An investigation of endoparasites and the determinants of parasite infection in European hedgehogs (*Erinaceus europaeus*) from Denmark

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A B S T R A C T

The European hedgehog population is declining in Europe. It is therefore important to investigate the causes for the decline and monitor the general health of the species. We investigated the endoparasite occurrence in 299 dead European hedgehogs. Of these, endoparasites were detected in 69% of the individuals tested. We identified *Crenosoma striatum*, *Capillaria aerophila* (syn. *Eucoleus aerophilus*), *Capillaria* spp., coccidia, *Cryptosporidium* spp., *Brachylaemus* spp. and *Capillaria hepatica*. We also examined the hedgehogs for *Giardia* spp. and *Echinococcus multilocularis* but all were negative. Coccidia (n = 7, 2.5%) and *Cryptosporidium* spp. (n = 14, 5.2%) were only detected in individuals from Zealand, Lolland and Jutland south of the Limfjord. Single cases of *Brachylaemus* spp. (n = 1, 0.4%) and *Capillaria hepatica* (n = 1, 1.1%) were exclusively discovered in Jutland south and north of the Limfjord, respectively. These results indicate a regional difference in endoparasite species carried by European hedgehogs in Denmark. This stresses the need for hedgehogs to be cared for locally when admitted to wildlife rehabilitation centres, and to be released within their area of origin, to prevent spread of endoparasite infections among hedgehogs. Additionally, we explored the following possible determinants of parasite infection in the hedgehogs: sex, age, mortality category (in-care, natural and roadkill), infection with MRSA, genetic heterozygosity, month of death, geographical location and habitat type, and found that only age had a statistically significant effect on endoparasite prevalence, as we detected a lower occurrence of endoparasites in juvenile hedgehogs (<1 year) compared to the other age classes. However, pairwise comparisons of geographical regions did show significant differences in endoparasite occurrence: Zealand vs. Jutland south of the Limfjord and Zealand vs. Falster. We conclude that, in line with previous studies of European hedgehogs throughout their range in Western Europe, endoparasite infections are common and a natural part of their ecology.

1. Introduction

Research from several western European countries reports decline, or concerns for decline, in the population of European hedgehogs (*Erinaceus europaeus*) (Holsbeek et al., 1999; Huijser and Bergers, 2000; Doncaster et al., 2001; Rondinini and Doncaster, 2002; SoBH, 2011; Krange, 2015; SoBH, 2015; van de Poel et al., 2015; Hof and Bright, 2016; Müller, 2018; SoBH, 2018; Williams et al., 2018; Taucher et al., 2020). It is therefore essential to study the causes of the decline to improve the conservation initiatives directed at this species. Examining

for parasite infections in European hedgehogs could potentially refine one of the actions for the preservation of the species, which is the treatment of sick and injured hedgehogs taken into care at wildlife rehabilitation centres all over Europe.

The European hedgehog can host several endoparasite species with varying clinical significance (Mullineaux and Keeble, 2016; Bexton and Couper, 2019). Infections with the lungworms *Crenosoma striatum* and *Capillaria aerophila* (syn. *Eucoleus aerophilus* (Moravec, 2000)) are commonly reported in hedgehogs (Schütze, 1980; Barutzki et al., 1984; Laux, 1987; Majeed et al., 1989; Keymer et al., 1991; Epe et al., 1993;

Reeve, 1994; Reeve and Huijser, 1999; Poglayen et al., 2003; Egli, 2004; Beck, 2007; Gaglio et al., 2010; Pfäffle, 2010; Haigh et al., 2014b; Rautio et al., 2016), while other lungworm species such as *C. tenuis* occur more rarely (Schütze, 1980; Saupe, 1988; Feliu et al., 2001). Table 1 provides an overview of endoparasites previously detected in European hedgehogs.

Parasites can have significant impacts on the dynamics of wildlife populations (Irvine, 2006). However, scarcely any studies have considered in detail, which impact parasite infections have on hedgehogs including influence on the dynamics of their host populations. This is presumably due to the lack of comprehensive background information on the individual animals.

Despite numerous publications on parasites in the European hedgehog, only few have investigated potential determinants of parasite infection by comparing parasite prevalence to e.g. age classes, sex, body condition or habitat type (Majeed et al., 1989; Reeve and Huijser, 1999; Egli, 2004; Pfäffle, 2010; Haigh et al., 2014b; Rautio et al., 2016). Comparing parasite occurrence with different determinants could provide important insights into how endoparasites influence the populations of European hedgehogs. Studies on Soay sheep from a free-living island population indicate that parasite mediated selection maintains genetic variation in the population by removing less heterozygous individuals (Coltman et al., 1999). A study on California sea lions established that inbreeding caused a wider range of helminth infections in the individuals (Acevedo-Whitehouse et al., 2003).

Consequently, the aim of this study is to examine endoparasites in hearts, lungs, livers and gastrointestinal tracts of European hedgehogs originating from Denmark, to investigate the potential determinants of endoparasite infection in this species. Subsequently, we will compare the presence of endoparasites in relation to the ecological factors age, sex, mortality category (in-care, natural and roadkill), time of year, geographical location and rural or urban habitat types, prevalence of MRSA and the genetic heterozygosity of each individual to describe potential determinants of endoparasite infection in European hedgehogs.

2. Materials and methods

2.1. Sample collection

The samples were obtained as part of a nationwide citizen science project in Denmark, The Danish Hedgehog Project, where people were encouraged to collect dead hedgehogs during May to December 2016. The volunteers were asked to provide details on the date and location of the find, and deliver the hedgehog carcasses to one of 26 collection stations, distributed nationally. A total of 299 dead hedgehogs representing all regions of the country were included in the study. The samples consisted primarily of road-killed hedgehogs, but also individuals found dead in the wild, plus individuals that died in care at Danish hedgehog rehabilitation centres. The hedgehog carcasses were stored at −20 °C until necropsied. During necropsy, hearts, lungs, intestines and liver tissues were collected. Occasionally, due to the nature of the collection including traumatic injury, predation or decomposition, some individuals did not contain all organs or sufficient amounts of faeces for the complete set of tests. Moreover, the age of the hedgehogs, their sex and locations were registered (data presented in Table 3, Fig. 1 and Supplementary material 1). The age of the individuals was determined through counting of periosteal growth lines in transverse sections of the lower jaws, as described in Rasmussen et al. (in prep.), Haigh et al. (2014a) and Morris (1970).

2.2. Parasitology

2.2.1. Organ parasite counts

The hearts and lungs of 276 hedgehogs were examined. Hearts were opened with scissors and examined for heartworms under a dissection

microscope. Lungs were cut open along the bronchi and bronchioles and microscopically examined for lungworms using a dissection microscope (Leica DMRB, Leica Microsystems A/S, Brønshøj, Denmark). Subsequently, the lungs were flushed several times with tap water into a conical glass and left to sediment for 20 min. Afterwards, the supernatant was removed and the sediment was transferred to glass slides for microscopic identification and enumeration of lungworms (Leica DMRB, Leica Microsystems A/S, Brønshøj, Denmark).

The livers of 94 hedgehogs were macro- and microscopically examined for abscesses, helminths and eggs of *C. hepatica*, using a dissection microscope (Leica DMRB, Leica Microsystems A/S, Brønshøj, Denmark). The livers were placed on a petri dish and back-illuminated with a strong light source to detect potential internal lesion or cysts, which are invisible from the liver surface. Abscesses were separated from the surrounding liver material, transferred to a microscopic slide, and microscopically examined for protoscolices, including those from *Echinococcus multilocularis* (Leica M125, Leica Microsystems A/S, Brønshøj, Denmark).

2.2.2. Faecal egg and oocyst count

Faeces from 276 hedgehogs were examined by flotation technique to identify parasite eggs and coccidian oocysts. The intestine was cut open along its length, and faeces was collected by scraping the intestine. Parasitic eggs and/or coccidian oocysts per gram of faeces (FEC/FOC) were calculated using a modification of the McMaster technique previously described by Roepstorff and Nansen (1998) with a sensitivity of 5 FEC/FOC. Samples consisting of 4 g faeces from each individual was suspended in 56 mL tap water, sieved through gauze, and 10 mL was centrifuged at $178 \times g$ for 10 min. The supernatant was removed, the pellet was re-suspended in 3 mL of flotation fluid (saturated saline with glucose, 50 g/100 mL, specific gravity 1.27 g/mL) and a disposable McMaster chamber was filled with the mixture.

Another 2 g faeces was analysed for trematode eggs by sedimentation. Faeces was diluted in 56 mL tap water placed in a plastic cup, stirred and sieved through gauze into a conical flask containing 250 mL 0,1% Teepol-water solution (Glucose Salt Teepol™ Broth, Teepol, UK) and left to sediment for 10 min. The supernatant was discarded, and an additional 250 mL Teepol-solution was added after which the sample was left to sediment for 10 min. The supernatant was discarded, and the sediment was collected in a 10 mL tube with a drop of malachite green added. The sample was transferred to a petri dish and examined microscopically for trematode eggs.

Faecal smears from 194 animals were examined for *Cryptosporidium* oocysts using a modified Ziehl-Neelsen technique (Henriksen and Pohlenz, 1981) based on staining of oocysts with Carbolfuchsin and acid destaining of the background. Subsequently, oocysts were microscopically identified based on the red colour against a green background as well as the size and shape.

Additionally, faeces samples from 74 hedgehogs were analysed for *Cryptosporidium* oocysts and *Giardia* cysts as previously described by Maddox-Hyttel et al. (2006) using immunofluorescence assay (IFA). Samples consisting of 1 g faeces per individual were suspended by vortexing in 3.5 mL 0.01% Tween 20 (Santa Cruz Biotechnology Inc, Texas, USA) and were filtered through multilayered 20-thread gauze. Another 3.5 mL 0.01% Tween 20 was added, and the fluid was pressed through the gauze. Afterwards, 3.5 mL flotation fluid (saturated saline with glucose specific gravity = 1.13 g/mL) was added to the filtrate and centrifuged at $53 \times g$ for 3 min. The supernatant was transferred to a clean tube and washed three times using MQ water and subsequently centrifuged at $1540 \times g$ for 10 min to obtain a final sample volume of 2 mL. For each sample, a 10 μL subsample was placed in a well on a 3-well Teflon printed diagnostic slide (Immuno-Cell Int., Belgium). The slide was airdried, fixed for 5 min with acetone and added 25 μl anti-*Cryptosporidium* fluorescein isothiocyanate (FITC)-labelled antibody mix according to the manufacturer's description (Crypto-Cell IF test, Cellabs, Australia). *Cryptosporidium* oocysts and *Giardia* cysts were identified

Table 1
Overview of endoparasite species previously found in European hedgehogs.

Class	Species	Country	Anatomical location in European hedgehogs	References
Enoplea (roundworms)	*Crenosoma striatum*	Portugal, United Kingdom, Italy, Ireland, Germany, Finland, Czech Republic, Spain, Switzerland, New Zealand	Lungs	Schütze (1980); Timme (1980); Laux (1987); Boag and Fowler (1988); Keymer et al. (1991); Epe et al. (1993); Giannetto et al. (1993); Reeve and Huijser (1999); Feliu et al. (2001); Liesegang and Lehmann (2003); Poglayen et al. (2003); Egli (2004); Epe et al. (2004); Pantchev et al. (2005); Gaglio et al. (2010); Pfäffle (2010); Haigh et al. (2014b); Pfäffle et al. (2014); Hofmannova et al. (2016); Rautio et al. (2016); Allen et al. (2020); Barradas et al. (2020)
	Capillaria aerophila syn. *Eucoleus aerophilus*	United Kingdom, Germany, Finland, Czech Republic, Switzerland, New Zealand	Lungs	Schütze (1980); Timme (1980); Laux (1987); Majeed et al. (1989); Keymer et al. (1991); Epe et al. (1993); Reeve and Huijser (1999); Egli (2004); Gaglio et al. (2010); Pfäffle (2010); Pfäffle et al. (2014); Rautio et al. (2016)
	Capillaria tenuis syn. *Eucoleus tenuis*	Portugal, Spain, United Kingdom	Lungs	Boag and Fowler (1988); Feliu et al. (2001)
	Capillaria erinacei syn. *Aonchotheca erinacei*	Italy, United Kingdom, Ireland, Portugal, Spain, Slovakia, Switzerland	Intestinal tract	Romashov (1980); Boag and Fowler (1988); Giannetto et al. (1993); Feliu et al. (2001); Poglayen et al. (2003); Egli (2004); Gaglio et al. (2010); Haigh et al. (2014b)
	Capillaria spp. found in intestinal tract	United Kingdom, Ireland, Germany, Finland, Czech Republic, Switzerland, New Zealand	Intestinal tract	Timme (1980); Keymer et al. (1991); Reeve and Huijser (1999); Liesegang and Lehmann (2003); Pfäffle (2010); Pfäffle et al. (2014); Rautio et al. (2016)
	Haemonchus contortus	Sardinia, Italy	Intestinal tract	Poglayen et al. (2003); Gaglio et al. (2010)
	Spirura rytipleurites seurati	Italy, Portugal, Spain	Stomach	Giannetto et al. (1993); Giannetto and Trotti (1995); Feliu et al. (2001); Poglayen et al. (2003)
	Physaloptera clausa	Italy, Czech Republic, Portugal, Spain	Stomach	Feliu et al. (2001); Poglayen et al. (2003); Pfäffle et al. (2014)
	Capillaria ovoreticulata	United Kingdom, Germany	Intestinal tract	Reeve and Huijser (1999); Gaglio et al. (2010)
	Porrocaecum spp. larvae	Portugal, Spain	Digestive tract	Feliu et al. (2001)
	Gongylonema	Sicily, Italy	Oesophagus	Giannetto et al. (1993)
	Capillaria hepaticum syn. *Calodium hepaticum*	Portugal, Spain	Liver	Feliu et al. (2001)
	Pterygodermatites plagiostoma	Portugal, Spain	Stomach	Feliu et al. (2001)
	Trichinella spp.	Belgium, Czech Republic	Muscles	Famerée et al. (1982); Hofmannová and Juránková (2019)
Trematodes (flatworms, flukes)	*Brachylaemus erinacei*	Italy, United Kingdom, Germany, Czech Republic, Portugal, Spain, Switzerland	Intestinal tract, bile ducts	Schütze (1980); Timme (1980); Laux (1987); Keymer et al. (1991); Epe et al. (1993); Giannetto et al. (1993); Reeve and Huijser (1999); Feliu et al. (2001); Poglayen et al. (2003); Egli (2004); Gaglio et al. (2010); Pfäffle (2010); Pfäffle et al. (2014)
	Isthmiophora melis	Czech Republic	Liver, gall bladder, intestine, and pancreatic ducts	Hildebrand et al. (2015)
	Nephrotrema truncatum	Austria	Liver, gall bladder, intestine, and pancreatic ducts	Löwenstein et al. (1991)
	Dicrocoelium dendriticum	Sicily, Italy	Liver	Giannetto et al. (1993); Poglayen et al. (2003)
	Brachylecitum aetechini	Italy	Liver, gall bladder, intestine, and pancreatic ducts	Giannetto et al. (1993); Poglayen et al. (2003)
	Brachylecitum mackoi	Elba, Italy	Liver, biliary ducts	Casanova and Ribas (2004)
Cestodes (flatworms)	*Rodentolepis erinacei* syn. *Hymenolepis erinaceus*	Germany, Czech Republic, United Kingdom	Intestines	Schütze (1980); Timme (1980); Boag and Fowler (1988); Keymer et al. (1991); Pantchev et al. (2005); Pfäffle et al. (2014)
	Mesocestoides spp.	Sicily, Italy	Digestive tract	Giannetto et al. (1993); Poglayen et al. (2003)
Acanthocephalans (thorny-headed or spiny-headed worms)	*Plagiorhynchus cylindraceus*	Germany, Czech Republic, New Zealand, United Kingdom	Intestines, body cavity	Pfäffle (2010); Skuballa et al. (2010); Pfäffle et al. (2014)
	Nephridiorhynchus major	Italy, Czech Republic, Portugal, Spain	Digestive tract	Giannetto et al. (1993); Feliu et al. (2001); Poglayen et al. (2003); Pfäffle et al. (2014)
	Oliganthorhynchus erinacei syn. *Echinorhynchus erinacei*	United Kingdom	Digestive tract	Gaglio et al. (2010)
	Prosthoryhnchus spp.	United Kingdom, Portugal, Spain	Intestines	Keymer et al. (1991); Feliu et al. (2001)
Protozoans (single-celled eukaryotes)	*Cryptosporidium parvum*	United Kingdom, Czech Republic, The Netherlands, Denmark, Germany	Digestive tract	Sturdee et al. (1999); Enemark et al. (2002); Dyachenko et al. (2010); Krawczyk et al. (2015); Hofmannova et al. (2016); Sangster et al. (2016)
	Cryptosporidium hominis	The Netherlands	Digestive tract	Krawczyk et al. (2015)
	Cryptosporidium erinacei	Czech Republic	Digestive tract	Hofmannova et al. (2016)

Table 1 (*continued*)

Class	Species	Country	Anatomical location in European hedgehogs	References
	Cystoisospora spp.	Germany, Czech Republic	Digestive tract	Epe et al. (1993); Epe et al. (2004); Pantchev et al. (2005); Hofmannova et al. (2016)
	Cystoisospora rastegaivae	Germany, Sicily Italy, Czech Republic	Intestines	Schütze (1980); Giannetto et al. (1993); Hofmannova et al. (2016)
	Eimeria ostertargi	Germany	Intestines	Schütze (1980)
	Eimeria perardi	Germany	Intestines	Schütze (1980)
	Toxoplasma gondii	Czech Republic, Austria	Brain	Sixl et al. (1989); Hofmannová and Juránková (2019)
	Sarcocystis spp.	Austria	Intestines	Löwenstein et al. (1991)
	Giardia duodenalis	Austria, The Netherlands	Intestines	Löwenstein et al. (1991); Krawczyk et al. (2015)
	Leishmania infantum	Spain	Spleen, skin	Munoz-Madrid et al. (2013); Alcover et al. (2020)

based on the fluorescence green colour, size and shape and were quantified by epifluorescence microscopy and expressed as oocysts or cysts per gram faeces (OPG, CPG).

2.3. Data analysis

2.3.1. Habitat classification analysis

Habitat types for each hedgehog were extracted within a 500 m radius around where a hedgehog was found. This area is roughly equivalent to a large hedgehog home range (Reeve, 1994). The habitat classes were defined using CORINE land cover data with a 100×100 m resolution (CLC 2012; Version 18.5.1). CORINE land cover data describes habitat types derived from satellite imagery divided into: artificial surfaces, industry, agricultural areas, forest and semi-natural areas, wetlands and water bodies. For each area around which a hedgehog was found, habitat types were extracted in R (R Core Team, 2020) using the raster package (Hijmans, 2018). Afterwards, the habitat types were reclassified as "urban", "rural" or "other". Based on the habitat extraction in R, 81 habitat classified squares were allocated to each individual. For further calculations, focus was on the percentages of urban versus rural, excluding the "other" category. The categorization of "urban" or "rural" was based on the highest percentagewise representation of the two categories for each individual hedgehog from a total of 81 squares per individual.

2.3.2. Modelling determinants of parasite infection in hedgehogs

To investigate whether the overall presence of parasites in a hedgehog was associated with sex, age, habitat type, geographical region, mortality category (in-care, natural and roadkill), month of death, occurrence of MRSA, or the degree of inbreeding, we fitted generalized linear models (GLMs) in R with binomial errors and a logit link function. The response variable was the detection or not of parasites (coded as 1 and 0 respectively) and the explanatory variables were sex (female/male), age (0–16 years) (Method for age determination: Counting of periosteal growth lines in a transverse section of the lower jaw, as described in Rasmussen et al. (in prep.)), habitat (urban/rural), region (a 6-level categorical variable), mortality category (a 3-level categorical variable: in-care, natural, roadkill), month of death (May until November), occurrence or not of MRSA (coded as 1 and 0 respectively) (Rasmussen et al., 2019b), and degree of inbreeding (Observed individual heterozygosity (iH$_O$) with a continuous scale from 0 = completely inbred to 1 = no inbreeding) (Rasmussen et al., 2019c, 2020).

Individuals from the islands Samsø, Møn, Bogø and Thurø were excluded because we had fewer than five individuals for these regions. Furthermore, we excluded individuals that died in April (n = 1) and December (n = 2). Prevalence of MRSA was added as a response variable as the carriage of MRSA by the hedgehogs could indicate a weakened immune system, and the individuals included in the present study had already been tested for MRSA (Rasmussen et al., 2019b). All data used for the models can be accessed in Supplementary material 1.

We first fitted a maximal model including all explanatory variables (sex, age, habitat, region, mortality category, month of death, occurrence of MRSA, degree of inbreeding) and a two-way interaction between sex and age. We then sequentially removed non-significant terms, using the dropterm function in R and Chi-Square statistics to obtain a minimal adequate model where all remaining terms were significant (Crawley, 2013). We repeated this exercise for all observations with complete cases (no missing data), with individuals with known inbreeding coefficients and known occurrence of MRSA (N = 134) to verify the outcome. Finally, we calculated the equivalent of the R-squared value for generalized linear models, the D-squared (Guisan and Zimmermann, 2000), as a measure of the amount of deviance accounted for using the R function Dsquared taking the number of observations into account (package modEvA).

2.3.3. Further data analysis

For the variables with many classes (age, region, and month of death), we performed tests on the proportion of hedgehogs with endoparasites using R's core functions prop.test and pairwise.prop.test.

For each of these classes of variables, we tested the endoparasite occurrence of a particular class, e.g. age class one year, compared to the overall population average, as well as between classes, e.g. age class one year compared to age class two years. Using Pearson's chi-squared tests, we investigated whether the proportions of hedgehogs infected with endoparasites were the same in the various age, region and month of death classes, and if not, between which classes significant differences occurred. For instance, we tested the parasite prevalence in hedgehogs of <1 year of age compared to the overall parasite occurrence in the population as well as to all other age classes with more than one observation. This meant that individuals >6 years of age were excluded from these analyses due to the low sample size in these age classes. For the pairwise comparison of regions, individuals from the islands Samsø, Møn, Bogø and Thurø were excluded because we had fewer than five individuals for these regions. Furthermore, we excluded individuals that died in April (n = 1) and December (n = 2), as well as seven individuals categorised as found during summer, for the analyses of month of death.

3. Results

Information regarding sex, age, habitat type, mortality category and geographical location of the hedgehogs included in this study is provided in Table 3.

One or more species of endoparasites or eggs excreted from endoparasites were identified in 69% (207/299) of the 299 hedgehogs examined in our study (Table 2). In 161 (54%) of the hedgehogs, the lungworms *C. striatum*, *C. aerophila* or co-infections with these, were identified as larvae or adult nematodes in the lungs (*C. striatum* and *C. aerophila*) or as larvae in faeces (*C. striatum*). No heartworms were identified. Eggs/oocysts of at least one of the parasites *Cryptosporidium* spp., *Capillaria* spp. (5–2000 faecal egg counts (FEC)), mean 275 FEC), coccidia (5–635 faecal oocyst count (FOC), mean 227 FOC) (the genus was not identified) and *Brachylaemus* spp. eggs were identified in faeces

Table 2
An overview of the results from the endo-parasitological examinations of 299 hedgehogs including the methods. The term "number of individuals tested positive" indicates the total number of individuals tested positive for a specific parasite even though multiple methods were used and may have yielded the same positive results in some individuals.

Species of endoparasites detected	Individuals tested	Positives	Prevalence (%)	Methods (positive/total examined)
Lungs				
Crenosoma striatum worms	276	130	**47.1**	Lung + heart inspection (130/276)
Capillaria aerophila worms	276	10	**3.6**	Lung + heart inspection (10/276)
Intestinal contents				
Capillaria spp. eggs	277	143	**51.6**	Sedimentation (34/261), McMaster (139/275)
Crenosoma striatum larvae	277	89	**32.1**	Sedimentation (80/260), McMaster (39/276)
Coccidia	276	7	**2.5**	McMaster (7/276)
Cryptosporidium spp.	268	14	**5.2**	IFA (11/74), Ziehl Neelsen (3/194)
Giardia spp.	74	0	**0.0**	IFA (0/74)
Brachylaemus spp.	276	1	**0.4**	Sedimentation (1/260), McMaster (0/276)
Liver				
Echinococcus multilocularis	94	0	**0.0**	Liver inspection (0/94)
Capillaria hepatica	94	1	**1.1**	Liver inspection (1/94)
Co-infections				
Crenosoma striatum + *Capillaria* spp. + *Cryptosporidium* spp.	299	7	**2.3**	
Crenosoma striatum + *Capillaria* spp. + coccidia	299	3	**1.0**	
Crenosoma striatum + *Capillaria* spp.	299	105	**35.1**	
Crenosoma striatum + *Cryptosporidium* spp.	299	9	**3.0**	
Capillaria spp. + *Cryptosporidium* spp.	299	9	**3.0**	
Crenosoma striatum + coccidia	299	5	**1.7**	
Capillaria spp. + coccidia	299	4	**1.3**	

Table 3
Overview of the different variables used in the data analysis, including the distribution of individuals in each category.

Category	Number of individuals	Percentage of the 299 individuals represented
Total number tested	299	100
Endoparasite positive individuals	207	69
Mortality category		
Traffic	163	55
Naturally in the wild	63	21
In care	73	24
Sex		
Females	106	35
Males	153	51
Unknown	40	13
Age tested (0–16 years)		
Total	252	84
MRSA tested		
Total tested	158	53
mecC-MRSA positive out of 158 tested	98	62
mecC-MRSA negative out of 158 tested	60	38
Tested for individual observed heterozygosity (iH$_O$) (0.045–0.384)	106	35
Habitat type		
Urban	112	37
Rural	155	52
Unknown	32	11
Geographical location		
Bogø	1	0.3
Bornholm	11	3.7
Falster	14	4.7
Funen	36	12.0
Jutland north of the Limfjord	10	3.3
Jutland south of the Limfjord	112	37.5
Lolland	16	5.4
Møn	1	0.3
Samsø	1	0.3
Zealand	95	31.8
Thurø	1	0.3
Unknown	1	0.3

from 1 (0.4%) individual hedgehogs. Of these, *Capillaria* spp. eggs (including *C. aerophila*, which are morphologically similar) were most prevalent (Table 2). For *Cryptosporidium*, three hedgehogs excreted moderate oocyst numbers (100.000–1.000.000 FOC), while the remaining (n = 11) excreted low levels of oocysts (<100.000 FOC).

Of the 299 individuals tested, 113 hedgehogs (38%) hosted multiple endoparasite species (Table 2) such as a combination of *C. striatum* and *Capillaria* spp., but also *C. striatum*, *Capillaria* spp., coccidia and *Cryptosporidium* spp. See Supplementary material 1 for an overview of the complete data set including egg and oocyst counts.

Crenosoma striatum and *Capillaria* spp. were commonly found in individuals from all over Denmark (Fig. 1). However, coccidia (n = 7) and *Cryptosporidium* spp. (n = 14) were only detected in individuals from Zealand, Lolland and Jutland south of the Limfjord. The single hedgehog infected with *Brachylaemus* spp. originated from Jutland south of the Limfjord (Fig. 1).
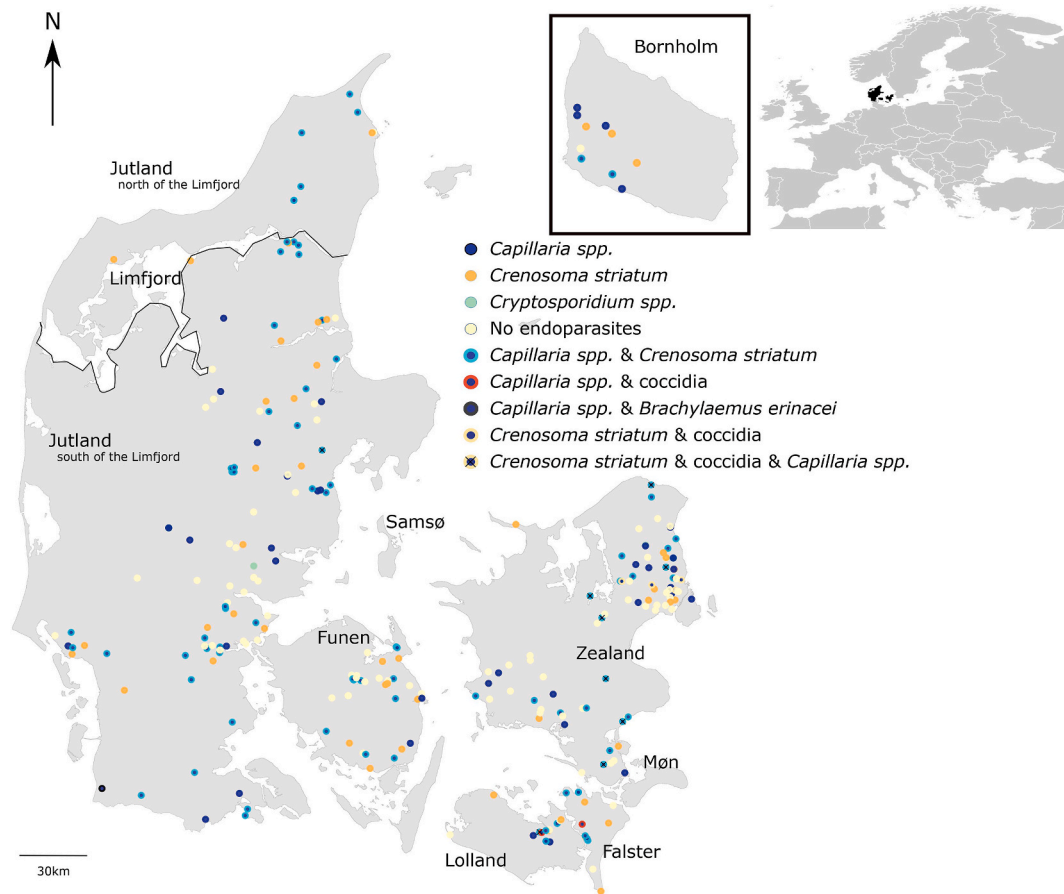
Fig. 1. A map representing Denmark and the geographical locations of the 299 dead European hedgehogs examined. Colours indicate the different species of endoparasites detected in each individual. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
An overview of the *dropterm* procedure for determining the best GLM to describe the data set, using only cases with known inbreeding coefficient (N = 134 individuals).

Model	dropterm	Overall AICc
Sex + Age + Habitat + Region + Mortality Category + Month of Death + Inbreeding + MRSA + Sex:Age	Habitat	102.21
Sex + Age + Region + Mortality Category + Month of Death + Inbreeding + MRSA + Sex:Age	Inbreeding	100.54
Sex + Age + Region + Mortality Category + Month of Death + MRSA + Sex:Age	Region	99.61
Sex + Age + Mortality Category + Month of Death + MRSA + Sex:Age	Mortality Category	99.02
Sex + Age + Month of Death + MRSA + Sex:Age	Month of Death	98.3
Sex + Age + MRSA + Sex:Age	Sex:Age	101.98 [+]
Sex + Age + MRSA	Sex	99.89
Age + MRSA	MRSA	108.91
Age [*]	–	145.99

[+] the number of rows the model is built on, changes from here and thus the AIC cannot be compared.

[*] the occurrence of parasites is significantly different between 0 year old and 1 year old hedgehogs, $p < 0.01$.

None of the abscesses isolated from livers contained material consistent with parasite infections caused by *E. multilocularis* or other tapeworms. However, *C. hepatica* eggs were identified in the liver of one hedgehog.

In the remainder of the paper, the term "endoparasite infection" refers to the detection of either adult lungworms or larvae, the oocyst stage of *Cryptosporidium* and coccidia or identification of parasite eggs (not adult worms) in faeces. The subsequent data analyses using GLMs investigating the possible determinants of endoparasite infection in European hedgehogs included the following variables (Table 3).

The minimal adequate model only retained the explanatory variable "Age" (Table 4). This is mostly explained by the difference in the proportion of <1 year old hedgehogs versus older individuals (Fig. 2). However, this final model only explained ~7% of the deviance found in our dataset (D-squared 0.074). In other words, when analysing data by

use of the GLM, the occurrence of parasites in hedgehogs was not well explained by sex, mortality category, infection with MRSA, individual genetic heterozygosity/the degree of inbreeding (iHO), month of death (season), geographical location or habitat type. The results from our model therefore indicate that European hedgehogs from Denmark often host endoparasites (69% occurrence) regardless of background (dying in the wild or in care), time of year, geographical location, habitat type, sex and general health condition. This was furthermore confirmed when exploring the distribution of *Capillaria* spp. and *Crenosoma striatum* between females and males, with *Capillaria* spp. in 53% of the females (n = 56/106) and 52% of the males (n = 79/153), and *Crenosoma striatum* in 59% of the females (n = 62/106) and 54% of the males (n = 83/153). Our data on the distribution of *C. striatum* in individuals from urban (51%, n = 57/112) or rural (57%, n = 87/152) areas emphasises the similarity found in endoparasite prevalence in hedgehogs from rural and
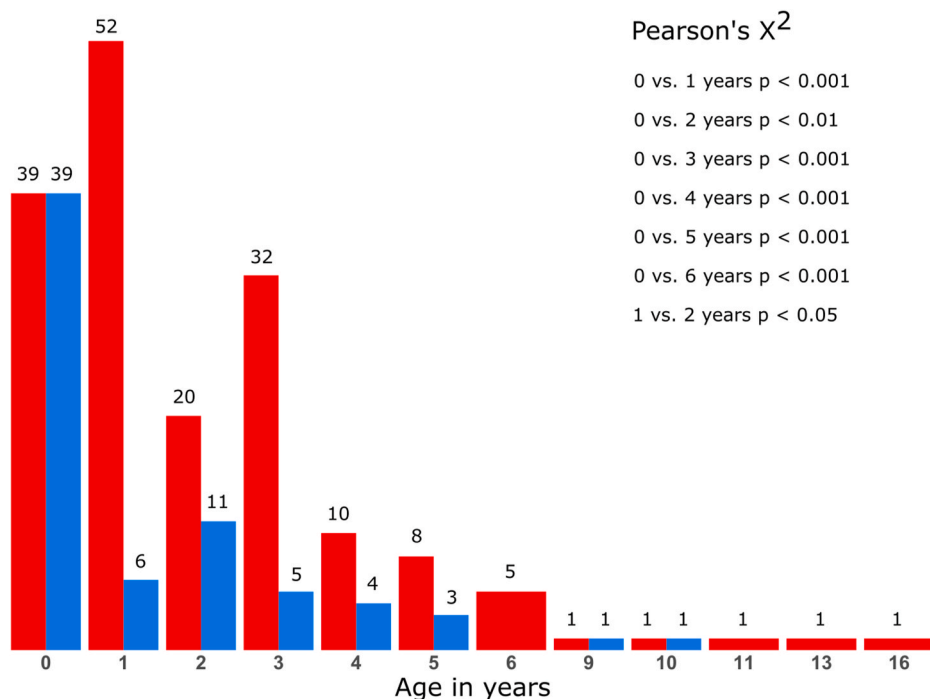
Pearson's X^2

0 vs. 1 years $p < 0.001$

0 vs. 2 years $p < 0.01$

0 vs. 3 years $p < 0.001$

0 vs. 4 years $p < 0.001$

0 vs. 5 years $p < 0.001$

0 vs. 6 years $p < 0.001$

1 vs. 2 years $p < 0.05$

Fig. 2. Overall parasite prevalence by age. Numbers on the x-axis indicate age in years. Numbers on top of the columns indicate number of individuals, in red for hedgehogs with endoparasites, in blue for hedgehogs without endoparasites. Statistically significant differences in proportions of hedgehogs with endoparasites versus without hedgehogs, were found between juveniles (<1 year) and age classes 1–6 years, and between hedgehogs of one year versus two years of age as shown in the upper right corner of the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
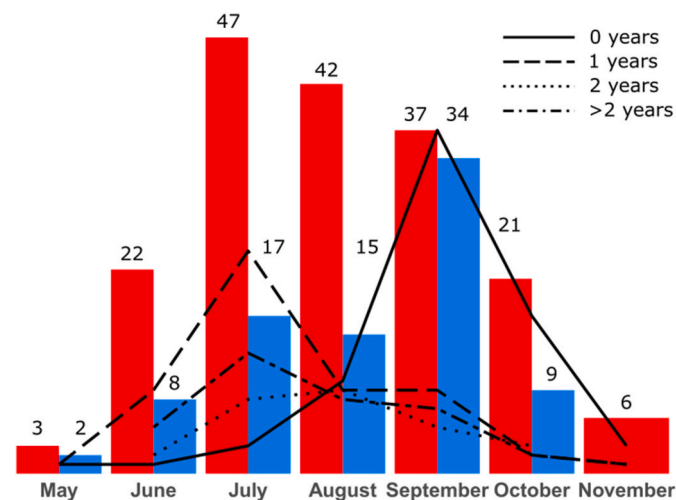

Fig. 3. Overall endoparasite prevalence by month. Numbers indicate number of individuals, in red for hedgehogs with endoparasites, in blue for hedgehogs without. The dead hedgehogs examined in the present study were collected from May to December 2016. Lines indicate the number of hedgehogs included in the study for various age classes per month. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Overall parasite prevalence by region. Numbers indicate number of individuals, in red for hedgehogs with parasites, in blue without. JNL denotes Jutland north of the Limfjord, and JSL abbreviates Jutland south of the Limfjord. Statistically significant differences in proportions of hedgehogs with endoparasites versus hedgehogs without endoparasites were found between Zealand and Jutland south of the Limfjord (JSL), and Zealand and Falster ($p < 0.05$ in both cases). We removed seven individuals from the analyses (Jutland north of the Limfjord (n = 1), Jutland south of the Limfjord (n = 4), Lolland (n = 1), Bornholm (n = 1)), as they were the only individuals found in April and December, and four were only categorised as collected in "Summer 2016". (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

urban areas.

However, age class does influence the prevalence of endoparasites in hedgehogs. The endoparasite occurrence of 50% in juvenile hedgehogs (<1 year of age) was significantly lower compared to older animals (from 1 to 6 years, $p < 0.01$, Pearson's chi-square test) (Fig. 2). We furthermore detected a difference in the number of hedgehogs carrying endoparasites for individuals of one compared to two years of age ($p < 0.05$, Pearson's chi-square test), with one-year old individuals showing a significantly higher occurrence of 90% (n = 52/58) compared to 65% in two-year old hedgehogs (n = 20/31). Additionally, our data on the occurrence of *C. striatum* indicates that the risk of infection increased with age (≤1 year: 46% and 2–6 years: 64%).
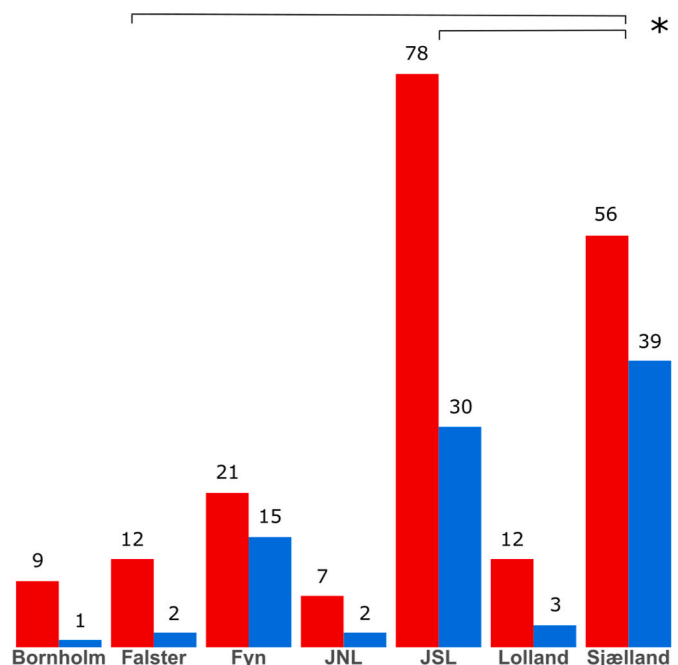
The hedgehog cadavers examined in the present study were collected from May to December 2016. Despite having observed no effect of month of death on endoparasite occurrence when analysing the whole data set by use of the GLM, we found that parasite prevalence in individuals dying in the month of September 2016 was significantly lower compared to the other months of collection, when performing pairwise comparisons of the different months of death, testing each month against the other (p < 0.001, Pearson's chi-squared) (Fig. 3).

Furthermore, when using pairwise group comparison to test the endoparasite prevalence found across the different regions of Denmark, we discovered significant differences between two groups of regions (Fig. 4). The 72% occurrence of endoparasites (n = 78/108) in individuals from Jutland south of the Limfjord (JSL) was significantly higher than the 59% (n = 56/95) in hedgehogs from Zealand (p < 0.05, Pearson's chi-square test). The 59% (n = 56/95) occurrence of endoparasites in hedgehogs from Zealand also differed significantly compared to the high occurrence of 86% (n = 12/14) in individuals from Falster (p < 0.05, Pearson's chi-square test). None of the regions showed significant differences when compared to the overall occurrence of endoparasites of 69% in the full sample set.

4. Discussion

The results demonstrate that the occurrence of endoparasites in Danish hedgehogs is high (69.0%), with *C. striatum* as the most prevalent lungworm (47.1%) and *Capillaria* spp. being most frequent in faecal samples (51.6%). The present study appears to be the first of its kind on endoparasites in hedgehogs from Denmark, leaving us unable to compare our data with historic data. Our results are generally in line with previous studies of endoparasite prevalence in European hedgehogs from other European countries, although we are aware that detection methods and sample materials may differ slightly between the studies. Endoparasite prevalence from previous research ranged from 55 to 79% (n = 38) in Finland (Rautio et al., 2016), 58–74% (n = 129) in Bayern, Germany (Esser, 1984), 64% (n = 498) in the Netherlands and UK (Reeve and Huijser, 1999), 66% (n = 42, lungworm only) in the UK (Majeed et al., 1989), up to 75% in the Czech Republic (*Capillaria* spp., n = 72) (Pfäffle et al., 2014), 90% (n = 135) in Switzerland (Egli, 2004), 91% (n = 21) in Ireland (Haigh et al., 2014b), 91% in the UK (n = 74) (Gaglio et al., 2010) and up to 93% (n = 232) in Leipzig, Germany (Laux, 1987).

The results of our study showed that the overall endoparasite prevalence was not correlated with sex, habitat type (urban/rural), time of death (season), infection with MRSA, individual genetic heterozygosity (inbreeding) (iHO), or mortality category. These findings stand in contrast to a range of previous studies investigating the possible factors influencing the prevalence and intensity of endoparasites infestation in European hedgehogs, which have shown inconsistent results. Research from Switzerland demonstrated that the parasite burdens for *C. striatum* were higher among urban-living hedgehogs than those living in rural and suburban habitats, and the author suggested a higher occurrence of slugs, snails and feeding stations in urban areas could be a possible explanation (Egli, 2004). The same study also observed that the mean intensity of *C. striatum* in hedgehogs increased from spring to autumn with concomitant decreasing body weight in the individual hedgehogs, and that the parasite burdens of *C. aerophila* was higher in spring compared to summer and autumn (Egli, 2004). In contrast, we found no overall effect on habitat type (urban/rural, e.g. prevalence of *C. striatum*: $n_{positive\ rural} = 57\%$, $n_{positive\ urban} = 51\%$) or season on the prevalence of endoparasites, apart from a lower endoparasite prevalence for individuals dying in the month of September compared to the other months between May and November 2016, which is likely explained by the higher inclusion of juvenile hedgehogs during September, having a lower parasite prevalence. A previous study investigating the negative influence of intestinal *Capillaria* spp. infections on the body condition of hedgehogs showed that increasing *Capillaria* spp. burden reduced the body condition (weight) of adult hedgehogs (Pfäffle, 2010). Pfäffle (2010) discovered that gonad sizes of the hedgehogs were positively affected by good body condition, which indicated that the parasite burden also indirectly decreased gonad size and therefore the reproductive success of the adult hedgehogs. Egli (2004) observed that the intensity of *C. aerophila* was higher in males compared to females. Haigh et al. (2014b) found that males had a significantly higher intensity of *C. striatum* than females as well as a tendency for males to have a higher burden of *C. erinacei* in the intestines and stomach than females. In contrast, Reeve and Huijser (1999) and Majeed et al. (1989) detected no interaction of sex on the prevalence of endoparasites in European hedgehogs. This is consistent with our results showing that sex did not influence the prevalence of endoparasites in the hedgehogs, with occurrences of *Capillaria* spp. in 53% of the females (n = 56/106) and 52% of the males (n = 79/153) and *Crenosoma striatum* in 59% of the females (n = 62/106) and 54% of the males (n = 83/153). When discussing the influence of age on endoparasite prevalence in hedgehogs the results from previous studies are also very contradictive, with no significant variation between the age and parasite load of hedgehogs detected (Haigh et al., 2014b), a higher occurrence in juveniles compared to adults (Rautio et al., 2016), and lastly, a lower infection rate in juvenile hedgehogs (Majeed et al., 1989; Schicht-Tinbergen, 1995). Our data on the occurrence of *C. striatum* also suggests that the risk of infection increased with age (<1 year: 46% and 2–6 years: 64%). Lastly, Reeve and Huijser (1999) discovered that the incidence of endoparasite infections varied significantly with age, where only 49% of the juveniles were infected (dependent young) compared to 85% subadults (independent juveniles in their first year of life) and 67% adults. The findings by Reeve and Huijser (1999) are similar to those of the present study, where we concluded that age did effect parasite prevalence. We observed that the endoparasite occurrence of 50% in juvenile hedgehogs (<1 year of age) was significantly lower compared to older animals (from 1 to 6 years). We furthermore detected a difference in the number of hedgehogs carrying endoparasites for individuals of one (90%, n = 52/58) compared to two years of age (65%, n = 20/31). The lower incidence of parasites seen in juvenile hedgehogs (<1 year of age) in most of the studies, including the present study, is likely explained by the shorter exposure time to vector species such as earthworms (*Capillaria* spp.), slugs and snails (*C. striatum*) due to the young age of the individuals. Additionally, the lower occurrence of parasites in hedgehog cadavers collected during the month of September observed in the present study, is likely due to the inclusion of juvenile hedgehogs in the samples (with a lower occurrence of parasites), as they usually become independent in September in Denmark (Rasmussen et al., 2019a).

We observed an occurrence of 3% coccidia (*Eimeria* and *Cystoisospora*) in the hedgehogs, all of which originated from Zealand (n = 4), Jutland south of the Limfjord (n = 1) and Lolland (n = 2). This occurrence is low compared to studies from other European countries showing infection rates of *Cystoisospora* spp. and *Eimeria* spp. between 4% (n = 2/47) in the UK (South and Haynes, 2018), 6% (n = 6/106) in Germany (Epe et al., 2004), 11% (n = 19/166) in a study with samples originating mainly from Germany (85% of samples), but also from Austria, France, Denmark, the Netherlands, Italy and Luxembourg (Pantchev et al., 2005) and 17.9% in hedgehogs from Germany (Raue et al., 2017). The differences in prevalence may be attributable to the sampling strategy which could be biased by only including sick animals which is not necessarily representable for the general population.

We identified *Cryptosporidium* spp. in faeces from 5% (n = 14) hedgehogs originating from Zealand (n = 11), Lolland (n = 1) and Jutland south of the Limfjord (n = 2). This prevalence is almost equivalent to 8% in the UK (n = 9/111) (Sangster et al., 2016), but low compared to Germany (30%, n = 56/188) (Dyachenko et al., 2010) and the Czech Republic (73%, n = 11/15) (Hofmannova et al., 2016). However, the German study was potentially biased as it was based on hedgehogs admitted to rehabilitation centres with clinical signs including diarrhoea and the study from the Czech Republic only

included 12 juveniles and 3 adults. The study design in the German and Czech Republic studies likely resulted in an overestimation of the prevalence compared to the total population. Both studies observed that juvenile hedgehogs were more frequently infected than older animals. In contrast, although only 14 animals were infected with *Cryptosporidium* in our study, an age-related infection tendency was indeed observed, with 8 out of 11 hedgehogs (with known age) infected (73%) being ≥1 year old (n = 6).

Brachylaemus spp. eggs were only identified in one individual (0.4%) from Jutland south of the Limfjord. This indicates that the occurrence of this endoparasite species is low in Denmark compared to the rest of Europe, with prevalences of 11% (n = 6/53), 41% (n = 16/39) and 53% (n = 18/34) in Italy, 16% (n = 42/256) in Yorkshire and Jersey and 55% (n = 41/74) in south Wales and in the east of England, UK (Reeve and Huijser, 1999; Poglayen et al., 2003; Gaglio et al., 2010).

The question is whether the inconsistent results found in studies on hedgehog parasitology are influenced by the variation in sample sizes or conditions of the hedgehogs, as most of the studies were based on dead individuals, with the majority dying in care at rehabilitation centres, as opposed to road-killed individuals and individuals dying of natural causes in the wild. One could argue that rehabilitated hedgehogs represent the weakest individuals in the population, as they require treatment to survive. This could potentially have caused a higher occurrence of endoparasites in the individuals examined. In our study, the samples consisted of a mixture of road-killed individuals (n = 163), individuals dying of natural causes in the wild (n = 63) as well as individuals dying at rehabilitation centres (n = 73). The varying degree of autolysis on the sampled road-killed individuals and individuals found dead in the wild could potentially have had an effect on parasite retrieval. Nevertheless, mortality category was not correlated with the occurrence of endoparasites in the present study.

We examined livers for *E. multilocularis* to determine whether the European hedgehog could be an intermediate host of this endoparasite species, which is a cause for public health concerns since it cannot be treated effectively in humans, and is therefore potentially lethal (Eckert and Deplazes, 1999). The life-cycle of *E. multilocularis* involves small rodents as intermediate hosts and wild or domestic canids as definitive hosts. Humans can act as aberrant intermediate hosts but must be infected through the ingestion of eggs excreted in the faeces of definitive hosts (Oksanen et al., 2016), which means that the potential infection of hedgehogs with this parasite is not a direct threat to humans. However, hedgehogs carrying *E. multilocularis* could potentially contribute to sustain the infection in the wild. This appears to be the first targeted investigation of *E. multilocularis* in European hedgehogs. We did not detect any *E. multilocularis* in the 94 hedgehog livers from individuals originating from all over Denmark, examined in the study. We therefore consider it unlikely that European hedgehogs are hosts of *E. multilocularis.*

Hedgehogs are not expected naturally to cross between the different geographical regions of Denmark, which are separated by sea. However, in a study on the genetics of Danish hedgehogs in which many of the same individuals were investigated (Rasmussen et al., 2020), there were some tendencies for an admixture of genes representing different geographical regions in some individuals, indicating that there must be cases of translocation of hedgehogs to other regions in Denmark, either by hedgehog carers or citizens in general. Nonetheless, research into the prevalence of MRSA in Danish hedgehogs revealed geographical differences in *spa*-types (Rasmussen et al., 2019b). The largest diversity of *spa*-types was detected in Jutland, the only region of Denmark connected to the European mainland, likely causing a flow of bacteria (and potentially also parasites) between these areas. This could explain why the only hedgehog infected with *Brachylaemus* spp. was found in an individual from Jutland south of the Limfjord. When applying pairwise group comparison to test the endoparasite occurrence found in each geographical region of Denmark against the other, we discovered that the prevalence of endoparasites in individuals from Zealand was

significantly lower than that of individuals from Jutland south of the Limfjord and Falster. However, none of the regions showed significant differences when compared to the overall parasite occurrence of hedgehogs in Denmark. The connectivity of Jutland south of the Limfjord to mainland Europe could in principle explain the higher occurrence of endoparasites found in this region compared to Zealand, which is an island. However, Falster is also an island, but the higher prevalence of endoparasites found here compared to Zealand could be the effect of a relatively low sample size from this area (n = 14). We consider it surprising that infections with *Cryptosporidium* spp. and coccidia were only detected in hedgehogs from Zealand, Lolland and Jutland south of the Limfjord, as they appear to be commonly found in previous studies from other countries, and that no cases of *Giardia* spp. were identified, as these infections have previously been diagnosed in hedgehogs with prevalences of 11.0% (Krawczyk et al., 2015) and 33.3% (Chilvers et al., 1998). However, our best explanation for this is that the island structure of Denmark presents a barrier to the spread of parasite infections in hedgehogs. The difference in parasite prevalence between regions of Denmark has previously been observed for *Angiostrongylus vasorum* infection in red foxes (Lemming et al., 2020) and *Toxoplasma gondii* in feral mink (Sengupta et al., 2021) where the prevalence was significantly higher in animals from Zealand (red fox 37.0–37.5%, mink 67.5%) compared Jutland (red fox 1.7–2.3%, mink 23.8%). The findings of our analysis on the geographical impact on hedgehog endoparasite occurrence should therefore be taken into consideration when discussing issues such as translocation of hedgehogs to prevent inbreeding, as the results stress the importance of only rehabilitating individuals in the geographical region in which they were found, and to release rehabilitated individuals as close to the original location as possible to prevent the spread of infections to other regions. Even if the individuals have received treatment for one type of infection, they may spread others.

Given that previous research into the connection between parasite burdens and genetic heterozygosity and survival in other mammal species have shown that inbreeding caused a wider range of helminth infections (Acevedo-Whitehouse et al., 2003) or a more severe and therefore deadly parasite burden (Coltman et al., 1999), we had expected to find an effect on parasite prevalence and diversity in more inbred individuals. However, there could be a number of potential explanations for the lack of connection between genetic heterozygosity and endoparasite occurrence in the present study. Firstly, it could be due to the relatively low sample size (n_{iHO} = 106 individuals, Table 3) and secondly, it may be that the Danish population of hedgehogs represented in our samples is generally highly inbred (Rasmussen et al., 2020) and to such a degree that the effect of inbreeding is present in all individuals examined and thirdly, that inbreeding is simply not a determinant of parasite infestation in European hedgehogs.

It is still common practice at several hedgehog rescue centres in Europe to treat all admitted individuals with anthelminthic drugs regardless of parasite burdens and reasons for admission. In many cases, the endoparasite species have not even been determined before treatment is administered. This should be a cause for concern given that endoparasite infections are so common, generally sublethal, and are therefore a natural part of hedgehog ecology. In a publication describing causes of death in European hedgehogs admitted to two large wildlife rehabilitation centres in Portugal, it was estimated that infectious and parasitic disease only accounted for the direct cause of death in 8.1% of the 248 hedgehogs (Garcès et al., 2020). The overtreatment and lack of targeted drug therapy could result in increased resistance among parasite species against anthelminthic drugs (Kaplan and Vidyashankar, 2012; Shalaby, 2013; Sangster et al., 2018), but could also potentially disturb a balance in the parasite-host relationship leaving the hedgehogs more exposed to new infections (Pedersen and Antonovics, 2013; Pedersen and Fenton, 2015; Carlsson et al., 2018). Furthermore, the Hygiene Hypothesis (Strachan, 1989) may also apply to hedgehogs, with the risk of causing a rise in autoimmune reactions and disorders by insisting that the many hedgehogs annually taken into care at hedgehog rehabilitation centres should

be completely parasite-free. Therefore, we strongly encourage hedgehog rehabilitators and their veterinarians only to administer anthelminthic drugs to hedgehogs diagnosed with endoparasite burdens high enough to directly compromise their health state, causing clinical signs of disease induced by endoparasites.

We conclude that, in line with previous studies of the European hedgehog throughout its range in Western Europe, that endoparasite infections are common in hedgehogs from Denmark and are a natural part of their ecology.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.10.005.

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