INTRODUCTION

Toxoplasma \((T. gondii)\) is considered one of the most important and successful parasites worldwide, primarily due to the low host specificity of all three of its developmental stages (tachyzoites, sporozoites in sporulated oocysts, bradyzoites in tissue cysts) (Dubey, 2014). The protozoan parasite is able to infect all mammals and birds and is the causative agent of human toxoplasmosis, one of the most frequently occurring parasitic zoonosis worldwide (Tenter, Heckeroth, & Weiss, 2000). It is assumed that one-third of the worldwide human population is chronically infected (Saadatnia & Golkar, 2012). In Germany, 55% of the population show antibodies against \(T. gondii\) (Wilking, Thamm, Stark, Aebischer, & Seeber, 2016). Although most infections remain asymptomatic in healthy individuals, infection in immuno-compromised persons can be followed by a life-threatening course of disease and in particular primary infection during pregnancy can lead to severe consequences for the unborn child (McLeod, Tubbergen, Montoya, & Petersen, 2014). Horizontal transmission can occur through accidental ingestion of the parasite, for example, through consumption of raw or

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**Abstract**

Consumption of game in Germany has increased during the past 10 years. Wild boar (\(Sus scrofa\)), roe deer (\(Capreolus capreolus\)) and red deer (\(Cervus elaphus\)) are the most frequently hunted and consumed game animals in Germany, yet information on the occurrence of zoonotic pathogens in these animal species is scarce. To better estimate the public health risk emanating from handling and consumption of game, this study investigated seroprevalences of \(Toxoplasma gondii\) in game hunted in the German federal state of Brandenburg during two hunting seasons from 2017 to 2019. \(Toxoplasma gondii\)-specific antibodies were detected in 24.4% (44/180, 95% CI: 18.4%–31.4%) of wild boar, 12.8% (16/125, 95% CI: 7.5%–20%) of roe deer and 6.4% (3/47, 95% CI: 1.3%–17.5%) of red deer using a commercial ELISA kit. Seroprevalences were similar in the two hunting seasons. Correlation between sex and seropositivity could not be observed. A rise in seroprevalence was seen with increasing age in all studied game species. Observed seroprevalences suggest that \(T. gondii\) is endemic in the sylvatic environment in the German federal state of Brandenburg and imply that game could represent a relevant source for human \(T. gondii\) infection.

**KEYWORDS**

epidemiology, game, Germany, serology, Toxoplasma, zoonosis
undercooked meat containing infective tissue cysts or of raw plant-based food or water contaminated with sporulated oocysts. Scallan et al. (2011) estimated that 50% of the *T. gondii* infections in the USA are foodborne. Due to the high disease burden associated with human toxoplasmosis, *T. gondii* ranks among the top of the most important foodborne pathogens in the USA and the Netherlands (Havelaar et al., 2012; Scallan, Hoekstra, Mahon, Jones, & Griffin, 2015; WHO, 2015). In Europe, consumption of raw or undercooked meat, including game, has been identified as one of the most important risk factors for toxoplasmosis (Cook et al., 2000; Kapperud et al., 1996). Indeed, consumption of raw venison and meat of wild boar has already been identified as probable infection source in cases of acute toxoplasmosis (Carme et al., 2002; Choi et al., 1997; Ross et al., 2001; Sacks, Delgado, Lobel, & Parker, 1983). In addition, potential smear infections during or after evisceration of carcasses or handling of raw meat have to be considered (Dubey, 1991; McDonald et al., 1990).

According to the German hunting association, consumption of game in Germany has increased during the past 10 years (German Hunting Association, 2019a) and as in other European countries, there is a growing trend towards consumption of undercooked game with a pink or bloody core (Richomme et al., 2010). Wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) are the most frequently hunted and consumed game animals in Germany, yet information on the prevalence of *T. gondii* in these species is scarce. To better estimate the public health risk emanating from handling and consumption of game, this study aimed to assess the prevalence of *T. gondii* in wild boar, roe deer and red deer in the German federal state of Brandenburg.

### 2 | MATERIALS AND METHODS

Sampling was performed in the established hunting seasons (October until January) of 2017/2018 and 2018/2019 during driven hunts organized by the German Federal Forest Service and the Frankenförder Forschungsgesellschaft mbH. Access to the hunting grounds was kindly provided by the German Institute for Federal Real Estate (BlmA).

Using the Epitools Epidemiological Calculator (Sergeant, 2018), a required sample size of 316 roe deer, 274 red deer and 288 wild boar was determined based on published prevalences of 29% for roe deer (Hedeggott, Steinbach, Pohl, & Frantz, 2018), 25% for wild boar (Lutz, 1997) and 24% for red deer (Witkowski et al., 2015) and the average annual hunting bag of Brandenburg between 2007/8 and 2017/18 (71,076 wild boar, 9,414 red deer and 68,820 roe deer) (German Hunting Association, 2019b), as estimate for the population size. For this study, we had access to a total of 125 roe deer, 180 wild boar and 47 red deer in seven counties of the German federal state of Brandenburg.

All animals were legally hunted for human consumption and made available for sampling post-mortem as part of the cooperation with the German Federal Forest Service. No animal was killed for the purpose of providing samples. Animal age was determined by hunters based on tooth eruption, tooth replacement, tooth wear and where available on antler development and physical appearance. In this study, the animals were divided into three age groups: juveniles (<1 years old), yearlings (1–2 years old) and adults (>2 years old).

Blood samples were obtained from the abdominal or thoracic cavity in 50 ml centrifugation tubes without additives and stored for 24–72 hr at 4°C for coagulation. After centrifugation at 1,600 g for 10 min at 4°C, sera were collected and stored at −20°C until analysis. Most sera were slightly haemolytic. *T. gondii*-specific antibodies were detected using the commercially available ELISA kit ‘ID Screen® Toxoplasmosis Indirect Multi-species’ (ID.Vet), which has been approved for the detection of *T. gondii*-specific antibodies in livestock and pets by the Licensing Authority of the Friedrich-Loeffler-Institut, Germany (FLI-B 550). It has also been utilized in *T. gondii* seroprevalence studies in wildlife, including wild boar, roe deer and red deer (Racka, Bartova, Budikova, & Vodrazka, 2015; Witkowski et al., 2015). The ELISA was performed and analysed in accordance with the manufacturer’s instructions. The detected optical densities (OD$_{450}$) were converted to S/P ratios $\frac{S/P = 100 \cdot \left(OD_{\text{sample}} - OD_{\text{negative control}}\right)}{OD_{\text{positive control}} - OD_{\text{negative control}}}$ and interpreted as follows: $S/P \leq 40\%$ negative, $S/P \geq 50\%$ positive, $40\% < S/P < 50\%$ doubtful. To assess a possible association between seroprevalence and sex, age or hunting season, the Fisher’s exact test was performed using the software SPSS v.21 (SPSS Inc.). A value of $p \leq .05$ was considered statistically significant. Additionally, odds ratios (ORs) were determined to assess the strength of correlation. The Haldane–Anscombe correction was used for odds ratio calculation when one of the cells had zero value.

### Impacts

- **Seroprevalence of *Toxoplasma gondii*** in red deer, roe deer and wild boar in the German federal state of Brandenburg was assessed during the hunting seasons 2017/2018 and 2018/2019.
- *Toxoplasma gondii*-specific antibodies were found in 24.4% of wild boar (*Sus scrofa*), 12.8% of roe deer (*Capreolus capreolus*) and 6.4% of red deer (*Cervus elaphus*) using a commercial ELISA kit. Detected seroprevalences of both hunting seasons were similar. An increase in seroprevalence with age was observed in all studied game species.
- The seroprevalences indicate that *T. gondii* is endemic in the sylvatic environment of the German federal state of Brandenburg. The data point to the possibility that meat from these game species could be a relevant source of human *T. gondii* infection.
3 | RESULTS

Serological examination revealed *T. gondii*-specific antibodies in 12.8% of roe deer (16/125, 95% CI: 7.5%–20%), 6.4% of red deer (3/47, 95% CI: 1.3%–17.5%) and 24.4% of wild boar (44/180, 95% CI: 18.4%–31.4%) (Table 1). Two sera of wild boar yielded doubtful results and were considered as negative for further analyses. Seroprevalences in both hunting seasons were similar and no statistical significant differences were observed (Table 1; *p* = .43–1). There was no correlation between sex and seropositivity (Table 1; *p* = .26–1). In all examined game species, seroprevalences increased with age. However, the increase in seroprevalence with age was only statistically significant for roe deer (Table 1; *p* = .004).

4 | DISCUSSION

Consumption of game in the German population has been on the rise in the past 10 years (German Hunting Association, 2019a), However, information on the occurrence of zoonotic pathogens in wild animals that are frequently hunted for human consumption is generally scarce in Germany.

In this study, observed seroprevalences of *T. gondii* in the investigated game species varied between 6.4% in red deer to 24.4% in wild boar. These results are in line with other European studies, where seroprevalences between seven and 60% in cervids and five and 57% in wild boar have been observed in the past (European Food Safety Authority, 2013; Heddergott et al., 2018; Jokelainen, Velstrom, & Lassen, 2015; Racka et al., 2015; Wallander, Frossling, Vagsholm, Uggl, & Lunden, 2015), further implying the endemic nature of *T. gondii* in European sylvatic environments.

The observed seroprevalence of 24.4% among wild boars in this study falls into the mid-range of seroprevalence estimates reported in former German studies (15%–33%) (Lutz, 1997; Rommel, Sommer, & Janitschke, 1967; Tackmann, 1999). Moreover, it is also comparable to the pooled estimated seroprevalences published for the Northern-Baltic region (33%), Europe (26%) and also globally (23%)

| Game species | Variable | Category | No. positive/No. tested | Seroprevalence in % (95% CI) | *p*-value | OR (95% CI) |
|--------------|----------|----------|-------------------------|-----------------------------|-----------|-------------|
| Wild boar    | Total    |          | 44/180                  | 24.44 (18.36–31.39)         |           | Reference   |
|              | Hunting season | 2017/2018 | 28/116                  | 24.14 (16.68–32.96)         | 1         | Reference   |
|              |          | 2018/2019 | 16/64                   | 25.00 (15.02–37.40)         | 1.05 (0.52–2.13) |          |
|              | Age      | <1 year old | 10/67                   | 14.93 (7.40–25.74)          | .08       | Reference   |
|              |          | 1–2 years old | 19/77                   | 24.68 (15.56–35.82)         | 1.87 (0.80–4.36) |          |
|              |          | >2 years old | 11/32                   | 34.38 (18.57–53.19)         | 2.99 (1.11–8.05) |          |
|              | Sex      | Male      | 19/70                   | 27.14 (17.20–39.10)         | .275      | Reference   |
|              |          | Female    | 21/106                  | 19.81 (12.70–28.68)         | 0.667 (0.33–1.35) |          |
| Roe deer     | Total    |          | 16/125                  | 12.80 (7.50–19.95)          | .43       | Reference   |
|              | Hunting season | 2017/2018 | 8/51                    | 15.69 (7.02–28.56)          | 0.65 (0.23–1.87) |          |
|              |          | 2018/2019 | 8/74                    | 10.81 (4.78–20.20)          | .004      | Reference   |
|              | Age      | <1 year old | 1/48                    | 2.08 (0.05–11.07)           |          |                   |
|              |          | 1–2 years old | 3/23                   | 13.04 (2.78–33.59)          | 7.05 (0.69–71.95) |          |
|              |          | >2 years old | 12/52                   | 23.08 (12.53–36.84)         | 14.1 (1.76–113.22) |          |
|              | Sex      | Male      | 3/41                    | 7.32 (1.54–19.92)           | .256      | Reference   |
|              |          | Female    | 13/83                   | 15.66 (8.61–25.29)          | 2.35 (0.63–8.77) |          |
| Red deer     | Total    |          | 3/47                    | 6.38 (1.34–17.54)           |           | Reference   |
|              | Hunting season | 2017/2018 | 2/27                    | 7.41 (0.91–24.29)           | 1         | Reference   |
|              |          | 2018/2019 | 1/20                    | 5.00 (0.13–24.87)           | 0.66 (0.06–7.81) |          |
|              | Age      | <1 year old | 0/19                    | 0.00 (0.00–17.65)           | .399      | Reference   |
|              |          | 1–2 years old | 1/9                    | 11.11 (0.28–48.25)          | 6.88 (0.25–186.69) |          |
|              |          | >2 years old | 2/19                   | 10.53 (1.30–33.14)          | 5.57 (0.25–124.20) |          |
|              | Sex      | Male      | 1/22                    | 4.55 (0.12–22.84)           | 1         | Reference   |
|              |          | Female    | 2/24                    | 8.33 (1.03–27.00)           | 1.91 (0.16–22.66) |          |

Note: For statistical analyses, the Fisher’s exact test was performed. Statistical analyses considering age were performed using three age groups (<1, 1–2, >2 years old). The age of four wild boar and two roe deer, as well as sex of four wild boar, one red deer and one roe deer was not recorded and could therefore not be included in the analysis.

*a*The Haldane–Anscombe correction was used for odds ratios considering age of red deer.
in two recent systematic reviews and meta-analyses (Olsen et al., 2019; Rostami et al., 2017).

Compared to our results, a recent study from Heddergott et al. in (2018) reported a higher seroprevalence of 29% in roe deer sampled in the German federal state of Thuringia. Previous European reports for T. gondii in roe deer also vary with the lowest prevalences found in Italy (13%) (Gaffuri et al., 2006) and the Czech Republic (14%) (Hejíček, Literák, & Nezval, 1997) and highest prevalences reported from Belgium (52%) (De Craeye et al., 2011) and France (60%) (Aubert et al., 2010).

To our knowledge, this is the first study reporting the occurrence of T. gondii-specific antibodies in red deer in Germany. The observed seroprevalence of 6.4% is similar to the reported seroprevalence in Norway (7.7%) (Vikoren, Tharaldsen, Fredriksen, & Handeland, 2004), but relatively low compared to other European studies from Poland and the Czech Republic, where 24.1% (Witkowski et al., 2015) and 45% (Bartova, Sedlak, Pavlik, & Literak, 2007) of the animals have been found to be seropositive, respectively. However, the relatively small sample size of red deer in this study resulted in a large confidence interval and more animals should be investigated to obtain more representative data. Sampling will continue during future hunting seasons. Noteworthy, irrespective of the sample size, we obtained comparable prevalence in each hunting season.

In general, seroprevalences for all game species reported in different European studies vary substantially. A variety of factors can influence T. gondii seroprevalence such as local differences in the environmental oocyst contamination, which is also dependent on the density of felids in the sampled area. Further, the sampling strategy and application of different serological test systems, cut-off values and sample types, can be a source for heterogeneity (Olsen et al., 2019; Rostami et al., 2017).

Seroprevalence for T. gondii in all studied game species increased with age as was seen in many other studies investigating wildlife and humans (Berger-Schoch, Bernet, Doherr, Gottstein, & Frey, 2011; Deksn & Kirjusina, 2013; Heddergott et al., 2018; Jokelainen, Näreaho, Hälli, Heinonen, & Sukura, 2012; Laforet et al., 2019; Rostami et al., 2017; Wallander et al., 2015; Wilking et al., 2016). However, we only found statistical significance for this observation for roe deer. In wild boar, we and others could not statistically confirm an age-dependent seroprevalence (Gauss et al., 2005; Jokelainen et al., 2015; Olsen et al., 2019) and the lifelong persistence of antibodies in wild boar is still under debate (Opsteegh, Swart, Fonville, Dekkers, & Giessen, 2011).

Detected prevalences in this study imply that T. gondii is endemic in the sylvatic environment in the German federal state of Brandenburg from where the parasite could be transmitted to humans. Although the detection of T. gondii-specific antibodies in different game animals indicates exposure to the parasite, it does not prove the existence of infective T. gondii-tissue cysts in meat derived from these animals. However, individual studies on white-tailed deer already showed detection of infective tissue cysts in up to 62% of seropositive animals (Dubey et al., 2004; Lindsay, Blagburn, Dubey, & Mason, 1991). Additionally, studies showing detection of T. gondii DNA in 7% as well as isolation of infective T. gondii strains in 48% of seropositive wild boar (Gazzonis et al., 2018; Richomme et al., 2009) suggests that a substantial proportion of seropositive animals in this study may have carried infective tissue cysts.

All animals investigated in this study were intended for human consumption. However, game meat is not examined for the presence of T. gondii during official meat inspections in Germany and many other countries and could therefore represent a relevant source for human T. gondii infection, especially if consumed frequently and not cooked thoroughly. As precautionary measures, the German Federal Institute for Risk Assessment recommends that game and raw game meat products made from it should therefore undergo proper heating (core temperature of at least 72°C for 2 min) prior to consumption. As potential smear infections and cross-contamination during evisceration of carcasses by the hunters as well as during processing of raw game meat by consumers are conceivable (Kapperud et al., 1996), general hygienic procedures should be followed.

Future studies on T. gondii seroprevalence in game should be extended to other regions in Germany to investigate temporal and spatial distribution patterns as have already been performed in Finland (Jokelainen et al., 2010), Sweden (Wallander et al., 2015) and Estonia (Remes et al., 2018). As game becomes a more and more popular meat source, an increase in the number of farmed game can be expected and should therefore also be included in the studies. To better emanate the public health risk from T. gondii in game, molecular methods should additionally be applied for the direct detection of the parasite and to identify prevailing genotypes.

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CONFLICT OF INTEREST
The authors declare they have no conflicts of interest.

ETHICAL STATEMENT
The study only involved animals killed by licensed hunters according to German regulations (German Hunting Act) with permission of the German Federal State of Brandenburg and the corresponding hunting authority. All animals were killed during the established hunting season on a regular wildlife management basis (population control). No animal was killed for the purpose of providing samples.

ORCID
Nadja Seyhan Bier https://orcid.org/0000-0002-1215-7246
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