Geuthner et al., 2014; Koethe et al., 2015; Schares et al., 2018), although intravenous infection with tachyzoites was utilized which limits applicability of the study results to field infections. Thus, there is a need for research into the influence of different strains and infection doses on the organ distribution of *T. gondii* in turkeys and chickens including edible organs, for both natural routes of infection, i.e. oocyst or cyst ingestion.

Comparative infections with representative strains of different clonal lineages are a prerequisite to judge the potential for *T. gondii* transmission by poultry meat. Accordingly, the aim of the study was to develop a model to simulate two naturally possible ways of infection of *T. gondii* in chickens and turkeys. For that reason, we infected chickens and turkeys with two well-described laboratory strains and one field strain of *T. gondii* belonging to two distinct clonal lineages (type II and III). The aim of the study was to investigate the influence of these three representative *T. gondii* strains, applied in different infective doses, on the distribution of *T. gondii* in several organs and tissues of chickens and turkeys. Additionally, the impact of the varied parameters on the seroconversion of infected animals was examined.

2. Material and methods

2.1. Parasites

In this study, three different strains belonging to two of the four major clonal lineages of *T. gondii* were used for animal infection, in particular the *T. gondii* strains ME49 (type II; Lunde and Jacobs, 1983; ToxoDB RFLP-Genotype 1), a field strain from the Czech Republic (type II strain CZ-Tiger; Juránková et al., 2013; ToxoDB RFLP-Genotype 3), and NED (type III; Dardé et al., 1992; ToxoDB RFLP-Genotype 2).

2.1.1. Tachyzoites

For infection of mice, ME49 and NED tachyzoites were cultured in VERO cells cultivated with Iscove's Modified Dulbecco's Medium (IMDM, PAA, Pasching, Austria), 5% fetal calf serum, 1% penicillin/streptomycin and 1% amphotericin B as described before (Geuthner et al., 2014) at 37 °C, 5% CO_2. For both ME49 and NED strains, the number of cell culture passages before mouse infection has not been tracked, both are long-term used laboratory strains. The supernatant of cell culture was centrifuged at 2000 × *g* for 5 min to harvest the parasites. The pellet was resolved in 1 mL PBS solution and tachyzoites were counted using a Neubauer chamber. For intraperitoneal infection of mice, tachyzoites were diluted in 100 µL sterile isotonic 0.9% sodium chloride solution (B. Braun Melsungen AG, Melsungen, Germany).

2.1.2. Oocysts

Six cats (ME49, CZ-Tiger, NED, n = 2 per strain) were orally infected with *T. gondii* tissue cysts from 1 to 2 mice brains per animal as described by Schares et al. (2018). Feces of experimentally infected cats were collected starting at day 3 after infection and *T. gondii* oocysts were obtained by flotation in saturated saline solution (specific gravity 1.20 g/mL). After sporulation at room temperature, oocysts were stored in 2% potassium dichromate at 4 °C. For infection dose preparation, sporulated oocysts were washed twice with faucet water before dosing.

2.1.3. Tissue cysts

For generating tissue cysts, Balb/c and CD1 mice were infected with 1000–2000 tachyzoites intraperitoneally (strains ME49 and NED), or 10–100 oocysts or tissue cysts orally (strain CZ-Tiger), respectively. Mice passage was done for all three *T. gondii* strains separately, with the actual infection dose depending on the amount of infectious material available. At least four weeks *post infectionem* (p.i.), mice were killed by cervical dislocation. Brains were dissected, and a squash preparation of every brain was examined for tissue cysts under light microscope.

Per chicken or turkey, the tissue amount of one positive mouse brain was used for infection, simulating the natural ingestion amount in case of preying on infective rodents. Therefore, for the infection of a group of six chickens or turkeys, respectively, six positive mouse brains and isotonic 0.9% sodium chloride solution (B. Braun Melsungen AG, Melsungen, Germany) were mixed to yield 6 mL of a brain homogenate. 1 mL of the homogenate was applied to each bird of the group to ensure a similar infection dose within each group.

2.2. Animals and husbandry

All animal trials were conducted at the Institute of Parasitology, Leipzig University, Germany and were approved by the responsible authority (Landesdirektion Leipzig, Germany, trial no. TVV 29/10).

2.2.1. Poultry

Commercially available turkeys (BUT B.I.G. 6, n = 78) and chickens (ISA JA 757, n = 78) were purchased as day-old poults. Chickens and turkeys were reared in separate groups for one week (chickens) or three weeks (turkeys) before experimental infection. The birds were kept on litter comprised of wood shavings. Chickens obtained conventional starter and grower feed for chickens without anticoccidials *ad libitum*. Because conventional starter and grower feed for turkeys without anticoccidials were not available, turkeys were receiving poultry feed for pet poultry without anticoccidials (deuka Wild-und Ziergeflügelfutter, Deutsche Tiernahrung Cremer, Duesseldorf, Germany) *ad libitum*. The feed was enriched with powdered milk and supplements (Korvimin ZVT + Reptil, WDT, Garbsen, Germany) to match the recommended nutritional values for growing turkeys. Animals had free access to water.

2.2.2. Cats

Specific pathogen-free cats were housed in disinfected, tiled rooms and were fed with commercially available canned cat food and dry food. Water was available *ad libitum*.

2.2.3. Mice

For all experiments, Balb/c and CD1 mice were acquired from the experimental center (Medizinisch-Experimentelles Zentrum), Leipzig

Table 1
Study design for chicken and turkey experiments (identical groups were formed for both poultry species, i.e. there was each of the given groups for chickens and for turkeys, respectively. NC, uninfected negative control).

Study group (n = 6 per group)	*T. gondii* strain	Infective stage	Infection dose
Cy-M	ME49	cysts	1 mouse brain equivalent
O-M1		oocysts	1×10^3
O-M2			1×10^5
O-M3			1×10^6
NC-M	–	–	–
Cy-T	CZ-Tiger	cysts	1 mouse brain equivalent
O-T1		oocysts	1×10^3
O-T2			1×10^5
O-T3			1×10^6
NC-T	–	–	–
Cy-N	NED	cysts	1 mouse brain equivalent
NC-N		–	–
O-N1	NED	oocysts	1×10^3
NC-N	–	–	–

University, Germany. They were kept on wood shavings and autoclaved hay. Commercially available rodent feed and water were provided *ad libitum*.

2.3. Infection and study design

2.3.1. Infection of poultry

78 chickens and 78 turkeys, raised for 9–10 days (chickens) or 21–26 days (turkeys), respectively, were included into the study.

For each of the type II strains ME49 and CZ-Tiger, 24 chickens and 24 turkeys were infected (see Table 1). Chickens and turkeys were divided into 4 groups each (n = 6). Per animal, either 1 mL of mouse brain homogenate, or 1 mL of water containing 1×10^3, 1×10^5, or 1×10^6 oocysts were orally administered by using an animal feeding needle into the crop.

For infection with NED strain, 12 chickens and turkeys were allocated into 2 groups (n = 6) each (see Table 1). They were infected with 1 mL mouse brain homogenate or water containing 10^3 oocysts as described above.

The remaining 18 chickens and 18 turkeys stayed uninfected as a negative control group (n = 6 per strain). All chickens were slaughtered at 5 weeks p.i. All turkeys were slaughtered at 8 weeks p.i. The time point of slaughtering was determined on the basis of previously published data (Geuthner et al., 2014).

2.3.2. Serological examinations

For screening of *T. gondii*-specific antibody concentrations, blood samples were drawn from the wing vein prior to infection and weekly after infection until slaughtering. Blood was centrifuged at 2500 × *g* for 15 min and serum samples were stored at – 20 °C until analyzing. Two different assays were performed to assess the serum antibody concentration; for turkey sera, a kinetic ELISA (KELA) was employed as previously described (Geuthner et al., 2014; Koethe et al., 2011), while an immunofluorescent antibody test (IFAT) was performed for chicken sera as described by Maksimov et al. (2011).

2.3.3. Sample processing

Animals were slaughtered after intramuscularly applied anesthesia with ketamine (25 mg/kg) and xylazine (1 mg/kg). During dissection, tissues samples were taken from 16 different organs/locations, including edible parts (heart, breast muscle, drumstick, thigh muscle, liver, gizzard) that may be purchased for human consumption, as well as non-edible parts (glandular stomach, pancreas, spleen, kidneys, brain, retina, gonads, intestine, lung, bone marrow).

Tissues were homogenized immediately after slaughter using commercial household blenders (La Moulinette, Tefal Groupe SEB, Offenbach, Germany) or mortar and pestles for brain, gonads, and bone marrow. The whole organ homogenate was stored at − 20 °C, except for muscles; for this tissue up to 300 g sample material per bird was taken from the different locations, and stored at − 20 °C.

25 mg tissue per sample or 10 mg spleen tissue were used for DNA extraction using the commercially available QIAamp DNA Mini Kit® (Qiagen, Hilden, Germany) following the manufacturer's instructions. As control of possible DNA contamination during extraction, after no more than three samples from infected birds, at least one sample of the negative control group was handled and carried along during the whole processing including PCR and gel electrophoresis. DNA concentration of samples was measured using a spectrophotometer (Nanodrop 2000c, Thermo Scientific, Waltham, MA, USA). For PCR, DNA from cell culture-derived ME49 tachyzoites served as positive control. Positive and negative controls (aliquot of currently used DNA elution buffer) were carried along in every PCR batch. PCR reactions were carried out as a direct PCR and followed by a nested PCR as described by Zöller et al. (2013) with minor modifications. For direct PCR, each reaction contained 1 U DreamTaq Green DNA Polymerase®, 10X DreamTaq Green Buffer® (Thermo Scientific, Mannheim, Germany), 200 µM of each dNTP (Fermentas, St. Leon Rot, Germany), 1.5 mM $MgCl_2$, 0.4 µM of each primer Tg1 (5′-AAA AAT GTG GGA ATG AAA GAG-3′) and Tg2 (5′-ACG AAT CAA CGG AAC TGT AAT-3′). DNA extract was added to obtain 80–200 ng template DNA, and reaction volume was adjusted to 25 µL by adding DNase-free water. For the subsequent nested PCR, PCR reactions were assembled as described above using primers Tgnested1 (5′-CGC TAATGT GTT TGC ATA GG-3′) and Tgnested2 (5′-GGC ACG TCT CTT GTT CTT CT-3′), 3 mM $MgCl_2$ and 2.5 µL of the direct PCR product as template. Both PCRs were performed in a peqSTAR thermocycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany) or a M × 3000P®Real-Time PCR System (Stratagene/Agilent Technologies, La Jolla, USA) under the following cycling conditions: preheating (2 min at 94 °C), amplification for 35 cycles (direct PCR) or 25 cycles (nested PCR) of 1 min at 94 °C, 40 s at 55 °C and 1 min at 72 °C and one final extension step of 5 min at 72 °C. The nested PCR products were analyzed by gel electrophoresis. Therefore, approximately 10 µL of the nested PCR products were applied on a 1.5% agarose gel, which was stained with ethidium bromide. Visualization of DNA bands took place with UV-light in positive samples at 375 bp fragment length.

2.3.4. Statistics

IBM SPSS Statistics Version 22 software package (IBM, New York, USA) was used for statistical analysis. All data were non-normally distributed and were therefore further analyzed by Kruskal–Wallis test followed by Mann-Whitney U test (infection group differences in number of animals positive per organ, sum of positive organs and seroconversion) and Friedman test (determination if any organs are more frequently positive than others). The Spearman rank correlation test was performed to explore possible correlations between the positivity status of individual organs. Group differences and correlations with P values of less than 0.05 were defined as statistically significant.

3. Results

3.1. Serology

3.1.1. Chickens

All chickens included into the study were tested negative for *T. gondii* antibodies on the day of infection (day 0). Each infected chicken was seroconverting not later than week four p.i. (see Table 2 for detailed seroconversion data by infection mode), except for one chicken of group Cy-N. Interestingly, chickens infected with the CZ-Tiger strain seroconverted significantly earlier than chickens of the two other

Table 2
Seroconversion of chickens over the complete time of study (Seropositive animals /infected animals (relative number of seropositive animals in %).

Infection mode[a]	Number and proportion of seroconverted animals stratified for time after infection					
	0 dpi	7 dpi	14 dpi[b]	21 dpi	28 dpi	35 dpi
Cy-M	0/6 (0%)	0/6 (0%)	0/6 (0%)[c]	5/6 (83.3%)	5/5 (100%)	4/5 (80.0%)
O-M1	0/6 (0%)	0/6 (0%)	0/6 (0%)[c]	5/6 (83.3%)	6/6 (100%)	6/6 (100%)
O-M2	0/6 (0%)	0/6 (0%)	1/6 (16.7%)[c, g]	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-M3	0/6 (0%)	0/4 (0%)	4/4 (100%)[d]	4/4 (100%)	4/4 (100%)	4/4 (100%)
M-total	0/24 (0%)	0/22 (0%)	5/22 (22.7%)[E]	20/22 (90.9%)	21/21 (100%)	20/21 (95.2%)
Cy-T	0/6 (0%)	0/6 (0%)	4/6 (66.7%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-T1	0/6 (0%)	0/6 (0%)	4/6 (66.7%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-T2	0/6 (0%)	1/6 (16.7%)	6/6 (100%)[h]	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-T3	0/6 (0%)	3/6 (50.0%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
T-total	0/24 (0%)	4/24 (16.7%)	20/24 (83.3%)[F]	24/24 (100%)	24/24 (100%)	24/24 (100%)
Cy-N	0/6 (0%)	0/6 (0%)	3/6 (50.0%)	3/5 (60.0%)	4/5 (80.0%)	4/5 (80.0%)
O-N1	0/6 (0%)	0/6 (0%)	0/5 (0%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
N-total	0/12 (0%)	0/12 (0%)	3/11 (27.3%)[E]	8/10 (80%)	9/10 (90%)	9/10 (90%)

[a] Abbreviatons of infection modes are illustrated in Table 1.
[b] Statistically significant differences between infection groups are indicated by a superscript letter (c–h), Mann-Whitney-U-test ($P < 0.05$)\. Different letters indicate statistically significant differences in the respective comparisons. [c,d] differences between ME49 infection modes; [E,F] differences between ME49, CZ-tiger and NED total seroconverted chickens; [g, h] differences between chickens infected with 10^5 oocysts of ME49, CZ-tiger and NED.

groups ($P = 0.008$ compared with strain NED, $P < 0.001$ compared with strain ME49), with 83.3% of the birds testing IFAT positive already 14 days after infection. For strains ME49 and CZ-Tiger, high-dose oocyst infections (groups O-M3, O-T3) yielded positive IFAT titers more often (100% of the chickens) at 14 days p.i. compared with the other infection modes of the respective strain. Seroconversion indicated a valid infection with *T. gondii*. Maximum reciprocal IFAT titers observed ranged from 800 to 51,200. *T. gondii*-specific antibodies were detectable over the complete period of the experiment. Only in one chicken (group Cy-M), an IFAT titer had decreased below the cut-off value at the point of slaughtering. Chickens of the negative control groups remained seronegative for the entire study.

3.1.2. Turkeys

All turkeys included in the study were tested negative for *T. gondii*-specific antibodies on the day of infection except for two turkeys with antibody levels just above the cut-off value.

Seroconversion was first observed one week p.i., when it was seen in 50% of ME49-infected, 58.3% of CZ-Tiger infected, and 33% of NED-infected turkeys, respectively. All infected animals showed a seroconversion not later than two weeks p.i. (see Table 3). The serum-antibody concentrations remained above the cut-off over the entire experiment in all animals except for one turkey of group O-T1 that showed concentrations below the cut-off in week five p.i. (see Table 3). The *T. gondii* strain did not significantly influence the time point of seroconversion of turkeys, this was true for both infective stages (cysts and oocysts). In contrast, 83.3% of moderate-dose oocyst infected turkeys (groups O-M2, O-T2), and 91.7% of high-dose oocyst infected turkeys (groups O-M3, O-T3) seroconverted 1 week p.i. irrespective of the strain. With 22% (of the groups Cy-M, Cy-T, Cy-N) for tissue cyst infection ($P = 0.001$ compared with moderate-dose and high-dose oocyst infections) and 27.8% (of the groups O-M1, O-T1, O-N1) for low-dose oocyst infection ($P = 0.003$ compared with moderate-dose and high-dose oocyst infections), respectively, significantly fewer animals revealed a seroconversion after or at 1 week p.i. in these groups. Interestingly, all turkeys of groups Cy-M and O-M1 seroconverted in week 2 p.i. only, i.e. significantly ($P = 0.002$) later than O-M2 and O-M3 animals. For CZ-Tiger and NED strains, the infection mode (infective stage and dose) did not clearly influence the point of seroconversion.

3.2. Animal health

3.2.1. Chickens

Five animals died before the end of the experiment. Two chickens of

Table 3
Seroconversion of turkeys the complete time of study.

Infection mode[a]	Number and proportion of seroconverted animals stratified for time after infection								
	0 dpi	7 dpi[b]	14 dpi	21 dpi	28 dpi	35 dpi	42 dpi	49 dpi	56 dpi
Cy-M	0/6 (0%)	0/6 (0%)[c]	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-M1	0/6 (0%)	0/6 (0%)[c]	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-M2	0/6 (0%)	6/6 (100%)[d]	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-M3	0/6 (0%)	6/6 (100%)[d]	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
M-total	0/24 (0%)	12/24 (50%)	22/22 (100%)	22/22 (100%)	22/22 (100%)	22/22 (100%)	22/22 (100%)	22/22 (100%)	22/22 (100%)
Cy-T	0/6 (0%)	2/6 (33.3%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-T1	0/6 (0%)	3/6 (50%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	5/6 (83.3%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-T2	0/6 (0%)	4/6 (66.7%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-T3	1/6 (16.7%)	5/6 (83.3%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
T-total	1/24 (4.2%)	14/24 (58.3%)	24/24 (100%)	24/24 (100%)	24/24 (100%)	23/24 (95.8%)	24/24 (100%)	24/24 (100%)	24/24 (100%)
Cy-N	1/6 (16.7%)	2/6 (33.3%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
O-N1	0/6 (0%)	2/6 (33.3%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
N-total	1/12 (8.3%)	4/12 (33.3%)	12/12 (100%)	12/12 (100%)	12/12 (100%)	12/12 (100%)	11/11 (100%)	11/11 (100%)	11/11 (100%)

[a] Abbreviatons of infection modes are illustrated in Table 1.
[b] Statistically significant differences between infection groups are indicated by a superscript letter ([c, d]), Mann-Whitney-U-test ($P < 0.05$). Different letters indicate statistically significant differences.

group O-M3, one chicken of group Cy-N, and one chicken of group O-N1 died during the study. However, in all four chickens, the pathological findings did not provide evidence for acute toxoplasmosis as the cause of death. In one chicken of group Cy-M, which died three weeks p.i., histopathological results suggested an acute heart failure as the cause of death due to a non-purulent myocarditis, most probably caused by *T. gondii*.

The remaining animals appeared clinically healthy throughout the study.

3.2.2. Turkeys

Five animals died during the study due to several reasons; all of them seemingly unrelated to the experimental *T. gondii* infection. Two turkeys of group O-M3 deceased of *E. coli* septicemia seven and ten days p.i., respectively. Because of cannibalism or skeletal problems, respectively, two turkeys of group NC-T and one turkey infected of group O-N1 had to be euthanized during the study.

All remaining turkeys seemed clinically healthy until the end of the experiment.

3.3. PCR

3.3.1. Chickens

Regarding type II strains, *T. gondii* DNA was detected in 90.5% (19/21) of all ME49-infected and 95.8% (23/24) of all CZ-Tiger-infected animals (Table 4). Over all infection modes, 76.2% of the ME49-infected animals and 87.5% of the CZ-Tiger-infected animals tested positive for *T. gondii* DNA in at least one edible organ. *T. gondii* DNA was detectable in 30.0% (3/10) of all NED infected chickens. The NED strain induced a significantly lower number of total positive organs (1.9%) ($P < 0.001$) and positive edible organs (1.3%) ($P = 0.005$ compared with strain ME49, $P < 0.001$ compared with strain CZ) compared with both type II strains

There were no marked differences in the type of positive organs between the different type II strain infected groups. However, the total number of positive organs overall infection groups was notably ($P = 0.016$) higher in CZ-Tiger infected groups (27.9%) than in ME49 group (16.4%). Besides, the infection mode (parasite stage and dose) influenced the total number of positive organs per group significantly (see Table 4).

Looking at the frequency of T. gondii positivity for different organ type (see Table 5), in ME49-infected chickens, *T. gondii* DNA was most commonly detected in hearts (38.1%) and brains (23.8%), thigh muscles (23.8%), drumsticks (23.8%), glandular stomach (23.8%) and lungs (23.8%). In the CZ-Tiger groups, most frequently heart (70.8%), brain (54.2%), gonads (45.8%) and gizzard (41.7%) were tested positive, whereby heart was notably ($P = 0.009$) more often affected than all other analyzed organs except the brain (Fig. 1).

For each of the three PCR-positive NED-infected chickens, only one organ tested positive. In group Cy-N, one animal's liver and another animal's bone marrow contained *T. gondii* DNA. In group O-N1, *T. gondii* DNA was found in one heart.

3.3.2. Turkeys

T. gondii DNA positive animals were found for all three infection strains used. Regarding ME49 infection, 95.5% (21/22) of the animals featured *T. gondii* DNA positive organs. Every organ type tested positive from at least one turkey in the ME49 infected groups except for kidney. For CZ-Tiger infected groups, 87.5% (21/24) were PCR positive. Infection with the NED strain resulted in 36.4% *T. gondii* DNA positive animals (5/11). Edible organs were often tested positive in all three strains, with 54.6% of all ME49 infected birds, 62.5% of all CZ-Tiger infected animals, and 36.4% of all NED infected turkeys, respectively (see Table 4).

Regarding individual organs, brain was tested *T. gondii* positive most frequently for all three infection strains with 68.2% of all brains in ME49 infected, 62.5% in CZ-Tiger infected, and 27.3% in NED infected groups.

Total frequencies of positive tested organs for all strains are shown in Fig. 1. In general, comparable to our chicken experiments, turkeys infected with ME49 as well as with CZ-Tiger showed distinctly higher proportions of positive organs (18.8% and 11.7%, respectively, of all analyzed organ samples) than turkeys infected with NED (5.7%).

The numbers of edible organs tested positive after infection with tissue cysts showed comparable results over all strains (ME49, 6.3%; CZ-Tiger, 4.2%; NED, 3.1%) (see Fig. 1, Table 5).

3.3.3. Comparison of results chicken and turkeys

T. gondii DNA was found in none of the uninfected animals of both host species.

ME49 infection in turkeys led to a lower variation in the tissue distribution of *T. gondii* DNA over all analyzed organs compared with the two other infection strains. As shown in Fig. 1, this phenomenon could not be observed in chickens.

In both tested host species, hearts were more often positive after infection with the CZ-Tiger strain than after ME49 infection (i.e., in chickens 70.8% vs. 38.1% or in turkeys 37.5% vs. 13.6%, respectively) (see Table 5), while NED strain induced positive results for only one chicken heart (1/10) and no turkey heart.

For chicken brain analysis, markedly more positive findings

Table 4
T. gondii DNA positive findings depending on parasite stage and dose used for infection. Overall infection groups for each strain. Listed as positive animals /infected animals, in brackets: (positive organs per infection mode /positive edible organs per infection mode).

	Infection strain	Infection mode				
		Tissue cysts[a]	1×10^3 oocysts[a]	1×10^5 oocysts[a]	1×10^6 oocysts[a]	Total[a]
Turkeys	CZ-Tiger	4/6 (6.3%^{b,d}/4.2%)	5/6 (8.3%^{f}/4.2%)	6/6 (15.6%/10.4%^{h})	6/6 (16.7%^{c}/6.3%)	21^{l}/24 (11.7%^{j}/16.7%)
	ME49	5/6 (24.0%^{e}/6.3%)	6/6 (24.0%^{g}/11.5%)	6/6 (12.5%/2.1%^{i})	4/4* (24.0%/4.7%)	21^{l}/22 (18.8%^{j}/16.7%)
	NED	3/6 (6.3%^{d}/3.1%)	2/5* (5.0%^{f}/1.3%)	NA	NA	5^{m}/11 (5.7%^{k}/6.1%)
Chicken	CZ-Tiger	6/6 (26.0%^{T}/8.3%^{N,V})	5/6 (17.7%^{X}/5.2%^{N})	6/6 (25.0%/8.3%^{N})	6/6 (42.7%/18.8%^{O})	23^{Z}/24 (27.8%^{Z}/27.1%^{Z})
	ME49	4/5* (6.3% $^{R, U}$/2.5%^{P})	5/6 (13.5%^{X}/7.3%)	6/6 (20.8%/7.3%)	4/4* (26.6% ^{S}/12.5%^{Q})	19^{Z}/21 (16.4%§/7.1%§)
	NED	2/5* (2.5%^{U}/1.3%^{W})	1/5* (1.3% ^{Y}/1.3%)	NA	NA	3§/10 (1.9%§/1.3%§)

NA = not applicable.

* Smaller group sizes due to deceased animals during the study period.

[a] Statistically significant differences between infection groups are indicated by a superscript letter ($^{b-z, \S, \$, \#}$), Mann-Whitney-U-test ($P < 0.05$). Different letters indicate statistically significant differences in the respective row/column. $^{b, c}$ differences between turkeys infected with CZ-Tiger; $^{d, e}$ differences between tissue cyst infected turkeys overall strains; $^{f, g}$ differences between 10^3 oocyst infected turkeys overall strains; $^{h, i}$ differences between 10^5 oocyst infected turkeys overall strains; $^{j, k, l, m}$ differences between turkeys overall infection modes and strains; $^{N, O}$ differences between chickens infected with CZ-Tiger; $^{P, Q, R, S}$ differences between chickens infected with ME49; $^{T, U, V, W}$ differences between tissue cyst infected chickens overall strains; $^{X, Y}$ differences between 10^3 oocyst infected chickens overall strains; $^{Z, \S, \$}$ differences between chickens overall infection modes and strains.

Table 5
T. gondii DNA positive organs for type II strains depending on parasite stage and dose used for infection.

	Tested organ	Infection mode				Total
		Cy-M/Cy-T[a]	O-M1/O-T1[a]	O-M2/O-T2[a]	O-M3/O-T3[a]	M/T[a]
Chickens	Heart	4. 8 %/ 16.7 %	4.8 %/ 8.3 %	19.0 %/ 20.8 %	9.5 %/ 25.0 %	38.1 %/ 70.8 %*
	Breast muscle	4.8 %/ -	4.8 %/ 8.3 %	4.8 %/ -	4.8 %/ 4.2 %	19.0 %/ 12.5 %
	Thigh muscle	- /4.2 %	14.3 %/ -	- / -	9.5 %/ 16.7 %	23.8 %/ 20.8 %
	Drumstick	- / -	4.8 %/ -	4.8 %/ -	14.3 %/ -	23.8 %/ -*
	Liver	- / 8.3 %	- / -	4.8 %/ -	- / 8.3 %	4.8 %/ 16.7 %
	Gizzard	- /4.2 %	4.8 %/ 4.2 %	- / 12.5 %	- / 20.8 %*	4.8 %/ 41.7 %*
	Glandular stomach	9.5 %/ 8.3 %	- /4.2 %	4.8 %/ 4.2 %	9.5 %/ 12.5 %	23.8 %/ 29.2 %
	Pancreas	- / 8.3 %	4.8 %/ 4.2 %	4.8 %/ 4.2 %	- / -	9.5 %/ 16.7 %
	Spleen	- / 8.3 %	4.8 %/ 8.3 %	4.8 %/ 8.3 %	4.8 %/ 8.3 %	14.3 %/ 33.3 %
	Kidney	- / -	4.8 %/ -	- / 4.2 %	9.5 %/ 4.2 %	14.3 %/ 8.3 %
	Brain	- / 12.5 %	4.8 %/ 12.5 %	14.3 %/ 8.3 %	4.8 %/ 20.8 %	23.8 %/ 54.2 %*
	Retina	- / 8.3 %	- /4.2 %	4.8 %/ 12.5 %	4.8 %/ 4.2 %	9.5 %/ 29.2 %
	Gonads	4.8 %/ 12.5 %	- / 8.3 %	- / 8.3 %	- / 16.7 %	4.8 %/ 45.8 %*
	Intestine	- / -	4.8 %/ -	14.3 %/ 16.7 %	- / 12.5 %	19.0 %/ 29.2 %
	Lung	- /4.2 %	4.8 %/ 4.2 %	14.3 %/ -	4.8 %/ 8.3 %	23.8 %/ 16.7 %
	Bone marrow	- / 8.3 %	- / 4.2 %	- / -	4.8 %/ 8.3 %	4.8 %/ 20.8 %
Turkeys	Heart	- / 4.2 %	9.1 %/ 4.2 %	- / 20.8 %*	4.5 %/ 8.3 %	13.6 %/ 37.5 %
	Breast muscle	- / 4.2 %	13.6 %/ 4.2 %	- / -	- / -	13.6 %/ 8.3 %
	Thigh muscle	4.5 %/ -	9.1 %/ 4.2 %	- / 8.3 %	9.1 %/ -	22.7 %/ 12.5 %
	Drumstick	9.1 %/ 4.2 %	9.1 %/ -	- / 4.2 %	- / -	18.2 %/ 8.3 %
	Liver	4.5 %/ -	9.1 %/ -	4.5 %/ -	- / -	18.2 %/ -*
	Gizzard	9.1 %/ 4.2 %	- / 4.2 %	4.5 %/ 8.3 %	- / 16.7 %	13.6 %/ 33.3 %
	Glandular stomach	9.1 %/ -	- / -	9.1 %/ -	- / 4.2 %	18.2 %/ 4.2 %
	Pancreas	9.1 %/ -	4.5 %/ -	- / -	- / -	13.6 %/ -
	Spleen	4.5 %/ -	4.5 %/ -	4.5 %/ -	- / -	13.6 %/ -
	Kidney	- / -	- / -	- / -	- / -	- / -
	Brain	13.6 %/ 8.3 %	13.6 %/ 12.5 %	22.7 %/ 20.8 %	18.2 %/ 20.8%	68.2 %/ 62.5 %
	Retina	- / -	4.5 %/ -	- / -	4.5 %/ -	9.1 %/ -
	Gonads	13.6 %/ -	4.5 %/ -	- / -	- / 4.2 %	18.2 %/ 4.2 %
	Intestine	- / -	9.1 %/ -	4.5 %/ -	- / 4.2 %	13.6 %/ 4.2 %
	Lung	9.1 %/ -	4.5 %/ 4.2 %	4.5 %/ -	- / 8.3 %	18.2 %/ 12.5 %
	Bone marrow	18.2 %/ -	9.1 %/ -	- / -	4.5 %/ 8.3 %	27.3 %/ -*

[a] Abrreviatons of infection modes are illustrated in Table 1.
* Indicates statistically significant differences between the amounts of positive organs of the respective infection group Mann-Whitney-U-test ($P < 0.05$).

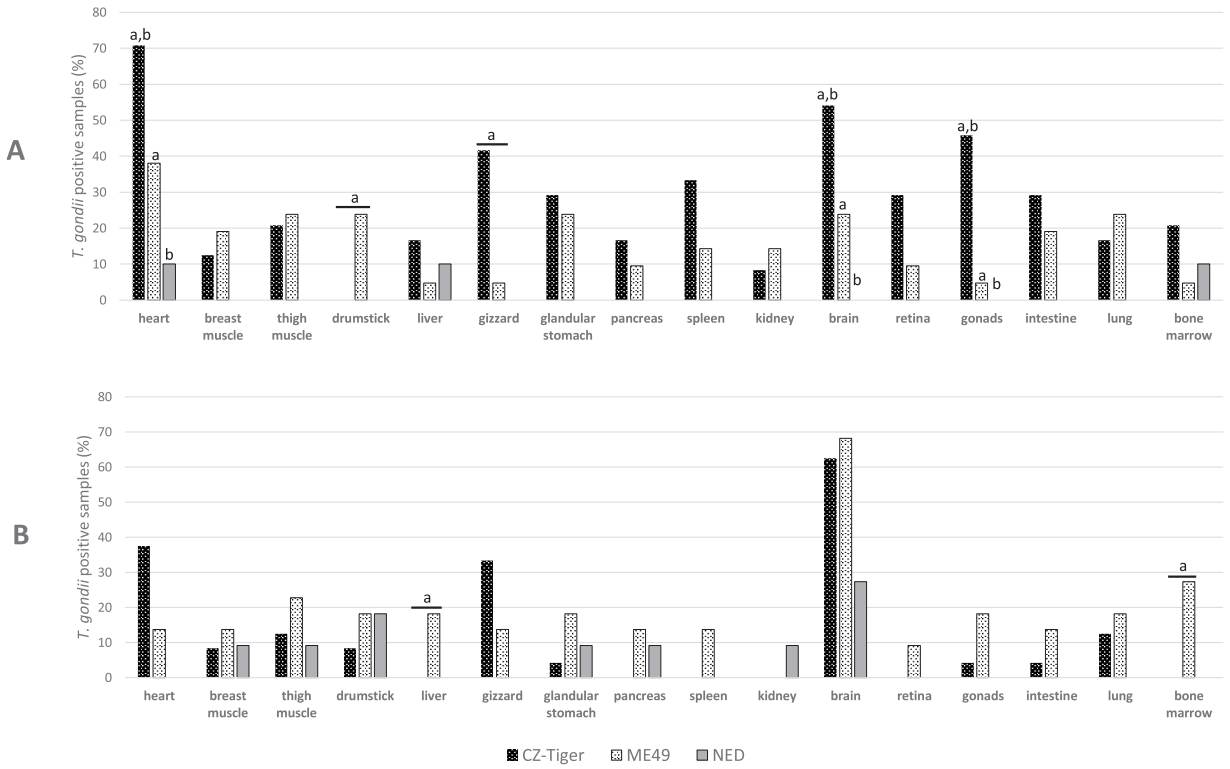

Fig. 1. Frequencies of organs tested T. gondii positive, over all infectious modes, by infection strain. (A) chickens (B) turkeys.a,b identical indices indicate statistically significant differences between the respective groups Mann-Whitney-U-test ($P < 0.05$).

occurred in CZ-Tiger infections (54.2%) than in ME49 infections (23.8%); while NED infection did not result in detection of *T. gondii* DNA in any of the chicken brains. In turkeys, the percentages of positive brains were similar between both type II strains (68.2% for ME49, and 62.5% for CZ-Tiger). A lower proportion of the brains were tested positive in NED infected turkeys (27.3%) but still clearly more than in chickens.

Gizzard was one of the most frequently *T. gondii* positive organs except heart and brain for CZ-Tiger infected chickens (41.7%) as well as turkeys (33.3%). Distribution of the single organs depending on the infection mode and tested bird species showed no significant strain-related differences. No differences in positive rates detected in the different tested organs (see Table 5) except in heart for medium level CZ-Tiger oocyst infected turkeys (CZ-Tiger 20.8%; ME49 0%) and in gizzard for high level CZ-Tiger oocyst strain infected chickens (CZ-Tiger 20.8%; ME49 0%).

The infection with ME49 revealed similar percentages of total positive samples in turkeys (18.8%) and chickens (16.4%). In chickens, but not in turkeys infected with ME49 or CZ-Tiger, the total numbers of positive samples as well as of positive edible samples increased with an increasing oocyst infection dose (see Table 4). However, there was no consistent tendency of higher numbers of positive samples in chickens or turkeys, when ME49 infection groups were compared.

4. Discussion

The aim of the present study was to investigate the influence of three different *T. gondii* strains of two clonal lineages and different infection doses and routes on the distribution of *T. gondii* in several organs and tissues of chickens and turkeys. Additionally, the impact of the infection strain on the seroconversion of infected animals should be examined. The organs were screened for *T. gondii* stages by PCR, which is not able to prove viability of the pathogen but is suitable to check a high amount of tissue samples for the presence and distribution of *T. gondii* and compare the different poultry groups. The study results could show an influence of the infection strain and dose on the seroconversion of the infected animals. However, the serologic data from the two bird species cannot be compared directly because different analysis methods were used for detection of *T. gondii* antibodies in the different species which was reported to result in varying outcome even for a single species (Dubey, 2010; Dubey et al., 1993a, 1993b; Schares et al., 2018), but a tendency is still noticeable.

Chickens are described as resistant hosts to clinical toxoplasmosis in general (Dubey, 2010). In the present study one chicken showed clinical symptoms after infection with ME49 tissue cysts. Clinical toxoplasmosis in chickens was previously described after infection with oocysts of the highly virulent type I GT1 strain (Dubey et al., 1993b), but to our knowledge there are no descriptions of clinical toxoplasmosis in chickens after infection with type II or III strains (Dubey, 2010). On the other hand, none of the infected turkeys showed clinical symptoms associated with toxoplasmosis during the study. This observation is in accord complying with former studies in turkeys (Bangoura et al., 2013; Dubey et al., 1993a; Geuthner et al., 2014; Sedlak and Franti, 2000; Zöller et al., 2013) although fatal toxoplasmosis in wild turkeys has been described previously (Howerth and Rodenroth, 1985; Quist et al., 1995).

One week after infection with ME49 or NED, no chicken tested seropositive though some chickens seroconverted as early as one week p.i. in the medium- and high-dose oocyst infected CZ-Tiger strain groups. Different authors (Biancifiori et al., 1986; Sedlak and Franti, 2000) described similar findings of seroconversion as late as two weeks p.i. in chickens after infection with oocysts of various amounts of oocysts of a type I or type III strain. To our knowledge, this is the first study investigating serological data of turkeys after infection with different *T. gondii* strains and infection modes. Our results show that turkeys reliably develop a measurable serologic response to type II and type III infections, irrespective of infection dose and route, and that antibody concentrations may drop several weeks after infection. Turkeys showed similar dose-depending serological responses as previously described for other non-avian (Forbes et al., 2012) and avian species after infection with various *T. gondii* strains, e.g. OV51/95 in partridges (Martínez-Carrasco et al., 2004), K21 in ducks (Bartova et al., 2004), RH in chickens (Maksimov et al., 2018). Seroconversion in turkeys infected with tissue cysts or oocysts of the CZ-Tiger strain and medium and high doses of ME49 oocysts took place one week after infection already. Results of previous studies (Bangoura et al., 2013; Sedlak and Franti, 2000) investigating infection of turkeys with oocysts of different strains support these findings.

To our knowledge, there is only one experiment (Dubey et al., 1993a) comparable to our study using ME49 oocyst infections in turkeys, describinga seroconversion on day 14 p.i. which was the earliest investigated time point.

One turkey of the Cy-N and O-T3 group, respectively, showed a seroconversion as early as the day of infection. The KELA-values of the two animals barely exceeded the cut-off and stayed on this low level until 14 dpi or even dropped under the cut-off. Koethe et al. (2011) described similar findings when infecting turkeys with *Eimeria* spp. and *Hammondia hammondi* where 10% of the infected animals showed low KELA-values above the cut-off in the *T. gondii* assay. Contact of turkeys in example in the hatchery with other apicomplexan protozoa than *T. gondii* cannot be ruled out definitively but are deemed highly unlikely since the animals were stabled as day-old poults. Two weeks after infection an intense immune response with strong increases of the KELA-values was observed in both turkeys similar to the 0 dpi seronegative turkeys. Therefore, both turkeys were kept in the study.

Infection with tissue cysts of NED did not yield seroconversion in one chicken over the entire time of the study. Schares et al. (2018) additionally analyzed the serum with MAT and TgSAG1-ELISA$_{SH}$, detecting a seroconversion. Furthermore, the bone marrow of the respecting chicken was found positive for *T. gondii* DNA, thus a valid infection of this animal can be assumed. Nonetheless, these findings in single turkeys and chickens indicates that the used methods are sensitive and specific, but performance of multiple tests may be advisable to explain disagreement between adverse findings. In birds, the availability of serological tests is much more restricted than in mice or humans, thus the tests that proved to be highly reliable in previous studies were chosen (Geuthner et al., 2014; Koethe et al., 2011, 2015; Schares et al., 2018).

In our hands, type III NED strain infections yielded a lower percentage of positive birds and positive individual organs in turkeys as well as in chickens compared to both utilized type II strains, regardless of the infection mode. This fact is in accordance with results from own previous NED tachyzoite infection of chickens (Geuthner et al., 2014), which also confirmed low *T. gondii* tissue stage quantities in positive organs after tachyzoite infection (Koethe et al., 2015; Schares et al., 2018). These reproducible findings underline the low virulence of the NED strain in chickens and turkeys. However, during passage in mice to obtain the tissue cysts for poultry infections, the NED strain showed high virulence in mice in our laboratory contrary to descriptions by Dardé (1996) (data not shown).

Infection with ME49 did not reveal a consistent tendency of higher numbers of positive samples following application of higher infection doses in chickens as well as turkeys, whereas such trend was noticeable after infections with CZ-Tiger in both host species. A possible explanation for this observation is the often noted altered pathogenicity of *T. gondii* strains after variable passages in cell culture (Saraf et al., 2017). The standard laboratory ME49 strain used in this study was maintained in cell culture with an unknown number of passages before used in mouse infections. In contrast, strain CZ-Tiger was conserved and applied as oocysts for infections of mice and cats. Therefore, it is hypothesized that ME49 showed a decreased pathogenicity by cell culture attenuation in contrast to the CZ-Tiger strain in this study.

The trend of a dose-dependent effect after oocyst infection with CZ-Tiger appears much clearer in chickens than in turkeys. It can be assumed that this is related to the distinct organ size differences, especially for muscles, between the two poultry species upon examinations. Accordingly, the concentration of tissue cysts in the same amount of sample material should be lower in turkeys given the same infection dose. Thus, the probability of finding *T. gondii* using PCR in a restricted portion of the homogenized tissue might be lower in turkeys than in chickens. This may have caused a more variable dose-effect relation curve considering the limited animal numbers analyzed per group. However, the general trend of a positive dose-effect correlation was visible in our experiments.

Brain and heart are described as the predilection sites for *T. gondii* in many species (Bartova et al., 2004; Esteban-Redondo et al., 1999; Gisbert Algaba et al., 2018; Juránková et al., 2014; Koethe et al., 2015; Schares et al., 2018). In general, *T. gondii* DNA was found most frequently in heart and brain compared with all tested organs of both studied host species, with brain being the most targeted organ in turkeys, and heart in chickens respectively, regardless of the infection strain. These observations are partly differing from previous studies (Biancifiori et al., 1986; Dubey et al., 1993b; Kaneto et al., 1997; Koethe et al., 2015; Sedlak and Franti, 2000) that are employing a variety of analytical methods. Our results are based on sample analysis by conventional PCR, utilizing a small amount of homogenized tissue for DNA extraction. The comparison of our data with results from Koethe et al. (2015) and Schares et al. (2018), who partly analyzed up to 100 g of the current sample material with MC-PCR and pepsin-digestion RT PCR on acidic pepsin digested chicken tissue (PD-RT PCR), show a much higher proportion of *T. gondii* positive tissues than found in the present study. This outcome indicates that in this study the number of positive organs in both host species is clearly underestimated due to methodical limitations in sample size used for DNA extraction. Nonetheless, considering the high amount of analyzed samples to obtain comprehensive systemic distribution data on the bird organisms, the PCR used was a pragmatic approach allowing to screen all samples.

As of today, little is known on the distribution of *T. gondii* in chickens and especially in turkeys after infection with tissue cysts. The current study could show that the infection with tissue cysts compared with low and medium dose oocyst infections are resulting in similar abundance of *T. gondii*-positive tissues in chickens and turkeys. However, the observations of group differences regarding the number of positive organs in animals infected with tissue cysts have to be interpreted with caution. Tissue cysts for infection were not enumerated before use, and 6 total mice brains per 6 chickens or turkeys were pooled and applied evenly to the birds of each group. The rationale behind was that poultry as omnivores are likely to catch mice on farms and then consume a whole brain, so could the selected infection regimen simulated the natural infection process. Hence, the amount of fed tissue cysts between the study groups possibly varied. Nevertheless, infections with tissue cyst numbers resembling natural conditions caused detectable *T. gondii* infections in tissues of turkeys and chickens. Consequently, a risk for human infection with *T. gondii* due to consumption of chicken and turkey meat can be anticipated. This is emphasized by our finding that at least half of the type II strain infected animals featured *T. gondii* PCR positive edible organs. Similar or even higher rates of detection were described in turkeys before (Bangoura et al., 2013; Geuthner et al., 2014; Zöller et al., 2013).

In terms of human *T. gondii* infection risk related to consumption of undercooked poultry products, it should be noted that gizzard – which is consumed regularly by humans – was the third most often PCR positive organ after heart and brain in CZ-Tiger infected animals in this experiment. Gizzard was not tested in other experimental infections of poultry except in previous studies of our working group (Bangoura et al., 2013; Geuthner et al., 2014; Zöller et al., 2013). Our currently detected high proportion of *T. gondii* positive gizzards in 41.7% of the chickens and 33.3% of the turkeys may be credited to the morphology of the organ since a gizzard is a well-circulated muscle, and indicates a potential consumers' risk. Nonetheless, it should be mentioned, that the risk is maybe decreased in conventionally raised chickens and turkeys in Europe, due to the common practice of feeding anticoccidials. *T. gondii* DNA was detected in a considerable proportion of the infected animals, including a high number of their gonads. Though literature is divided on a potential vertical transmission of *T. gondii* in birds (Biancifiori et al., 1986; Khademi et al., 2018; Pande et al., 1961), our current and previous data (Bangoura et al., 2013) indicate that there may be a need for further investigation.

5. Conclusion

In conclusion, our study demonstrated the impact of the infection strain and doses on seroconversion as well as on the amount of *T. gondii* positive organs in chickens and turkeys. We could show lesser virulence of the type III strain NED in poultry in contrast to type II strains. Nevertheless, the risk for human infection with *T. gondii* after consumption of products from type III infected animals cannot be excluded. Our data show clear evidence that heart and brain are possibly not the only predilection sites for *T. gondii* in turkeys and chickens, but also organs like gizzard that pose a risk for human *T. gondii* infection. Furthermore, the study could give further evidence that the second natural route of infection with tissue cysts will lead to *T. gondii* in different organs in poultry.

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