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ORIGINAL PAPER

Prevalence of *Toxoplasma gondii* and *Cryptosporidium* in Feral and Farmed American Mink (*Neovison vison*) in Denmark

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Abstract
Purpose To investigate the prevalence of *Cryptosporidium* spp. infection and *Toxoplasma gondii* antibodies in farmed and feral mink in Denmark.
Methods We examined meat juice from 235 feral mink and 306 farmed mink for *T. gondii* antibodies, and faecal samples from 113 feral mink and 166 farmed mink for *Cryptosporidium* oocyst excretion. Meat juice was analysed using a commercial indirect enzyme-linked immunosorbent assay and oocyst excretion was identified by a modified Ziehl–Neelsen method.
Results All farmed mink tested sero-negative, while 53.6% of feral mink were *T. gondii* sero-positive. The probability of being sero-positive for *T. gondii* was not associated with recent escapes from farms ($p = 0.468$), but was significantly higher for male feral mink (64.2%) than female feral mink (42.5%) ($p = 0.0008$). Only one feral mink and four farmed mink (2.4%) excreted *Cryptosporidium* oocysts.
Conclusion Farmed mink were all *T. gondii* sero-negative, whereas approximately half the feral mink were sero-positive. *Cryptosporidium* prevalence in farmed and feral mink were low. Overall, the public health risk of transmission of these two parasites via mink in Denmark is low.

Keywords Feral mink · Farmed American mink · *Neovison vison* · *Toxoplasma gondii* · *Cryptosporidium* · Prevalence

Introduction

The American mink (*Neovison vison*) is a semiaquatic species belonging to the family Mustelidae. Farmed American mink were introduced to Denmark in the 1930s and have since been bred for their fur [1] with around 17 million skin produced annually [2]. At the time of this study, Denmark had one of the largest mink fur productions globally with around 800 commercial mink farms and 2–3 million breeding females [3]. Occasionally, farmed mink escape unintentionally when handled, which has resulted in a feral mink populations being common and wide spread in Denmark [4, 5]. Consequently, the feral mink are regarded as an invasive species [6] and thus 5000–6000 animals are trapped every year by hunters as part of the wildlife population management in Denmark [7].

Despite the large number of both farmed and feral mink in Denmark, very little is known about their parasitic infections, which potentially could have an impact on public health and animal welfare [8]. The protozoan parasites *Toxoplasma* and *Cryptosporidium* are both single-celled zoonotic genera infectious to both mink [9], and humans [10], among others. *Toxoplasma* consists solely as *Toxoplasma gondii* [10], while the *Cryptosporidium* genus consist of several species and subspecies [11].

Toxoplasma gondii has a two-host life cycle, where felids sheds oocysts in faeces, and warm-blooded species act as intermediate hosts infected with tissue cysts. Tissue cysts are immediately infectious upon ingestion [12] of raw- and undercooked meat or tissues. *Toxoplasma gondii* infection can cause human toxoplasmosis occasionally leading to ocular disease [13], and congenital toxoplasmosis which

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can cause abortion, neonatal death, or foetal abnormalities [14, 15]. Previously symptomatic *T. gondii* infections in mink have been documented on two American farms. The symptoms included deaths, deformity, stillborn puppies, and reduced feed intake [16, 17]. In 1994, a Danish study observed 3% *T. gondii* sero-positive mink originating from five mink farms. Nevertheless, none had clinical signs [18]. The number of farmed mink in Denmark has increased markedly, since, nonetheless, no recent studies on *T. gondii* infections in Danish mink exist.

In contrast to *T. gondii*, *Cryptosporidium* infections in mink in Denmark have to our knowledge, never been investigated. *Cryptosporidium* infections, both zoonotic and species-specific species, have previously been identified in farmed mink in China (1.7–22.9%) [19, 20], Czech Republic (6%) [21], and Spain (24%) [22]. The *Cryptosporidium* parasite has a direct life cycle, where transmission between hosts occur without the need of an intermediate host. Some *Cryptosporidium* species are relatively host specific, while others can infect several animal species [11]. Infection occurs when animals ingest oocyst-contaminated faeces, feed or water. *Cryptosporidium* infection can cause cryptosporidiosis, including self-limiting diarrhoea in immunocompetent humans, but can be severe and life threatening in immunocompromised humans [23].

The aim of the present study is to investigate the prevalence of two parasitic infections in farmed and feral mink in Denmark, based on the sero-prevalence for *T. gondii* and the oocyst excretion for *Cryptosporidium* spp.

Materials and Methods

Farmed American mink (*Neovison vison*), submitted for diagnostic examination to Centre for Diagnostic, Technical University of Denmark, (DTU-CfD), and pelted cadavers from a pelt-processing centre were included in the study. Data on sex, age and postal code of farm location were noted for farmed mink. Feral American mink trapped in nature by The Danish Nature Agency or local hunters were submitted to DTU-CfD as part of the Danish wildlife surveillance. Data on sex, length of the body without tale (cm) and place of origin were obtained for feral mink. The place of origin was noted as postal code. The length of the feral mink was obtained to separate the much smaller wild-born mink from the escapees (mink caught in the wild, but born on farm) as done by Pagh et al. [4]. The length of the body for adult escapees is ≥ 40 cm for females and ≥ 44 cm for males, while wild-born adult females are ≤ 39 cm and male ≤ 43 cm [4].

Antibodies against *T. gondii* were examined in meat juice collected from the heart. The heart was removed from all mink and frozen at −20 °C for min. 48 h. Occasionally, hearts were unsuitable for examination due to trauma or decomposition. Subsequently, hearts were placed in a plastic funnel (CC Plast A/S, Hillerød, Denmark) at room temperature a described by Nielsen [24]. Meat juice was collected in a 10 ml tube during thawing and placed at −20 °C until further analysis. The collected meat juice was subsequently examined in duplicate for IgG antibodies against *T. gondii* using the ID Screen® Toxoplasmosis Indirect Multi-species ELISA kit (ID.vet, France). The optical density (OD) was read at 450 nm. Results were evaluated by calculating the *S*/*P*% (sample/positive percentage) = (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, 41–69% doubtful, and ≥ 70% positive, following manufactures guidelines.

Cryptosporidium oocyst excretion was examined by a modified Ziehl–Neelsen method [25]. Faeces were collected from rectum and stored at 4 °C until further analysis. Occasionally, faeces were unavailable due to trauma or decomposition. The Ziehl–Neelsen method is based on staining of the oocysts in faecal smears with the dye carbol-fuchsin (Merck Life Science A/S, Søborg, Denmark) after which the background is decolorized with acid and re-stained with malachite green (Merck Life Science A/S, Søborg, Denmark). The oocysts appear red against a green background. All samples were microscopically examined and semi-quantified as negative, low (1–5 oocysts/field of view), moderate (5–25 oocysts/field of view) or massive (> 25 oocysts/field of view) excretion. All faecal samples was considered positive for *Cryptosporidium* if at least one oocyst was observed microscopically and considered negative if no oocysts were observed. Genomic DNA from the positive *Cryptosporidium* samples was extracted using QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions following oocyst purification as described in Petersen et al. [26]. Partial nucleotide sequences of 18S rDNA and HSP70 gene of *Cryptosporidium* isolates were obtained by PCR as previous described by Langkjær et al. [27].

The prevalence of *Cryptosporidium* infection and the sero-prevalence of *T. gondii* was calculated together with the 95% confidence intervals. Difference in prevalence was determined by a binary logistic regression model with *T. gondii* infection as the dependent variable and sex, region of origin undefined (Fig. 1) and feral mink categories (escapees/wild mink, see result section) as independent variables. The variables are analysed independent. A p value below 0.05 was considered significant.

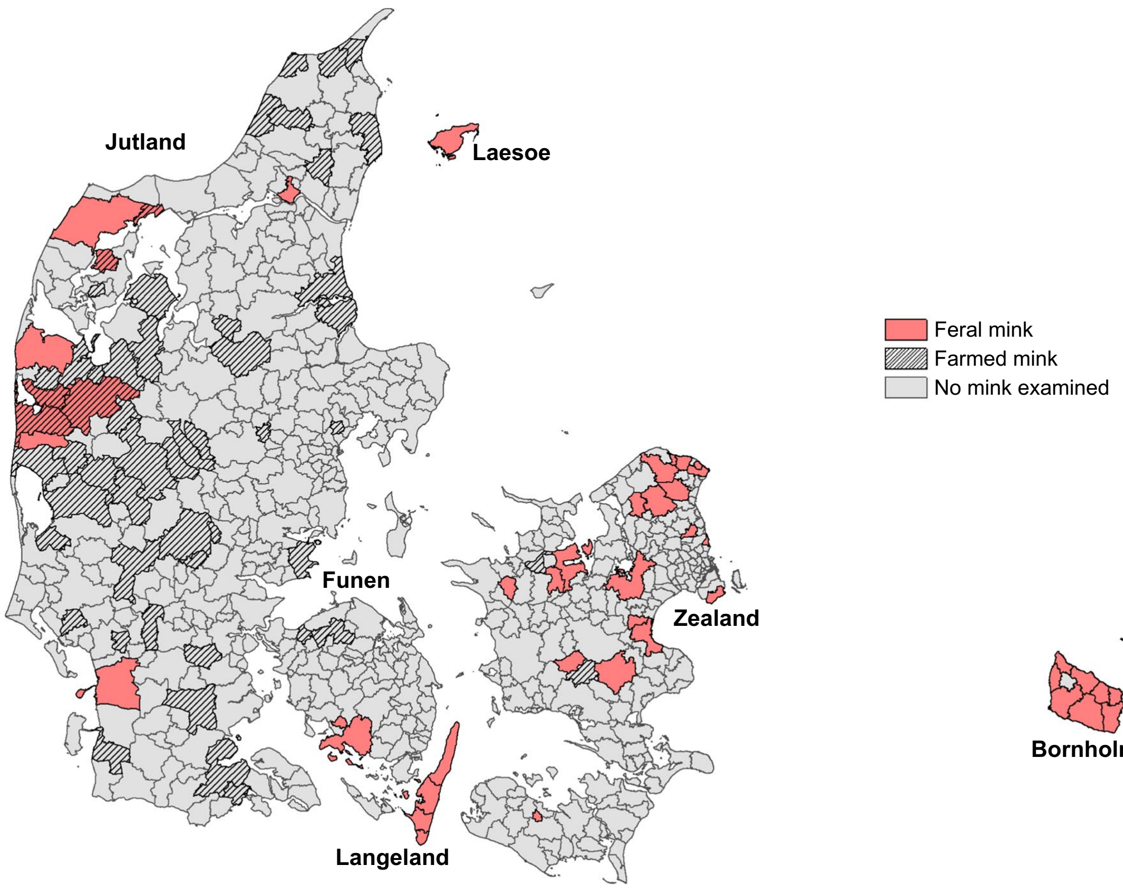

Fig. 1 Place of origin by postal area for farmed mink (scattered) and feral mink (pink). Grey indicate that no mink were sampled from this postal area

Results

In total, 376 farmed mink and 240 feral mink were included in the study. The farmed mink originated from 103 different farms distributed throughout 60 postal codes (Fig. 1). The feral mink originated from 45 postal codes (Figs. 1, 2).

Toxoplasma gondii Antibodies

In total, 235/240 feral mink and 306/376 farmed mink were analysed for *T. gondii* antibodies. All farmed mink tested sero-negative, while 53.6% [126/235; 95% CI 47.0–60.0] of feral mink were *T. gondii* sero-positive. Four feral mink tested doubtful, and were classified sero-negative in the above interpretation of results (Table 1).

The probability of being sero-positive for *T. gondii* was significantly higher for male feral mink (64.2%) than female feral mink ($p = 0.0008$). Sex was not recorded for two mink (Table 1).

Sero-positive feral mink originated from 31/45 postal codes located in both Jutland, the peninsula of Denmark, and islands (Fig. 2). The prevalence was significantly different ($p < 0.0001$) between the four different regions of Denmark, see Table 1. The odds for testing seropositive were higher in mink from Bornholm and Zealand, than mink from Funen (including the island Langeland and small islands south of Funen) and Jutland (including the island Laesoe).

Of the feral mink, escapees constituted 43.8%, while the remaining mink were categorised as wild-born mink. Sero-positive feral mink occurred both among escapees (56.3%) [58/103; 95% CI 46.2–66.1] and wild-born mink (51.4%) [67/130; 95% CI 42.6–60.4], with almost equal prevalence (Table 1). The probability of being sero-positive for *T. gondii* was, therefore, not associated with recent escapes from farms ($p = 0.468$). The length of the body was not recorded for three mink and these were, therefore, excluded from the above analysis.

Cryptosporidium Oocysts

In total, 113/240 feral mink and 166/376 farmed mink were examined for *Cryptosporidium* oocysts. Only one feral mink excreted *Cryptosporidium* oocysts, while four farmed mink (2.4%) [95% CI 0.7–6.1] excreted oocysts. All five

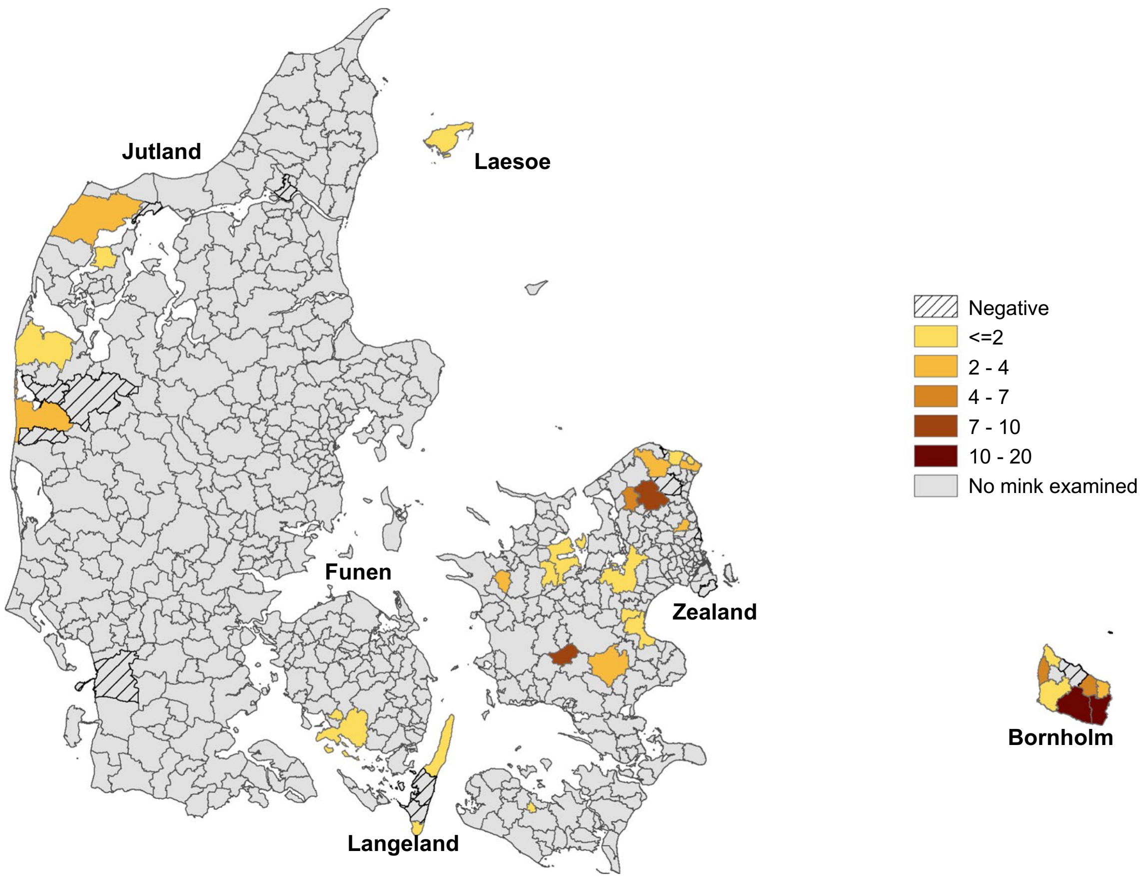

Fig. 2 Place of origin for feral mink examined for *Toxoplasma gondii* antibodies and number of positive by postal areas indicated by different colours

Table 1 *Toxoplasma gondii* results from feral mink presented by region of origin (Feral/Escapee/wild or geographical location) and sex

Origin of mink	*N* examined	Female		Male		Total sero-positive	
		Pos/*n*	Pos-% [95% CI]	Pos/*n*	Pos-% [95% CI]	*N*	Pos-% [95% CI]
Feral mink	235[a]	48/113	42.5 [33.2–52.1]	77/120	64.2 [54.9–72.7]	126	53.6% [47.0–60.0]
Escapees[b]	103	15/44	34.1 [20.5–49.9]	43/59	72.9 [59.7–83.6]	58	56.3 [46.2–66.1]
Wild mink[b]	130	33/69	47.8 [35.7–60.2]	34/61	55.7 [42.5–68.5]	67	51.4 [42.6–60.4]
Bornholm	96	18/42	42.9 [27.7–59.0]	39/54	72.2 [58.4–83.5]	57	59.4 [48.9–69.3]
Funen	7	1/4	25.0 [0.6–80.6]	2/3	66.7 [9.4–99.2]	3	42.9 [9.9–81.6]
Jutland[c]	52	7/32	21.9 [9.3–40.0]	5/19	26.3 [9.2–51.2]	12	23.8 [12.5–36.8]
Zealand[d]	80	22/35	62.9 [44.9–78.5]	31/44	70.5 [54.8–83.2]	54	67.5 [56.1–77.6]

[a]Five feral mink were not tested for *T. gondii*

[b]Two feral mink were not categorised, since body length was not measured

[c]Sex was not recorded for one mink from Jutland

[d]Sex was not recorded for one mink from Zealand

positive faecal samples were categorized as low excretion. The *Cryptosporidium*-positive farmed mink originated from only two different farms. The farm prevalence was 20.0% [1/5; 95% CI 2.2–71.6] and 10.3% [3/29; 95% CI 2.2–27.4], respectively. Three of the positive farmed mink were juvenile, while age was unknown for the last one. *Cryptosporidium* oocyst species identification was attempted with PCR on extracted oocysts from the positive faecal samples. However, probably due to the low number of oocysts in the samples, the PCR was unsuccessful.

The *Cryptosporidium*-positive feral mink (1.0%) [95% CI 0.02–5.4] originated from the island of Bornholm.

Discussion

This is the first large investigation of *T. gondii* sero-prevalence and *Cryptosporidium* oocysts excretion in feral- and farmed mink in Denmark.

A likely transmission route for *T. gondii* infections in farmed mink is via the feed [28] as mink are carnivores. Another route could be via cat faeces if cats have access to the mink cages. However, our results demonstrate that all farmed mink were *T. gondii* sero-negative. Danish farmed mink have previously tested positive for *T. gondii* antibodies (3.0%) [18], as have farmed mink from the United States [16, 17], Poland (13.9%) [28] and China (8.6%) [29]. However, based on this study *T. gondii* infections in Danish farmed mink are likely absent, probably due the use of pre-treated (frozen, heated or acid treated) commercially produced mink feed [30] and thus if *T. gondii* tissue cysts are present they are inactivated.

More than half the feral mink (53.6%) in our study were *T. gondii* sero-positive. Among the positive feral mink were both escapees (most likely born on farms (43.8%)) and wild-born mink (55.1%). For feral mink, the *T. gondii* transmission route is primarily through diet, since they consume a wide range of wild animals including small mammals and birds [31], both of which can carry *T. gondii* cysts [12, 32]. Another route of exposure to *T. gondii* is ingestion of oocysts from the environment, including water bodies. The habitat of feral mink is highly associated with water streams [33]; therefore, ingestion of oocysts directly from contaminated water could also lead to *T. gondii* infection in feral mink [34]. The probability of being sero-positive for *T. gondii* was significantly higher for male than female feral mink. The differences in seroprevalence between sexes could relate to different food preferences. Birks and Dunstone [35] observed that males preyed more heavily upon lagomorphs than did females, while females preyed more upon fish and crustaceans. This was also observed on Iceland, where males consumed more medium-sized mammals than females [36]. In contrast to our study, Sepúlveda [37] observed equal *T. gondii* sero-prevalence between sexes in mink from Chile. *Toxoplasma gondii* can probably infect all warm-blooded animals, while fish appear as an insignificant host of *T. gondii* [38]. However, filter-feeding fish can act as biotic vectors for infective *T. gondii* oocysts [39]. The role of crustaceans in the transmission of *T. gondii* oocysts is unknown; however, oocysts have been observed in some species including bivalves [40]. Nevertheless, the differences in sero-prevalence could also relate to the age of the feral mink. Previous studies reported that increasing mink age increased the odds of positivity to *T. gondii,* probably as a result of higher probability of life time exposure in older animals [37, 41]. This has also been observed in other animal species including wild ungulates from Norway [42]. Unfortunately, age was not recorded for feral mink in our study and age-related sero-prevalences could not be confirmed.

Differences in *T. gondii* sero-prevalence were observed for feral mink from different regions of Denmark. Mink from the islands Bornholm and Zealand had higher sero-prevalence than mink from the island Funen and the mainland Jutland. The area-related prevalences are likely correlated to the type of prey, and the *T. gondii* prevalence in their prey animals.

The *Cryptosporidium*-positive mink originated from two farms with farm prevalence of 20.0% and 10.3%. Unfortunately, the species/genotypes could not be identified. From other studies, *C. canis*, the *Cryptosporidium* mink genotype [20], *C. ubiquitum* [21], *C. andersoni* [43] and *C. meleagridis* [44] has been identified in feral and farmed mink with *Cryptosporidium* mink genotype being the most prevalent. All these *Cryptosporidium* species has also been identified in humans [45–48]. Since many animal species may be infected with different *Cryptosporidium* species, there are several opportunities for potential transmission to mink, including mink from other farms and even from humans. However, *Cryptosporidium* infections are rare in Danish farmed mink, although the prevalence was 20.0% on one farm, suggesting that the infection may be established here.

Whether there is a risk of infection for humans with *Cryptosporidium* from mink in Denmark cannot be ruled out based on data from this study, but the risk is probably minimal as the positive mink originated from only two farms and in only one feral mink. The zoonotic risk of *T. gondii* infection from farmed mink in Denmark is insignificant, since all farmed mink were sero-negative. For the feral mink, despite the high prevalence of *T. gondii* the risk is also considered negligible, since mink is not consumed. However, pelting feral mink could pose a minor risk for especially pregnant women [49] due to the extensive contact with raw animal flesh during the procedure.

Conclusion

All farmed mink in Denmark tested sero-negative for *T. gondii* antibodies, and only four mink from two farms excreted *Cryptosporidium* oocysts. Hence, the risk for employees in the mink industry to become infected with *T. gondii* or *Cryptosporidium* infections when handling farmed mink is considered negligible. Even though 53.6% feral mink were *T. gondii* sero-positive, the risk when handling or pelting them is also considered negligible, since mink is not consumed.

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Author contributions Conceptualization, MES and HHP; methodology, MES and HHP; planning and sampling, SP and MC; validation, all authors; analysis, A-SS and HHP, writing–original draft preparation, MES and HHP; writing–review and editing, all other authors. All authors have read and agreed to the published version of the manuscript.

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Availability of data and material The data sets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study did not require official or institutional ethical approval as no animals were hunted for the purpose of this study. Certified hunters shot the mink or caught them in traps, and the samples were collected post mortem.

Consent to participate All farms involved in the study participated voluntarily.

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