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Sero-prevalence and risk factors of *Toxoplasma gondii* infection in wild cervids in Denmark

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ABSTRACT

Toxoplasma gondii is a zoonotic protozoan parasite capable of infecting possibly all warm-blooded animals including humans, and is one of the most widespread zoonotic pathogens known. Free-ranging wildlife can be valuable sentinels for oocyst contaminated environments, as well as a potential source for human foodborne infection with *T. gondii.* Here we aimed to determine the sero-prevalence of *T. gondii* in Danish wild deer populations and examine risk factors associated with increased exposure to the parasite. Blood samples were collected from 428 cervids (87 fallow deer (*Dama dama*), 272 red deer (*Cervus elaphus*), 55 roe deer (*Capreolus capreolus*) and 14 sika deer (*Cervus Nippon*) from 23 hunting sites in Denmark. The animals were shot during the hunting season 2017/2018, and screened for antibodies against *T. gondii* using a commercial ELISA kit. One hundred and five (24.5%) cervids were sero-positive. Sero-prevalence was significantly different between species ($p < 0.05$), with odds of sero-positivity being 4.5 times higher in roe deer than fallow deer, and 3.0 times higher in red deer than in fallow deer. A significant increase in sero-prevalence with age was observed, driven by a significant increase in risk in adult red deer compared to calves (OR: 13.22; 95% CI: 5.96–33.7). The only other significant risk factor associated with wild cervid *T. gondii* sero-positivity was fencing, with the highest exposure associated with deer from non-fenced hunting areas (OR: 2.21; 95% CI: 1.05–4.99). This study documented a widespread exposure to *T. gondii* in Danish cervids. Therefore the meat of the wild deer, in particular from roe deer and red deer, should be considered a significant risk of *T. gondii* infections to humans, if not properly cooked. Further, molecular studies to confirm the presence of infective parasitic stages in the muscles of deer used for consumption is recommended.

1. Introduction

Toxoplasma gondii is a zoonotic protozoan parasite, prevalent in most areas of the world (Tenter et al., 2000). The parasite has a complex life cycle, where possibly all warm-blooded animals, including humans, can act as intermediate hosts, but only felids (domestic and wild cats) are definitive hosts. The parasite completes its sexual life-cycle in the intestine of infected cats, which may then excrete millions of oocysts into the environment leading to exposure for other animals and humans. Approximately one third of the world's human population is estimated to be infected with *T. gondii* (Saadatnia and Golkar, 2012), and the parasite is ranked as the fourth globally most important food-borne parasites that poses a threat to public health according to FAO/WHO (FAO; WHO, 2014). Infection in humans can occur accidently by consumption of water, feces, vegetables or vegetation contaminated with sporulated oocysts derived from felid feces, or via the consumption of raw or undercooked meat containing tissue cysts (Dubey, 2016). In fact, consumption of raw or undercooked meat are identified as the central risk factor that predicted acute infection in pregnant women (Cook et al., 2000). *Toxoplasma gondii* infections in healthy humans typically remain asymptomatic or mild, while a first time exposure to the parasite in the early stages of pregnancy, may lead to congenital toxoplasmosis and cause severe disease in the fetus (Weiss and Dubey, 2009). *Toxoplasma gondii* also has importance in animal sector, causing significant economic losses in terms of abortion in farm animals, i.e. pigs, small ruminants, cattle and equids (Stelzer et al., 2019).

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While the role and infection status of domestic animals has been studied more extensively, relatively little is known about this parasite in wild animals, not least in wild deer species in Europe (Fanelli et al., 2020). Besides the importance of such knowledge for wildlife health, there is an important One Health perspective, since *T. gondii* is a zoonosis. Likewise, wildlife may act as sentinels, giving indication of contaminated or "risky" areas.

Wild ungulate population have increased in Europe, as well as the consumption and demand for wild game meat (Ramanzin et al., 2010) which is often promoted as a healthier or more sustainable alternative to other meat sources, e.g. cattle (Taggart et al., 2011). It is estimated that more than 7 million ungulates are harvested each year in Europe (Linnell et al., 2020). In Denmark, roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) are the most frequently hunted and consumed game animals (Vildtudbytte 2020). Several cases of *T. gondii* infections in humans have been reported in association with ingesting uncooked or undercooked game meat (Gaulin et al., 2020; Ross et al., 2001; Sacks et al., 1983).

In Denmark, a sero-prevalence of 28% in pregnant women has been reported for *T. gondii* (Lebech et al., 1993). A study on wild boars in Denmark, revealed that a substantial proportion of animals were *T. gondii* sero-positive (Laforet et al., 2019), while more than 50% of free-living mink (Sengupta et al., 2021) and raccoon dogs (Kjær et al., 2021) tested sero-positive for *T. gondii*. These studies suggest that *T. gondii* may be common and widespread, in particular in the continental part of Denmark. However, there has been no studies on the prevalence of *T. gondii* in wild cervids in Denmark, despite the large and growing populations. In Germany, the sero-prevalence of *T. gondii* infection in roe deer has been reported to 6.4% (Bier et al., 2020), while it was estimated to 32.0% in Sweden (Malmsten et al., 2011).

In Denmark, four species of wild cervids exist: Roe deer (*Capreolus capreolus*), fallow deer *(Dama dama*), sika deer (*Cervus Nippon*) and red deer (*Cervus elaphus*). Roe deer is by far the most common and is widespread throughout the whole country. Fallow deer is represented in all parts of Denmark and the population is increasing (Danmarks Jægerforbund 2019(a)). The red deer is primarily present in Jutland, and on Zealand.

To assess the importance of wild cervids as a potential source of human infections and to provide insights into which species and regions that may be particularly exposed to *T. gondii* contamination or infection in Denmark, this study set out to i) investigate the sero-prevalence of *T. gondii* in wild cervids hunted for consumption in Denmark, and ii) determine associated individual and area-specific risk factors, which may support future risk-based or sentinel surveillance of this parasite in Denmark.

2. Materials and methods

2.1. Collection of blood samples and laboratory analysis

Approximate sample size required for estimating the overall sero-prevalence was calculated using OpenEpi (Sullivan et al., 2009) to be 193–317 animals, based on assumed sero-prevalence estimates of 15%–29% as estimated for wild cervids in Europe by Fanelli et al. (2020), a 95% confidence level and estimated population sizes of 10,000–150,000 (Danmarks Jægerforbund 2019(a); Danmarks Jægerforbund 2019(b)). Blood samples were collected from 428 cervids: 87 fallow deer, 272 red deer, 55 roe deer and 14 sika deer from 23 hunting sites in Denmark, shot during the hunting season October to January 2017/2018, and were screened for antibodies against *T. gondii* using a commercial ELISA kit. The hunts were organized either by The Danish Nature Agency's or on private estates from 23 geographical locations (hunting sites). Efforts were made to achieve the broadest geographical coverage as well as equal number of samples from each of the 4 major regions of Denmark,

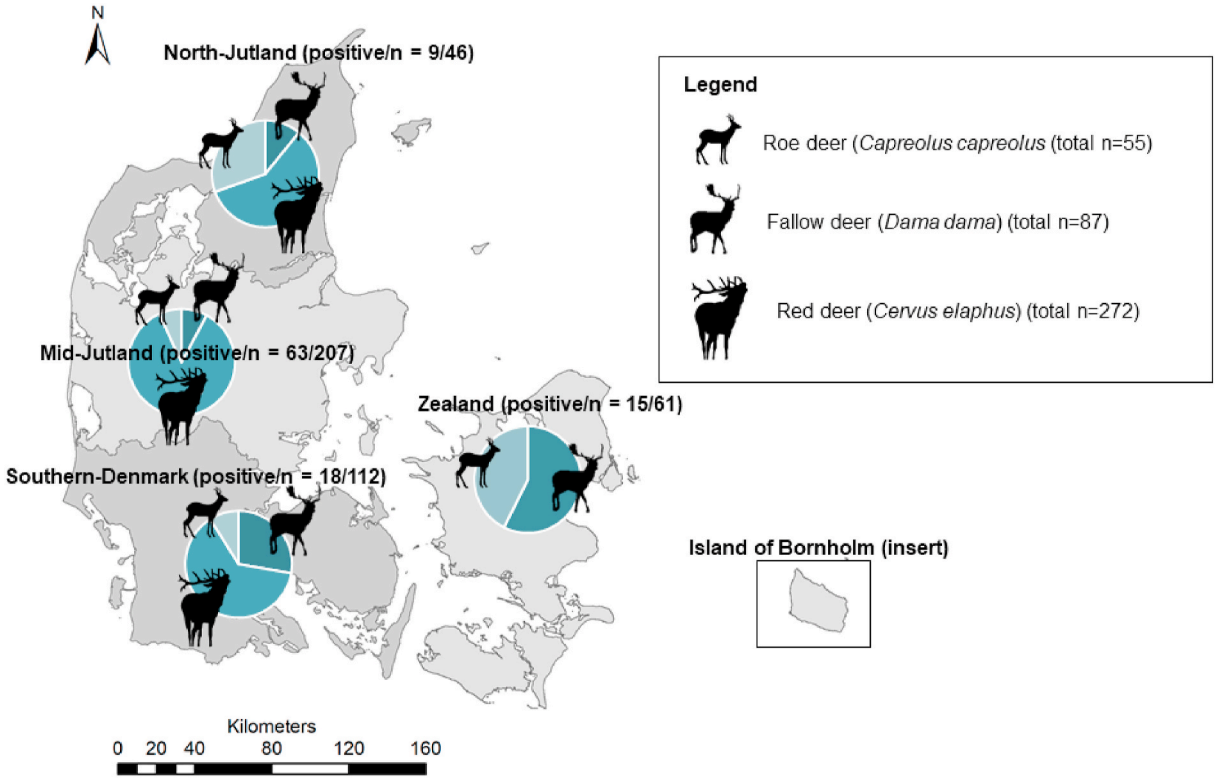

Fig. 1. The geographical distribution of wild cervids included in the study and the number of animals tested positive for antibodies against *Toxoplasma gondii* in the hunting season 2017–2018 in Denmark (n = 428). Shown by region and proportion of species sampled (roe deer, fallow deer or red deer) in each region. Pie charts indicate proportion of samples from each cervid species. Note Sika deer was only sampled in Mid-Jutland (n = 14), and hence not included in the map. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

namely; North-Jutland, Mid-Jutland, Southern-Denmark and Zealand (see Fig. 1). However, the number of animals sampled per location depended on the number of animals shot on the particular date. A few locations were visited twice due to several hunting days. No animals were shot for the purpose of this study. All animals were shot by certified hunters for consumption and made available for sampling post-mortem. For three animals, sex was not reported. The age was estimated by experienced hunters based on dental status (tooth eruption, tooth wear and tooth replacement), body size, and, where applicable antler growth (males). Animals were categorized into three age groups: calves (<1 year), yearlings (1–2 years) and adults (>2 years). Blood samples were collected within 1–5 h post-mortem using 50 ml falcon tubes, and stored immediately in cooler boxes with cooling elements, while transported to the laboratory. Once at the laboratory, blood samples were stored at 4 °C until sera were separated by centrifugation, collected in 2 ml eppendorf tubes and stored at −20 °C until further analysis. Collection of sera was done no later than 24 h after collection of blood. The undiluted serum samples were examined for IgG antibodies against *T. gondii* in duplicates using the ID Screen® Toxoplasmosis Indirect Multi-species ELISA kit following manufacture instructions (IDvet, Grabels, France). According to the manufacture, the ELISA kit is applicable for ruminants. However, the sensitivity and specificity of the commercial ELISA test used is not provided for cervids. However, validation of the ELISA kit has been done by immunoblot analysis of samples from sheep, goats, camelids and fallow deer testing positive or negative using the ELISA kit with 100% accordance between the two methods (Moskwa et al., 2018; Basso et al., 2020), and Formenti et al., (2015), have used this kit to evaluate anti-*T. gondii* prevalence in wild cervids.

The optical density (OD) was read at 450 nm. Cutoff values was calculated based on sample-to-positive percentage (S/P%) according to the formula: (mean OD of sample − mean OD of negative control)/(mean OD of positive control − mean OD of negative control) × 100. Samples with S/P%≤40% were considered negative, 41%≤S/P%≥49% were doubtful, and S/P%≥ 50% were positive, following the manufacturer's values for non-canine animals. Further validation of each ELISA plate were done as recommended by the manufacturer, where results were considered conclusive when: mean OD of positive controls >0.350, and the ratio of the mean OD values of positive and negative controls >3.

2.2. Environmental and management related data

Information related to deer management was obtained from the owners/managers using a questionnaire and conducted during and after blood sample collection by interviewing owners/managers to assess the following potential risk factors for exposure to *T. gondii* at the estate level: The questions posed were as follows: 1) Is the estate area fenced or not? (yes/no); 2) Is the estate area considered a dry or wet nature type? (dry/wet); 3) Are there any co-grazing with domestic animals (yes/no)? 4) What is the size of the estate area? (in m2); 5) Are the deer fed with supplementary feed (i.e. hay, pellets in periods food scarcity) (yes/no)?

Other hypothesized environmental risk factors related to climate and land use or land cover were extracted for each estate from remotely sensed resources. For land use, we used the 100 × 100 m land cover raster dataset obtained from the CORINE land cover inventory (https://land.copernicus.eu/paneuropean/corine-land-cover). This dataset is classified in 48 land cover types, but for this analysis they were aggregated to five major classes ('Artificial surfaces,' 'Agriculture,' 'Forest,' 'Wetlands' and 'Open water bodies') following the CORINE land cover nomenclature (Copernicus, 2015). The proportion of each of these land cover types, within a bufferzone of 10 km in diameter (with the geographical coordinates of each estates as the centroid) was extracted using the spatial analyst tool in ArcGIS. (ESRI, ArcGIS vs. 10.6 for Desktop). In Denmark, the domestic cat (*Felis domesticus*) is considered as the definitive host for *T. gondii*. As domestic cat density is assumed to be highest close to human settlements (in Denmark often suburban areas or residential summer house areas), we also measured the Euclidean distance in km from each estate to the nearest build-up/urban area. In combination with the proportion of the land cover type 'artificial area' in the buffer zones around each estate (extracted from the CORINE land cover data as described above), these serve as proxies for how much domestic cats may be able to access and contaminate the areas where the wild deer can become exposed in Denmark.

Meteorological data on annual (max., min. and average) temperature for the years 2017 and 2018, as well as total precipitation for each year were downloaded from the Danish Meteorological Institute web-site (www.dmi.dk), for each of the municipalities where the estates are located. Co-linearity between the climatic covariates were tested and ranked according to the goodness of fit, Akaike Information Criteria (AIC) (Hosmer and Lemeshow, 2000), where the best fitting one was selected for further modelling.

2.3. Statistical analysis and identification of risk factors

To assess risk factors potentially associated with sero-prevalence in Danish cervids, we first build univariate mixed effects logistic regression models for each species of cervids to investigate the relationship between *T. gondii* sero-positivity (yes/no) with individual level risk factors (age, sex) for each species separately. Secondly, we ran exploratory univariate mixed effects models using all sampled cervid species as the outcome, accounting for both individual and site-level independent variables, to select candidate variables for multivariate modelling. To account for clustering at the site-level, a location-specific random effect was included in all univariate models. In the models including all species, independent variables with $p \leq 0.20$ were retained as input to a final multivariate mixed effect logistic regression model. A two-level hierarchical modelling approach was chosen to account for clustering of animals (level 1) within estates (level 2). The dependent variable was the serological status of the animal (seropositive = 1, seronegative = 0), whereas as species, age and sex were the independent variables at the individual level, and climatic, land use and management factors) the independent variables at estate level with estate ID as a random effect in the model.

The final model was built using backward elimination, retaining all variables with $p \leq 0.05$ in the likelihood ratio test. All analysis was carried out in STATA (Stata version 14.2, College Station, TX, USA) and R Studio (Version 1.4.1106 ©2009–2021 RStudio, PBC).

3. Results

The geographical distribution of the sampled animals by region, cervid species and numbers tested positive in each of the four main regions in Denmark are visualized in Fig. 1. The distribution of tested cervid species and sero-prevalence is shown in Table 1.

3.1. Sero-prevalence of Toxoplasma gondii in Danish wild cervids

Of the 428 cervids examined for antibodies against *T. gondii,* 105 (24.5%, 95% CI: 20.5–28.9) were sero-positive. Only one female red deer had an S/P% value in the doubtful category (41%–49%). This animal was considered negative in the interpretation of the results. The sero-prevalence was highest in roe deer (32.7%, 95% CI = 21.8–45.9), followed by red deer (27.2%, 95% CI = 22.3–33.2) and fallow deer (13.8%, 95% CI = 8.0–23.1). Only one of the 14 sampled sika deer was sero-positive. Therefore, individual sero-prevalence was not estimated for sika deer, and subsequent individual species risk factor-analysis was not performed. Seroprevalence for all cervid species is summarized in Table 1.

Furthermore, at least one sero-positive animal originated from 21/23 hunting estates. The sero-prevalence ranged 9–58% positive cervids per site. Positive animals were found in all four regions (Fig. 1), with the

Table 1
Overview of Danish wild cervids sampled for *Toxoplasma gondii*, shot during the hunting season 2017–2018 and associated apparent sero-prevalence and confidence intervals (CI). Results from univariate logistic regression analysis for possible associations between positivity status and individual level risk factors sex and age, performed for each deer species separately, are shown in the last two columns. Information for Sika deer is only shown as total due to the low sample size. Significant associations highlighted in bold.

Cervid species	Variable	Category	Positive/No. tested	Sero-prevalence (95% CI)	OR* (95% CI**)	*P*-value
Fallow deer	**Total**		12/87	13.8 (7.34–22.9)		
	Age	Calves	1/25	4.0 (0.1–20.4)	1.00	ref
		Yearlings	6/34	17.7 (6.8–34.5)	–	0.142
		Adults	5/28	17.9 (6.1–36.9)	–	0.145
	Sex	Male	4/27	14.8 (4.2–28.2)	1.00	ref
		Female	8/60	13.3 (5.9–24.6)	–	0.8530
Red deer	**Total**		74/272	27.2 (22.0–32.9)		
	Age	Calves	7/103	6.8 (2.8–13.5)	1.00	ref
		Yearlings	14/61	23.0 (13.2–35.5)	**4.09 (1.59–11.42)**	**0.0045**
		Adults	53/108	49.1 (39.3–58.9)	**13.22 (5.96–33.7)**	**<.0001**
	Sex	Male	13/83	15.7 (8.1–25.3)	1.00	ref
		Female	60/187	32.1 (25.5–39.3)	**2.54 (1.34–5.13)**	**0.0061**
Roe deer	**Total**		18/55	32.7 (20.7–46.7)		
	Age	Calves	3/14	21.4 (4.7–50.8)	1.00	ref
		Yearlings	8/28	28.6 (13.2–48.7)	–	0.621
		Adults	7/13	53.9 (25.1–80.8)	–	0.089
	Sex	Male	3/18	16.7 (3.6–41.4)	1.00	ref
		Female	15/36	41.7 (25.5–59.2)	–	0.0759
Sika deer	**Total**		1/14	7.1 (0.4–30.5)	–	–
Total			**105/428**	**24.5 (20.5–28.9)**		

*OR: Odds ratio.

highest proportion found in Mid-Jutland (63/207).

3.2. Risk factors associated with Toxoplasma gondii sero-positivity

In the initial exploratory univariate mixed effects logistic regression models, implemented for each species of cervids individually, a significant increase in sero-positivity with age for red deer only (OR: 13.22; CI: 5.96–33.7) was observed (Table 1). Risk factors associated with *T. gondii* sero-positivity in the initial exploratory, univariate analysis (all cervid species) were mainly individual level risk factors (age, sex and species), as well as the estate level factors "average annual minimum temperatures", proportion of the land cover types ("artificial area" and "forest") and fencing practices (yes/no) (see Table 2 for details).

After the backward elimination procedure ($p < 0.05$ level) in the final multivariate, two-level hierarchical logistic regression model the following predictor variables were found to be significantly associated with *T. gondii* sero-positivity (yes, no): age-group (3 categories); species and fencing (yes/no) (Table 2). The random effect of estate ID was small, accounting only for 5% (rho = 0.049) of the variance in the model, but significant ($p = 0.051$ in LR test of rho = 0), and hence retained in the final model. The odds of sero-positivity was 4.5 times higher in roe deer than fallow deer, and 3.0 times higher in red deer than in fallow deer. The prevalence was significantly different between the age groups ($p < 0.0001$). The odds of sero-positivity in adults (3–5 years) was 10 times higher than in calves (<1 year), and 4 times higher than in yearlings (1–2 years). The only significant other risk factor associated with *T. gondii* sero-positivity in wild cervids in the multivariate analysis was fencing, with the highest exposure associated with deer from non-fenced hunting areas (OR = 2.21(1.05–4.99), $p = 0.037$). There was no interaction between the variables species and fencing ($p = 0.749$).

4. Discussion

With a growing large-herbivore population, it is important to have updated information on infection status of potential zoonoses is in Danish wildlife, as well as updated information of risk factors for wildlife infection. Wild deer may serve as sentinel species to identify *T. gondii* contaminated environments, as well as a potential source for human foodborne infection with *T. gondii*. Information on zoonotic infections in cervid species in Denmark is however scarce and to the best of our knowledge, this is the first nationwide survey of *T. gondii* sero-prevalence in Danish cervids.

Our results indicated a widespread exposure to *T. gondii* among wild cervid species across all four regions in Denmark (Fig. 1) with an overall apparent sero-prevalence of 24.5% (CI = 20.5–28.8) and demonstrated noteworthy sero-prevalences especially in roe deer and red deer. Sero-positivity indicates exposure, chronic infection, and/or the presence of *T. gondii* tissue cysts. It is therefore possible that sero-positive animals harbor viable tissue cysts (Dubey et al., 1970), but to ascertain the actual presence of *T. gondii* cysts in the tissue molecular tools or bioassays are required (Hamilton et al., 2015; Kuruca et al., 2017). The consumption of raw or inadequately cooked meat, in particular from roe deer and red deer which are frequently hunted for human consumption in Denmark, should therefore be considered a potential risk of *T. gondii* infections to humans in Denmark. .

Toxoplasma gondii sero-positive cervids have previously been observed in several other European countries with varying prevalence (Aubert et al., 2010; Bartova et al., 2007; Bier et al., 2020; De Craeye et al., 2011; Malmsten et al., 2011; Vikøren et al., 2004; Witkowski et al., 2015) implying an endemic nature of *T. gondii* in European cervids. The species specific prevalence observed in the present study is in agreement with recent estimates based on meta-analysis of the prevalence of *T. gondii* in roe deer and red deer in Europe (Fanelli et al., 2020), that found an overall sero-prevalence of 29% (95% CI: 23%–35%) in roe deer and 15% (95% CI: 10%–20%) in red deer.

The sero-prevalence in wild cervids significantly increased with age in our study (Table 2), driven largely by a significant higher prevalence in older animals in red deer (Table 1). This is supported by earlier findings in white-tailed deer (*Odocoileus virginianus*) (Vanek et al., 1996), reindeer (*Rangifer tarandus*) (Oksanen et al., 1997), moose (*Alces alces*) and roe deer (Vikøren et al., 2004). The higher seroprevalence in older animals reflects a cumulative likelihood for exposure to *T. gondii* and lifelong persistence of antibodies.

We also found that *T. gondii* sero-prevalence varied significantly between cervid species (7.1%–32.7%) with roe deer presenting the highest overall sero-prevalence. A higher sero-prevalence in roe deer than red deer has, likewise, been observed in several other European countries (Bier et al., 2020; Gauss et al., 2006; San Miguel et al., 2016; Vikøren et al., 2004; Witkowski et al., 2015). In our study, the observed differences in sero-prevalence between roe deer and red deer were

Table 2
Results from risk factor logistic regression analyses for possible associations between positivity status and individual and location (estate level) risk factors for all deer species, using a two-level hierarchical logistic regression model (with estate ID as random effect). Results from initial exploratory univariate logistic regression analyses, are shown in the first two columns. Results from the final multivariate logistic regression model (after a backward selection procedure) of predictor variables found to be significantly associated with overall *Toxoplasma gondii* sero-positivity in all sampled deer, are shown in the last two columns. Significant associations highlighted in bold ($P < 0.05$). σ^2; variance of location (estate) level random effect.

Variables	*T. gondii* sero-positivity of wild cervids			
	Univariate regression		Final multivariate regression model	
	OR*(95% CI**)	*P*-value	OR*(95% CI**)	*P*-value
Age (calves <1 year)	1.00	–	1.00	–
1-2 yrs (yearlings)	**4.09 (1.79–9.39)**	<0.001	**4.28 (1.86–8.85)**	<0.001
>3 years (adults)	**9.06 (4.35–20.29)**	<0.001	**10.41 (4.81, 21.55)**	<0.001
Species (Fallow deer)	1.00	–	1.00	–
Red deer	**2.46**	0.031	**4.49**	0.001
Roe deer	**(1.08–5.59)**	0.011	**(1.79–11.26)**	0.005
Sika deer	**3.26 (1.31–8.13)**	–	**3.00 (1.39–6.48)**	0.999
	-		**1.00 (0.09–9.60)**	
Sex (Male)	1.00	–		
Female	**2.05 (1.16–3.60)**	0.013	–	–
Fencing (yes)	1.00	–	1.00	–
No	**2.55 (1.08–5.99)**	0.032	**2.29 (1.05–4.99)**	0.037
Co-grazing (domestic animals) (no)	1.00			
Yes	0.76 (0.33–1.72)	0.506	–	–
Supplementary Feeding (yes)	1.00			
No	1.19 (0.51–2.77)	0.691	–	–
Estate size (km2)	1.00 (0.99–1.00)	0.601	–	–
Nature type (dry)	1.00			
Wet	0.78 (0.27–2.21)	0.641	–	–
Meteorological data:				
Annual precipitation 2017 (mm)	1.01 (1.00–1.01)	0.482	–	–
Annual precipitation 2018 (mm)	1.01 (1.00–1.01)	0.340	–	–
Max. annual temperature 2017 (C°)	1.58 (0.77–3.24)	0.210	–	–
Max. annual temperature 2018 (C°)	1.30 (0.74–2.29)	0.354	–	–
Min. annual temperature 2017 (C°)	0.84 (0.64–1.10)	0.214	–	–
Min. annual temperature 2017 (C°)	**0.57 (0.33–0.984)**	0.044	–	–
Corine land cover data:				
Artificial surfaces	0.93 (0.87–1.00)	0.067	–	–
Agriculture	0.99 (0.98–1.01)	0.641	–	–
Forest	1.01 (0.99–1.03)	0.073	–	–
Wetlands	0.96 (0.92–1.02)	0.156	–	–
Open waterbodies	0.92 (0.84–1.02)	0.147	–	–
Model random effects:				
σ^2 (random effect variance)	–	–	0.05	0.01–0.24

*OR: Odds ratio; **CI: Confidence interval.

attributed largely to a difference in the sero-prevalence in the younger animals across species (Table 1), while the sero-prevalences for adults and yearlings were similar across species. This contradicts findings in Norway and Germany, where different sero-prevalence was observed between red- and roe deer at all ages (Gauss et al., 2006). In our study, fallow deer had a general lower prevalence than roe- and red deer (Table 1), however only few studies have examined the *T. gondii* sero-prevalence in fallow deer simultaneously with red- and roe deer.

The observed differences in sero-prevalence between cervid species may be attributed to species difference in susceptibility to infection (Dubey et al., 1980; Vikøren et al., 2004; Williamson et al., 1980). Roe deer is known to be a comparatively more synanthropic species, using habitats where domestic cats are more likely to roam (Ferroglio et al., 2014; Vikøren et al., 2004). Furthermore, it has been demonstrated that when roe deer live in farmlands, which is the preferred feeding niche of the European roe deer, they display a partly similar feeding behavior as for wild boar (*Sus scrofa*), taking considerable amounts of on-the ground fruits and seeds, and increased intake of plant vegetative parts during winter (Tixier and Duncan, 1996). It should however be noted that for the detection of anti-*T. gondii* antibodies there is no reference standard serological test with 100% sensitivity and 100% specificity available. The performance of serological tests can vary between the type of samples and the selected cut-offs (Felin et al., 2017), and it is therefore likely that this have contributed to the heterogeneity detected in the seroprevalence between the animal species.

At the estate level, the only significant risk factor associated with the risk of being sero-positive, was fencing (OR: 2.29, CI: 1.05, 4.99), meaning that the cervids living in the fenced areas had a significantly lower risk of being infected than cervids in non-fenced areas. Hence, the foraging range of these animals was narrower than the free-living cervids, which may potentially decrease the likelihood of infection.

We also noted geographical variation in sero-prevalence within cervid species originating from different areas in Denmark (Fig. 1). Cervids are herbivores (browser and/or grazers) and presumably become infected by ingesting oocysts from the environment. Therefore, local differences in environmental oocyst contamination could explain differences in sero-prevalence within a species originating from different areas. Large variation in sero-prevalence between red deer originating from different areas has been observed in Spain with sero-prevalences ranging 0%–44.2% (Gauss et al., 2006). Moreover, location has previously been identified as a significant factor for *T. gondii* sero-prevalence in other studies including wild rabbits (Almería et al., 2004), red deer (Gauss et al., 2006) and roe deer in Spain (Gamarra et al., 2008). The regional differences might correlate with the humidity and temperature of the area, as these parameters determine if oocysts become infective (Dubey et al., 1970) demonstrating a geographical variation of parasite infestation under different climatic condition (Fanelli et al., 2020; Rostami et al., 2017). Gauss et al. (2006) observed that a lower prevalence in areas of Spain with mostly dry habitats compared to areas with a more humid mountainous area (Gauss et al., 2006). However, in our

study no significant association between variation in sero-prevalence and local climatic- or other environmental factors could be established, most likely because there is very little climatic variation across regions in Denmark. The observed differences in sero-prevalence between areas in Denmark is thus more likely dependent on geographical variation in the density of oocysts excreting felids in the area. In Denmark, felids is represented by the domestic cat, and cat density is assume to be highest close to human settlements as 1 out 5 Danish households own cats, and up to 71% of these allow their cats to roam all the time or on a regular basis (Sandøe et al., 2018). In Czech Republic, Hejlíček et al. (1997) observed higher prevalence in wild mammals from suburban areas where there was high density of domestic cats, and lower prevalence in areas extensively damaged by military activities where cats were less numerous (Hejlíček et al., 1997). However, in our study, no significant association between sero-positivity and distance to urban areas or proportion of build-up areas (as proxies for cat density) could be established. Furthermore, the sero-prevalence varied between species originating from the same area, indicating that the area related risk factors are of less importance in Denmark.

In an earlier Danish study considerably higher sero-prevalence was observed in extensively farmed wild boars (33.3%–63.6%) roaming the exact same fenced areas in the same time period (Laforet et al., 2019) as some cervids in this study, suggesting that the feeding behavior indeed is central. Wild boars are omnivores consuming both carcasses, plant material, and roots exposing them to infection with both oocysts from the environment and tissue cysts from cadavers. This likely explains the higher sero-prevalence compared to cervids from the same geographical area. This is supported by a study from Spain in domestic animals where *T. gondii* sero-prevalence was higher in animals feeding on the ground and at stubble fields compared to those that did not. Altogether, this points at a contaminated environment and an increased probability of ingestion of *T. gondii* oocysts in animals fed on the ground (Almería et al., 2004).

5. Conclusion

The observed prevalences in our study indicate that *T. gondii* is endemic in the wild cervid populations in Denmark. Although representing much smaller volumes of meat compared to that from farm animals, meat from wild cervids, should not be neglected as a potential source for human toxoplasmosis in Denmark. Taking into consideration the sanitary conditions when the animals are slaughtered under field conditions and the preparation in private kitchens, the risk of transmission of *T. gondii* from an infected animal should not be neglected. Future, molecular based studies should clarify the exact correlation between sero-positive status and the presence of viable *T. gondii* cysts in deer meat. Also, as farmed game meat is becoming increasingly popular, future studies should include farmed deer in Denmark to fully ascertain the risk of zoonotic transmission of *T. gondii* to humans in Denmark.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Almería, S., Calvete, C., Pagés, A., Gauss, C., Dubey, J.P., 2004. Factors affecting the seroprevalence of *Toxoplasma gondii* infection in wild rabbits (*Oryctolagus cuniculus*) from Spain. Vet. Parasitol. 123, 265–270. https://doi.org/10.1016/j.vetpar.2004.06.010.

Aubert, D., Ajzenberg, D., Richomme, C., Gilot-Fromont, E., Terrier, M.E., de Gevigney, C., Game, Y., Maillard, D., Gibert, P., Dardé, M.L., Villena, I., 2010. Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Vet. Parasitol. 171, 346–349. https://doi.org/10.1016/j.vetpar.2010.03.033.

Bartova, E., Sedlak, K., Pavlik, I., Literak, I., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in wild ruminants from the countryside or captivity in the Czech Republic. J. Parasitol. 93, 1216–1218. https://doi.org/10.1645/GE-1126R.1.

Basso, W., Sollberger, E., Schares, G., Küker, S., Ardüser, F., Moore-Jones, G., Zanolari, P., 2020. *Toxoplasma gondii* and *Neospora caninum* infections in South American camelids in Switzerland and assessment of serological tests for diagnosis. Parasites Vectors 13 (1), 256. https://doi.org/10.1186/s13071-020-04128-9.

Bier, N.S., Stollberg, K., Mayer-Scholl, A., Johne, A., Nöckler, K., Richter, M., 2020. Seroprevalence of *Toxoplasma Gondii* in Wild Boar and Deer in Brandenburg, Germany, 67. Zoonoses Public Health. https://doi.org/10.1111/zph.12702.

Cook, A.J.C., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E., Dunn, D.T., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. BMJ 321. https://doi.org/10.1136/bmj.321.7254.142.

Copernicus, 2015. CORINE Land Cover Nomenclature Conversion to Land Cover Classification System. *Available at*: https://land.copernicus.eu/eagle/files/eagle-related-projects/pt_clcconversion-to-fao-lccs3_dec2010.

Danmarks Jægerforbund, 2019a. Dåvildt (Dama dama). https://www.jaegerforbundet.dk/vildt-og-natur/artsleksikon/pattedyr/hovdyr/davildt/. (Accessed 17 January 2019).

Danmarks Jægerforbund, 2019b. Kronvildt (Cervus elaphus). https://www.jaegerforbundet.dk/vildt-og-natur/artsleksikon/pattedyr/hovdyr/kronvildt/. (Accessed 17 January 2019).

De Craeye, S., Speybroeck, N., Ajzenberg, D., Dardé, M.L., Collinet, F., Tavernier, P., Van Gucht, S., Dorny, P., Dierick, K., 2011. *Toxoplasma gondii* and *Neospora caninum* in wildlife: common parasites in Belgian foxes and Cervidae? Vet. Parasitol. 178, 64–69. https://doi.org/10.1016/j.vetpar.2010.12.016.

Dubey, J.P., 2016. Toxoplasmosis of animals and humans. In: Toxoplasmosis of Animals and Humans, second ed. Second Edition.

Dubey, J.P., Miller, N.L., Frenkel, J.K., 1970. The *Toxoplasma gondii* oocyst from cat to feces. J. Exp. Med. 132, 636–662.

Dubey, J.P., Thorne, E.T., Sharma, S.P., 1980. Experimental toxoplasmosis in elk (*Cervus canadensis*). Am. J. Vet. Res. 41.

Fanelli, A., Battisti, E., Zanet, S., Trisciuoglio, A., Ferroglio, E., 2020. A systematic review and meta-analysis of *Toxoplasma gondii* in roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) in Europe. Zoonoses Public Health. https://doi.org/10.1111/zph.12780.

FAO; WHO, 2014. Multicriteria-Based Ranking for Risk Management of Food-Born Parasites, Microbiological Risk Assessment Series No. 23. Rome.

Ferroglio, E., Bosio, F., Trisciuoglio, A., Zanet, S., 2014. *Toxoplasma gondii* in sympatric wild herbivores and carnivores: epidemiology of infection in the Western Alps. Parasites Vectors 7. https://doi.org/10.1186/1756-3305-7-196.

Formenti, F., Trogu, T., Pedrotti, L., Gaffuri, A., Lanfranchi, P., Ferrari, N., 2015. *Toxoplasma gondii* infection in alpine red deer (*Cervus elaphus*): its spread and effects on fertility. PLoS One 10. https://doi.org/10.1371/journal.pone.0138472.

Gamarra, J.A., Cabezón, O., Pabón, M., Arnal, M.C., Luco, D.F., Dubey, J.P., Gortázar, C., Almeria, S., 2008. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Vet. Parasitol. 153, 152–156. https://doi.org/10.1016/j.vetpar.2008.01.028.

Gaulin, C., Ramsay, D., Thivierge, K., Tataryn, J., Courville, A., Martin, C., Cunningham, P., Désilets, J., Morin, D., Dion, R., 2020. Acute toxoplasmosis among canadian deer hunters associated with consumption of undercooked deer meat hunted in the United States. Emerg. Infect. Dis. 26 https://doi.org/10.3201/eid2602.191218.

Gauss, C.B.L., Dubey, J.P., Vidal, D., Cabezón, O., Ruiz-Fons, F., Vicente, J., Marco, I., Lavin, S., Gortazar, C., Almería, S., 2006. Prevalence of *Toxoplasma gondii* antibodies

in red deer (*Cervus elaphus*) and other wild ruminants from Spain. Vet. Parasitol. 136, 193–200. https://doi.org/10.1016/j.vetpar.2005.11.013.
Hamilton, C.M., Kelly, P.J., Bartley, P.M., Burrells, A., Porco, A., Metzler, D., Crouch, K., Ketzis, J.K., Innes, E.A., Katzer, F., 2015. *Toxoplasma gondii* in livestock in St. Kitts and Nevis, west Indies. Parasites Vectors 8, 166.
Hejlíček, K., Literák, I., Nezval, J., 1997. Toxoplasmosis in wild mammals from the Czech Republic. J. Wildl. Dis. 33, 480–485. https://doi.org/10.7589/0090-3558-33.3.480.
Hosmer, D.W., Lemeshow, S., 2000. Applied Logistic Regression, second ed. John Wiley & Sons, Inc.
Kjær, L.J., Jensen, L.M., Chriél, M., Bødker, R., Petersen, H.H., 2021. The raccoon dog (*Nyctereutes procyonoides*) as a reservoir of zoonotic diseases in Denmark. Int. J. Parasitol. Parasites Wildl. 16, 175–182. https://doi.org/10.1016/J.IJPPAW.2021.09.008.
Kuruca, L., Klun, I., Uzelac, A., Nikolić, A., Bobić, B., Simin, S., Lalošević, V., Lalošević, D., Djurković-Djaković, O., 2017. Detection of *Toxoplasma gondii* in naturally infected domestic pigs in Northern Serbia. Parasitol. Res. 116, 3117–3123. https://doi.org/10.1007/s00436-017-5623-7.
Laforet, C.K., Deksne, G., Petersen, H.H., Jokelainen, P., Johansen, M.V., Lassen, B., 2019. *Toxoplasma gondii* seroprevalence in extensively farmed wild boars (*Sus scrofa*) in Denmark. Acta Vet. Scand. 61, 4. https://doi.org/10.1186/s13028-019-0440-x.
Lebech, M., Larsen, S.O., Petersen, E., 1993. Prevalence, incidence and geographical distribution of *Toxoplasma gondii* antibodies in pregnant women in Denmark. Scand. J. Infect. Dis. 25, 751–756. https://doi.org/10.3109/00365549309008574.
Linnell, J.D.C., Cretois, B., Nilsen, E.B., Rolandsen, C.M., Solberg, E.J., Veiberg, V., Kaczensky, P., van Moorter, B., Panzacchi, M., Rauset, G.R., Kaltenborn, B., 2020. The challenges and opportunities of coexisting with wild ungulates in the human-dominated landscapes of Europe's Anthropocene. Biol. Conserv. 244, 108500. https://doi.org/10.1016/j.biocon.2020.108500.
Malmsten, J., Jakubek, E.B., Björkman, C., 2011. Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in moose (*Alces alces*) and roe deer (*Capreolus capreolus*) in Sweden. Vet. Parasitol 177, 275–280. https://doi.org/10.1016/j.vetpar.2010.11.051.
Moskwa, B., Kornacka, A., Cybulska, A., Cabaj, W., Reiterova, K., Bogdaszewski, M., Steiner-Bogdaszewska, Z., Bien, J., 2018. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infection in sheep, goats, and fallow deer farmed on the same area. J. Anim. Sci. 96, 2468–2473. https://doi.org/10.1016/S1383-5769(97)00033-0.
Oksanen, A., Åsbakk, K., Nieminen, M., Norberg, H., Näreaho, A., 1997. Antibodies against Toxoplasma gondii in Fennoscandian reindeer - Association with the degree of domestication. Parasitol. Int. 46, 255-261.
Ramanzin, M., Amici, A., Casoli, C., Esposito, L., Lupi, P., Marsico, G., Mattiello, S., Olivieri, O., Ponzetta, M.P., Russo, C., Marinucci, M.T., 2010. Meat from wild ungulates: ensuring quality and hygiene of an increasing resource. Ital. J. Anim. Sci. 9, 3. https://doi.org/10.4081/ijas.2010.e61.
Ross, R.D., Stec, L.A., Werner, J.C., Blumenkranz, M.S., Glazer, L., Williams, G.A., 2001. Presumed acquired ocular toxoplasmosis in deer hunters. Retina 21, 226–229. https://doi.org/10.1097/00006982-200106000-00005.
Rostami, A., Riahi, S.M., Fakhri, Y., Saber, V., Hanifehpour, H., Valizadeh, S., Gholizadeh, M., Pouya, R.H., Gamble, H.R., 2017. The global seroprevalence of *Toxoplasma gondii* among wild boars: a systematic review and meta-analysis. Vet. Parasitol. 244, 12–20. https://doi.org/10.1016/j.vetpar.2017.07.013.
Sacks, J.J., Delgado, D.G., Lobel, H.O., Parker, R.L., 1983. Toxoplasmosis infection associated with eating undercooked venison. Am. J. Epidemiol. 118, 832–838. https://doi.org/10.1093/oxfordjournals.aje.a113701.
Saadatnia, G., Golkar, M., 2012. A review on human toxoplasmosis. Scand. J. Infect. Dis. 44, 805–814. https://doi.org/10.3109/00365548.2012.693197.
San Miguel, J.M., Gutiérrez-Expósito, D., Aguado-Martínez, A., Gonzá Lez-Zotes, E., Pereira-Bueno, J., Gómez-Bautista, M., Rubio, P., Ortega-Mora, L.M., Collantes-Fernández, E., Álvarez-García, G., 2016. Effect of different ecosystems and management practices on *Toxoplasma gondii* and *Neospora caninum* infections in wild ruminants in Spain. J. Wildl. Dis. 52, 293–300. https://doi.org/10.7589/2015-07-176.
Sandøe, P., Nørspang, A.P., Kondrup, S.V., Bjørnvad, C.R., Forkman, B., Lund, T.B., 2018. Roaming companion cats as potential causes of conflict and controversy—a representative questionnaire study of the Danish public. Anthrozoös 31, 459–473. https://doi: 10.1080/08927936.2018.1483870.
Sengupta, M.E., Pagh, S., Stensgaard, A.S., Chriel, M., Petersen, H.H., 2021. Prevalence of *Toxoplasma gondii* and cryptosporidium in feral and farmed American mink (*Neovison vison*) in Denmark. Acta Parasitol 66, 1285–1291. https://doi.org/10.1007/s11686-021-00409-0.
Stelzer, S., Basso, W., Benavides Silván, J., Ortega-Mora, L.M., Maksimov, P., Gethmann, J., Conraths, F.J., Schares, G., 2019. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: risk factors and economic impact. Food Waterborne Parasitol. https://doi.org/10.1016/j.fawpar.2019.e00037.
Sullivan, K.M., Dean, A., Soe, M.M., 2009. OpenEpi: a web-based epidemiologic and statistical calculator for public health. Publ. Health Rep. 124, 471–474. https://doi:10.1177/003335490912400320.
Taggart, M.A., Reglero, M.M., Camarero, P.R., Mateo, R., 2011. Should legislation regarding maximum Pb and Cd levels in human food also cover large game meat? Environ. Int. 37, 18–25. https://doi.org/10.1016/j.envint.2010.06.007.
Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30 https://doi.org/10.1016/S0020-7519(00)00124-7, 1217-5.
Tixier, H., Duncan, P., 1996. Are European roe deer browsers? A review of variations in the composition of their diets. Revue d'Ecologie (Terre Vie) 51, 3–17.
Vanek, J.A., Dubey, J.P., Thulliez, P., Riggs, M.R., Stromberg, B.E., 1996. Prevalence of *Toxoplasma gondii* antibodies in hunter-killed white-tailed deer (*Odocoileus virginianus*) in four regions of Minnesota. J. Parasitol. 82, 41–44. https://doi.org/10.2307/3284113.
Vikøren, T., Tharaldsen, J., Fredriksen, B., Handeland, K., 2004. Prevalence of *Toxoplasma gondii* antibodies in wild red deer, roe deer, moose, and reindeer from Norway. Vet. Parasitol. 120, 159–169. https://doi.org/10.1016/j.vetpar.2003.12.015.
Vildtudbytte, 2020 [in Danish] [WWW Document], 2020. Aarhus Univ. URL: https://fauna.au.dk/jagt-og-vildtforvaltning/vildtudbytte/, 1.21.21.
Weiss, L.M., Dubey, J.P., 2009. Toxoplasmosis: a history of clinical observations. Int. J. Parasitol. 39, 895–901. https://doi.org/10.1016/j.ijpara.2009.02.004.
Williamson, J.M.W., Williams, H., Sharman, G.A.M., 1980. Toxoplasmosis in farmed red deer (*Cervus elaphus*) in Scotland. Res. Vet. Sci. 29, 36–40. https://doi.org/10.1016/s0034-5288(18)32682-1.
Witkowski, L., Czopowicz, M., Nagy, D.A., Potarniche, A.V., Aoanei, M.A., Imomov, N., Mickiewicz, M., Welz, M., Szaluś-Jordanow, O., Kaba, J., 2015. Seroprevalence of *Toxoplasma gondii* in wild boars, red deer and roe deer in Poland. Parasite 22. https://doi.org/10.1051/parasite/2015017.