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Estimating parasite-condition relationships and potential health effects for fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) in Denmark

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ABSTRACT

Parasites can exert a substantial influence on the ecology of wildlife populations by altering host condition. Our objectives were to estimate single and multiparasite-condition relationships for fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) in Denmark and to assess potential health effects along the parasite burden gradient. Fallow deer hosted on average two endoparasite taxa per individual (min = 0, max = 5) while red deer carried on average five parasite taxa per individual (min = 2, max = 9). Body condition of both deer species was negatively related to presence of *Trichuris* ssp. eggs while body condition of red deer was positively related to antibodies of the protozoan *Toxoplasma gondii*. For the remaining parasite taxa (n = 12), we either found weak or no apparent association between infection and deer body condition or low prevalence levels restricted formal testing. Importantly, we detected a strong negative relationship between body condition and the sum of endoparasite taxa carried by individual hosts, a pattern that was evident in both deer species. We did not detect systemic inflammatory reactions, yet serology revealed reduced total protein and iron concentrations with increased parasite load in both deer species, likely due to maldigestion of forage or malabsorption of nutrients. Despite moderate sample sizes, our study highlights the importance of considering multiparasitism when assessing body condition impacts in deer populations. Moreover, we show how serum chemistry assays are a valuable diagnostic tool to detect subtle and sub-clinical health impacts of parasitism, even at low-level infestation.

1. Introduction

Parasites are the most abundant and diverse trophic group on the planet that live and reproduce at the expense of their hosts (Poulin et al., 2011). Parasite infection typically has direct negative effects on host body condition through loss of resources and damage to tissue (Medzhitov, 2009). However, parasites can also impact their host indirectly by stimulating costly immune responses (Schmid-Hempel, 2009) or by changing host behaviours (Heil, 2016). Through these impacts, parasites can exert a substantial influence on host fitness (Budischak et al., 2018), population dynamics of wildlife (Tompkins and Begon, 1999) and even ecosystem composition and function (Thomas and Renaud, 2007).

Despite the general notion that parasite infection negatively affects the body condition of their hosts, a recent meta-analysis revealed substantial variation in the strength and direction (i.e. positive, negative and null effects) of parasite–condition relationships (Sánchez et al., 2018). Positive parasite–condition relationships can occur when, for example, hosts that are in good condition use tolerance as a parasite defence strategy while hosts in poor condition are unable to (Medzhitov et al., 2012). Null effects, i.e. no apparent association between infection and host condition, are also common (Sánchez et al., 2018) and can occur when parasites fail to reach pathogenic threshold loads (Méthot and Alizon, 2014) or when hosts are asymptomatic carriers (Ogutu et al., 2010). Most studies that estimate parasite-condition relationships focus on single parasite species detected in the host, yet multiparasitism (i.e. the concurrent infestation of a single host with two or more parasite species), has long been considered the rule rather than the exception, especially in wildlife (Petney and Andrews, 1998). Therefore, host

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condition is likely influenced by cumulative effects and synergistic interactions between multiple parasite species (Vaumourin et al., 2015).

Deer species have expanded their range and increased in abundance across much of Europe and North America over the last few decades (Côte et al., 2004; Linnell et al., 2020). Changes in deer density impacts the spread of parasites across landscapes (Medlock et al., 2013), with consequences for disease transmission risk to livestock and among wild ungulates (Sorensen et al., 2014) and humans (Kilpatrick et al., 2014). The prevalence and diversity of parasites in European deer species are generally well described (Figueiredo et al., 2020; Gray et al., 2021; Santín-Durán et al., 2004). In addition, studies on parasite-condition relationships in deer are abundant, though largely focused on single parasite-host effects. For instance, treating wild reindeer (*Rangifer tarandus platyrhynchus*) in Svalbard with anthelmintics revealed negative relationships between infection load of the gastrointestinal nematode *Ostertagia gruehneri* and reindeer body condition and fecundity (Stien et al., 2002). For red deer (*Cervus elaphus*) in Spain, counts of the nematode *Elaphostrongylus cervi* negatively correlated with body condition of males (Vicente et al., 2007). Similarly, for red deer on the Isle of Rum, Scotland, infection with *Ostertagia* spp. had a negative effect on carcass weights of males and non-lactating females (Irvine et al., 2006). Because deer are hosts to a wide-variety of parasite species, they represent an ideal study system to evaluate the influence of multi-parasitism on host condition, which may manifest itself even at low infection levels due to cumulative impacts (Bordes and Morand, 2009).

The ecological impact of parasites on deer in Denmark has, thus far, not been investigated in detail with previous studies focusing primarily on parasite occurrence (Guildal, 1966; Nielsen et al., 2022; Stensgaard et al., 2022). Here, we estimated parasite-condition relationships for fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) sampled across four study sites in Denmark. Prevalence and intensity of endoparasite infection were quantified and effects on body condition were subsequently estimated for each endoparasite taxa separately and as a function of the total number of parasite taxa detected within individual deer. Potential health or nutritional impacts (e.g. inflammation, changes in total protein and serum globulin levels) along the parasite-condition gradients were evaluated using serum chemistry assays.

2. Material and methods

2.1. Study sites and deer sampling

This study included samples of 20 fallow deer (9 females and 11 males) and 21 red deer (9 females and 12 males), all of which were shot in collaboration with certified local hunters as part of the annual deer regulation at four different locations in Denmark (Fig. 1, Table S1 in Supporting Information). Three of the study sites were fenced estates including Dyrehaven (1000 ha), with estimated spring (before calving) densities of 23 red deer per 100 ha and 115 fallow deer per 100 ha. Tofte (4000 ha) had an estimated spring density of 10 red deer per 100 ha (no fallow deer present), and Høstemark (570 ha) had an estimated spring density of 25 red deer per 100 ha (no fallow deer were present in this study site). The study area at Gyldensteen strand was the only unfenced estate with fallow deer being the most common deer species. Only few red deer are present at Gyldensteen strand and none were included in this study. No natural predators were present in any of the study areas. All deer included in this study were sampled post-mortem between November 8th and December 12th in 2021, which is within the official hunting season in Denmark. Individual deer were classified into one of three age classes based on body- and/or antler size: juveniles (n = 2 fallow deer, n = 12 red deer), sub-adults (n = 12 fallow deer, n = 5 red deer), and adults (n = 6 fallow deer, n = 4 red deer).

Two different metrics of deer condition were estimated: one qualitative measure that assesses and scores the amount of muscle and subcutaneous fat at multiple locations on the body (body condition scores (BCS)) and one quantitative metric using mass-length ratios (MLR). BCSs

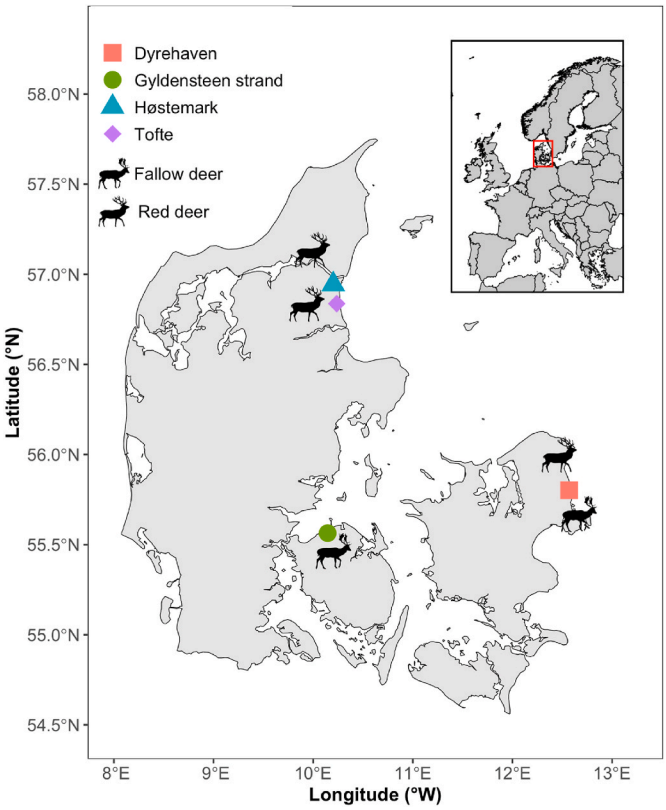

Fig. 1. Map of Denmark and its location within Europe (see red square in inset) showing the distribution of the four study sites (Dyrehaven – pink square; Gyldensteen strand – green circle, Høstemark – blue triangle; Tofte – purple diamond) where fallow deer (*Dama dama*; n = 20) and/or red deer (*Cervus elaphus*; n = 21) were sampled. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were estimated by visually inspecting and palpating ribs, spine, hips and the base of the tail bone to assess muscle mass and fat deposits and scoring the overall condition between 1, representing 'very poor condition' (cachexia or emaciated), and 5, representing 'very good condition' (fat) using half-unit increments (*sensu*: Audige et al., 1998). BCS of all deer were assessed by the same professional wildlife veterinarian to limit observer bias and maintain consistency. MLRs were calculated by dividing an individual's carcass weight (in kg after removal of organs) by body length (in cm and measured from the tip of the nose to the base of the tail bone). A complete overview of the body condition measurements is provided in Table S2 in Supporting Information.

From each individual deer, the liver was collected and placed in a Ziplock bag, and faecal pellets (>25 g) were collected from the rectum and transferred into a plastic container, both stored at 4-6 °C. Blood (60 ml) was drawn from each deer within 60 min after it was shot by making a small incision in the jugular vein using a sterile stainless-steel scalpel. Blood was collected in small plastic cups and directly pipetted into EDTA anticoagulant tubes (3 × 10 ml) and serum separator tubes (3 × 10 ml), which were placed in a portable electric cooler box and brought to the slaughterhouse. There, the blood in the serum separator tubes was centrifuged for 10 min at 2561×*g* after which the serum was pipetted into 5 ml cryotubes and kept cool at 4-6 °C. Within 24 h, the cooled serum samples were delivered to the Veterinary Diagnostic Laboratory, University of Copenhagen, Denmark for serum chemistry analyses (described below). Also, within 24 h, faecal samples, livers, serum samples and whole blood in the EDTA anticoagulant tubes were brought to the Centre for Diagnostics, Technical University of Denmark for parasitic, DNA and antibody analyses (described below).

2.2. Parasite analyses

Samples (faeces, whole blood, serum and liver) were screened for different types of endoparasites (helminth's and protozoa: Table 1) that have previously been observed in deer from Denmark (Guildal, 1966; Stensgaard et al., 2022) or that are known to occur in domestic and other wildlife species in Denmark (Laforet et al., 2019; Petersen et al., 2015, 2020; Rasmussen et al., 2021; Takeuchi-Storm et al., 2018). Parasites were identified to the genus level, unless otherwise stated.

Faecal samples from each individual deer were divided into three sub-samples. The first set of sub-samples (4 g) were analysed for eggs of Strongyles, *Trichuris* spp., *Strongyloides* spp., *Capillaria* spp., *Nematodirus* spp., and oocysts of *Eimeria* spp. using a modified McMaster technique with a sensitivity of 5 eggs/oocysts per g faeces (*sensu*: Petersen et al., 2020). Counts of eggs per gram of faeces (EPG) and *Eimeria* spp. oocysts per gram of faeces (OPG) were categorized into excretion levels of low (<100 EPG/OPG), moderate (100–1000 EPG/OPG) and high (>1000 EPG/OPG) based on the categorization used in the laboratory, where this method is accredited according to DANAK (Danish Accreditation Fund). The second set of faecal sub-samples (1 g) were used to isolate and enumerate *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts using immunofluorescent staining (IFA) following isolation as previously described by Petersen et al. (2015). Counts of *Giardia* cysts and *Cryptosporidium* oocysts in faeces were categorized into levels of low (*Giardia* <1000 cysts/g; *Cryptosporidium* <100.000 oocysts/g), moderate (*Giardia* 1000–5000 cysts/g; *Cryptosporidium* 100.000–1.000.000 oocysts/g) and high (>5000 cysts/g; *Cryptosporidium* >1.000.000 oocysts/g) based on the categorization used in the laboratory. The third set of faecal sub-samples (20 g) were used to screen for lungworm larvae using Baermann examination. Here, faecal pellets were placed in 20-thread gauze, immersed in tap water in a Baermann glass and incubated at room temperature for approx. 24 h. Subsequently, aliquots of approximately 2 ml containing the larvae were removed from the bottom of the Baermann glass by a Pasteur pipette and transferred to a counting chamber. The sediment was microscopically examined for first stage larvae (L1) of lungworm. Larvae detected in our samples were morphologically identified as *Dictyocaulus* spp. and *Muellerius* spp. and categorized into levels of low (1–5 larvae), moderate (5–25 larvae) and high (>25 larvae) excretion based on the categorization used in the laboratory.

Whole blood extracted from deer was tested for presence of DNA from *Babesia divergens* using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany) following the manufacturer's instructions. Amplification of the 70 kDa heat shock protein (*Hsp70*) gene was performed by PCR using primers previously described by Sprong et al. (2019). Reactions were performed in a total volume of 23 µl each containing 1 x buffer, 1.5 mM $MgCl_2$, 250 µM of each dNTP, 0.2 µM of each primer and 1-unit Amplitaq gold polymerase (Applied Biosystems, Massachusetts, USA). PCR conditions were: a denaturation step at 95 °C for 3 min followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C and 90 s at 72 °C, with a final extension step of 10 min at 72 °C. The PCR products were run on 1% agarose gel with ethidium bromide to control that PCR amplicons generated DNA fragments of expected size.

Serum samples were examined in duplicate for IgG antibodies against *Toxoplasma gondii* using the ID Screen Toxoplasmosis Indirect Multi-species ELISA kit (ID.vet, France) as also used in Stensgaard et al. (2022). The optical density (OD) was read at 450 nm. 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.

Finally, the liver of each individual deer was sliced into approx. 1.0 cm wide strips. Bile ducts in each strip were cut open and examined macroscopically for flukes of *Dicrocoelium dendriticum* and *Fasciola hepatica* and for any pathological changes (*sensu*: Mazeri et al., 2016).

2.3. Serum chemistry analyses

The analyses of the total serum protein concentration (biuret reaction), albumin (bromocresol green reaction) and the acute phase reactant iron (ferrozine reaction) were conducted using an automated spectrophotometrical analyser (Atellica®Solution; Siemens Healthineers, Germany). Analysis of the acute phase protein Serum Amyloid A (SAA) was done using an immunoturbidometric assay (SAA-LZ; Eiken Chemical; ADVIA, 1800; Siemens Healthineers, Germany). This assay has previously been described for use in white-tailed deer (Cray et al., 2019) and other mammals (Christensen et al., 2012; Jacobsen et al., 2006). Serum protein electrophoresis (SPE) was conducted using capillary electrophoresis (Minicap, Sebia Italia Srl, Italy) according to the manufacturer's instructions. Fraction limits for five protein fractions (albumin, alpha 1, alpha 2, beta, and gamma globulins) were based on conventions for mammalian species and performed by the same operator for all samples. Absolute values were determined by multiplying the total protein by the percent of each fraction identified by SPE. The analytical machines and assays were all part of daily and weekly internal laboratory quality controls and quarterly external quality controls. See Table S3 in Supporting Information for an overview of the values measured during the serum chemistry analyses.

Table 1
Overview of the presence, absence, and prevalence of endoparasite taxa screened for in samples taken from individual fallow deer (*Dama dama*; n = 20) and red deer (*Cervus elaphus*; n = 21) in Denmark.

Endoparasite taxa	Type	Region of infection	Sample screened	Fallow deer			Red deer		
				Negative (n)	Positive (n)	Prevalence (%)	Negative (n)	Positive (n)	Prevalence (%)
Capillaria spp.	Helminth	Intestines	Faeces	18	2	10.0	10	11	52.4
Dicrocoelium dendriticum	Helminth	Biliary system	Liver	20	0	0.0	21	0	0.0
Dictyocaulus spp.	Helminth	Lungs	Faeces	14	6	30.0	0	21	100.0
Fasciola hepatica	Helminth	Liver	Liver	20	0	0.0	21	0	0.0
Muellerius spp.	Helminth	Lungs	Faeces	20	0	0.0	12	9	42.9
Nematodirus spp.	Helminth	Intestines	Faeces	19	1	5.0	19	2	9.5
Strongyles	Helminth	Intestines	Faeces	5	15	75.0	1	20	95.2
Strongyloides spp.	Helminth	Intestines	Faeces	11	9	45.0	15	6	28.6
Trichuris spp.	Helminth	Intestines	Faeces	18	2	10.0	17	4	19.0
Babesia divergens	Protozoa	Red blood cells	Whole blood	17	3	15.0	13	8	38.1
Cryptosporidium spp.	Protozoa	Intestines	Faeces	20	0	0.0	21	0	0.0
Eimeria spp.	Protozoa	Intestines	Faeces	14	6	30.0	6	15	71.4
Giardia spp.	Protozoa	Intestines	Faeces	20	0	0.0	20	1	4.8
Toxoplasma gondii	Protozoa	Tissue	Serum	19	1	5.0	19	2	9.5

2.4. Statistical analyses

Single and multiparasite-condition relationships for deer were estimated at the population level using generalized linear mixed-effects models through the library 'glmmTMB' (Brooks et al., 2017) in the statistical software package R (R Development Core Team, 2023).

First, models were constructed considering each of the detected parasite taxa separately. Either the variable BCS (gaussian family) or MLR (beta family and logit link function) was fitted as the response variable and 'presence (1) or absence (0)' of specific parasite taxa detected in deer as the predictor variable. Separate models were developed for each condition metric and deer-parasite combination but only if specific parasite taxa were either present or absent in ≥2 individual deer. We also included 'days since the start of the sampling process' as a predictor variable in all models to account for any changes in BCS or MLR of deer over time for reasons other than parasitic burden. Preliminary regression analyses revealed that EPG and OPG of the various endoparasite taxa increased slightly over time within samples (i.e. 'days since the start of the sampling process') but no statistical relationships were detected ($p > 0.05$) and, therefore, not considered in further analyses. We then tested for differences in BCS and MLR between the different sexes, age classes and study sites for each deer separately using either ANOVA or t-tests. Due to limited sample size and risk of model overfitting, we did not consider these variables in our regression analyses as fixed effects but instead fitted them as nested random intercepts to indirectly account for any ecological effect and for unbalanced data across factor levels (Table S4 in Supporting Information).

Next, the 'sum of endoparasite taxa' detected in each individual deer was calculated. This variable was fitted as the predictor variable in a second set of generalized linear mixed effects models for each deer species separately and with either 'BCS' or 'MLR' as the response variable. As above, 'days since the start of the sampling process' was also included as a predictor variable and the variables: 'sex', 'age class' and 'study site' were fitted as nested random effects. We fitted a separate model with the same random structure but the interaction between 'sum of parasite taxa' and 'deer species' to assess if the strength and direction of the parasite-condition effects differed between fallow deer and red deer. We also calculated the marginal R^2 to assess the proportion of variance explained by the fixed effects (Nakagawa and Schielzeth, 2013). As in the single parasite analyses above, we also tested for differences in total parasite load (sum of endoparasite taxa) between sexes, age classes and study sites for each deer species separately using either ANOVA or t-tests. Again, due to low sample size, we did not consider these variables as fixed effects in our regression analyses but accounted for any ecological effect and for unbalanced data design by fitting them as nested random effects only (Table S4 in Supporting Information).

To test for any changes in serum chemistry along the multiparasitism-condition gradient, we applied k-means cluster analyses to categorize individuals into groups based on their (dis)similarity in total parasite burden and BCS or MLR. The k-means cluster analyses were performed for each deer species and condition metric separately in the R library 'factoextra' (Kassambara and Mundt, 2020). Because k-means cluster analysis is sensitive to the distribution and range in values of the input data (Steinley, 2006), we scaled and standardized the variables 'BCS', 'MLR' and 'sum of parasite taxa'. K-means cluster analysis iteratively classifies the input data into an increasingly larger number of groups (here 1–10) and we selected the optimal number of groups by identifying the cluster value with the highest coefficient value using silhouette width analysis (Lengyel and Botta-Dukát, 2019). Depending on the number of clusters identified, differences in serum parameter values between clusters were tested using either the Mann-Whitney *U* test (when the number of clusters was two) or Kruskal-Wallis Rank Sum Test, followed by Dunn's test for multiple comparisons (when the number of clusters was three or more). We relied on non-parametric tests here as assumptions of normality (Shapiro-Wilks Test) and equal variance (Levene's F Test) were not met.

3. Results

3.1. Endoparasite occurrence, prevalence and intensity

A total of 11 endoparasite taxa (out of 14 taxa screened for) were detected in deer, including seven helminths and four protozoans (Table 1). Prevalence levels varied between deer species and across parasite taxa (Table 1), and excretion levels of gastrointestinal parasites were low to moderate (Table 2). For fallow deer, prevalence was highest for the gastrointestinal helminths Strongyles (75%) and *Strongyloides* spp. (45%). For red deer, all 21 individuals tested positive for the lungworm *Dictyocaulus* spp. and nine red deer hosted the lungworm *Muellerius* spp. with a prevalence of 42.9%. The tick-borne protozoal parasite *B. divergens* was detected in both deer species and antibodies against *T. gondii* were found in one fallow deer and in two red deer. Liver flukes and the protozoan *Cryptosporidium* spp. were not detected in any of the deer sampled.

Multiparasitism in deer was common (Fig. 2) as we detected an average of two parasite taxa per individual fallow deer (min = 0, max = 5) and an average of five parasite taxa per individual red deer (min = 2, max = 9). Out of the 20 fallow deer tested, 13 hosted two or more endoparasites taxa, six individuals carried one parasite taxa and only one individual did not carry any of the endoparasites screened for in this study (Fig. 2). In contrast, all 21 red deer hosted two or more endoparasite taxa with one individual hosting a total of nine different parasite taxa (Fig. 2).

3.2. Parasite-body condition relationships

The average BCS of red deer and fallow deer differed between study sites ($p = 0.011$ and $p = 0.040$ respectively; Table S5 in Supporting Information), but not between sex ($p = 0.122$ and $p = 0.342$) and age classes ($p = 0.287$ and $p = 0.077$). The average MLR of red deer differed between study sites ($p = 0.024$), sex (0.015), and age classes ($p = 0.007$). For fallow deer, the average MLR did not differ between study sites ($p = 0.124$) or sex ($p = 0.256$), but a significant difference was evident between age classes ($p = 0.002$).

Relating BCS of fallow deer to single endoparasite taxa (Fig. 3a) revealed that individuals infected with *Trichuris* spp. ($p = 0.0123$) and *Capillaria* spp. ($p = 0.0128$) had lower BSC compared to individuals that did not. The remaining endoparasite taxa did not impact BCS of fallow deer ($p > 0.05$), though we were unable to test for an effect for *Nematodirus* spp. and *T. gondii* due to low prevalence (Table 1). BCS of red deer individuals was significantly lower with presence of *Nematodirus* spp. ($p = 0.007$) and *Trichuris* spp. ($p = 0.029$) (Fig. 3b), and we found a marginally significant positive effect on red deer BCS when individuals tested positive for antibodies of *T. gondii* ($p = 0.069$). For the remaining parasite taxa, we did not find an apparent association with red deer BCS ($p > 0.05$), though we were unable to test for effects for *Giardia* spp. due

Table 2
Excretion levels of gastrointestinal and respiratory parasites detected in fallow deer (*Dama dama*; n = 20) and red deer (*Cervus elaphus*; n = 21) in Denmark. Excretion levels were based on threshold levels of counts of larvae, eggs and (oo) cysts per gram of faeces (see text for details).

Endoparasite taxa	Fallow deer			Red deer		
	Low	Moderate	High	Low	Moderate	High
Capillaria spp.	2	0	0	11	0	0
Dictyocaulus spp.	3	1	2	5	5	11
Muellerius spp.	0	0	0	2	0	7
Nematodirus spp.	1	0	0	2	0	0
Strongyles	14	1	0	18	2	0
Strongyloides spp.	9	0	0	6	0	0
Trichuris spp.	1	1	0	3	1	0
Eimeria spp.	6	0	0	12	3	0
Giardia spp.	0	0	0	1	0	0

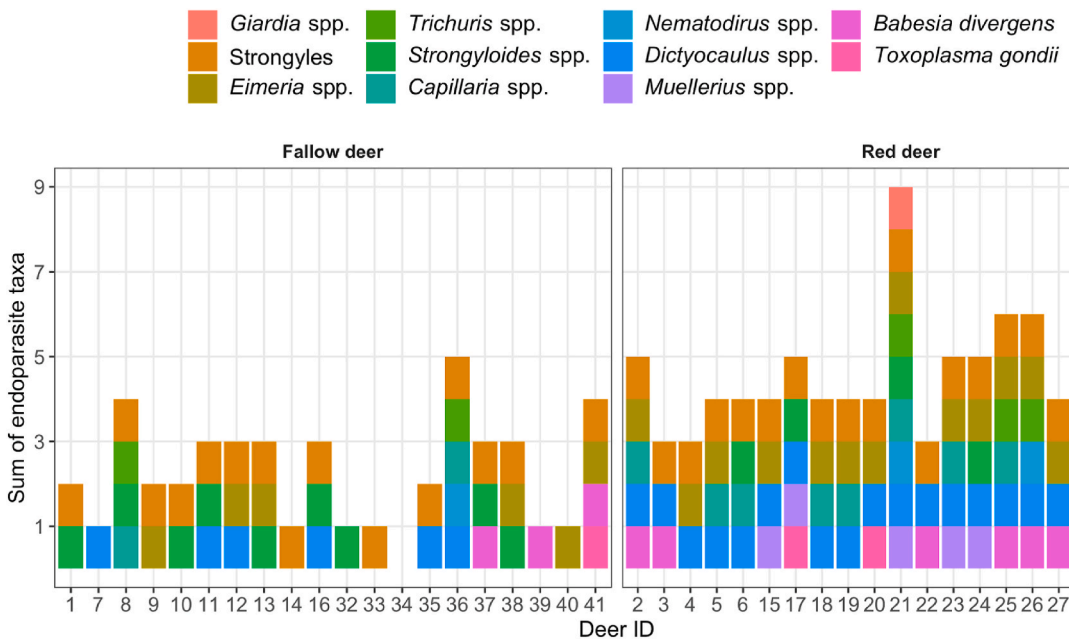

Fig. 2. Barplots showing the sum of endoparasite taxa detected in each individual fallow deer (*Dama dama*; n = 20) and red deer (*Cervus elaphus*; n = 21) sampled across four areas in Denmark. Note that individuals were also screened for presence of *Cryptosporidium* spp. and two species of liver flukes (Table 1), but none were detected and thus are not placed here. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to low prevalence and for *Dictyocaulus* spp. due to 100% prevalence (Table 1). Relating the condition metric MLR to each endoparasite taxa separately (Fig. S1 in Supporting Information) produced similar results as for BCS, except that here we did not detect a significant difference in MLR between red deer individuals that hosted *Trichuris* spp. and those that did not. Also similar to the BCS result, we found a marginally significant positive effect on red deer MLR when individuals tested positive for antibodies of *T. gondii* ($p = 0.066$).

Regressing BCS against the total number of endoparasite taxa detected across individual deer revealed strongly negative trends in both fallow deer and red deer (Fig. 4). The model predicted that an increase of one endoparasite taxa in fallow deer resulted in a decline of the BCS by 0.28 (SD: 0.09, $p = 0.013$), while for red deer an increase of one endoparasite taxa resulted in a decline of the BCS by 0.15 (SD: 0.06, $p = 0.04$). The interaction between the total number of endoparasite taxa and deer species was not significant ($p = 0.267$), suggesting that the strength of the effect did not differ between deer species. The parameter 'days since the start of the sampling process' had no effect on BCS of deer ($p > 0.05$ in all models), suggesting that the duration of our sampling period did not influence the parasite-condition relationship. The BCS model had a marginal R^2 value of 0.614. Similar results were found when regressing MLR against the total number of endoparasite taxa with strong negative relationships for both fallow deer and red deer (Fig. S2 in Supporting Information). The MLR model had a marginal R^2 value of 0.660.

The sum of endoparasite taxa detected in red deer and fallow deer differed between study sites (Fig. S3 in Supporting Information). We did not detect differences in the sum of endoparasite taxa between male and female fallow deer, but we did detect significantly higher endoparasite load in female compared to male red deer (Fig. S3 in Supporting Information). Juvenile fallow deer had higher endoparasite burden than adults and sub adults, but no differences in sum of endoparasite taxa between age classes were detected in red deer (Fig. S3 in Supporting Information).

3.3. K-means clustering and serum chemistry analyses

The k-means cluster analyses suggested that fallow deer individuals could best be classified into two groups, while red deer individuals could best be classified into four groups (Fig. S4 in Supporting Information) as a function of their BCS and total parasite load (Fig. 4). The same number of groups were selected based on k-means cluster analyses as a function of their MLR and total parasite load (Fig. S5 in Supporting Information).

Significant differences in serum chemistry between clusters based on BCS were detected for some of the parameters (Fig. 5). For both deer species, clusters of individuals with low BSC and high parasite burden had lower levels of iron (μmol L^{-1}) and total protein (g L^{-1}) in their serum than individuals with high BSC and low parasite burden. Similar differences in iron (μmol L^{-1}) and total protein (g L^{-1}) concentrations were detected between clusters based on MLR (Fig. S6 in Supporting Information). In fallow deer, gamma globulin levels (g L^{-1}) in serum of individuals with low BSC and high parasite burden were lower than of individuals with high BSC and low parasite burden. In red deer, the albumin-to-globulin ratio in serum of individuals with low BSC and high parasite burden were lower than of individuals with high BSC and low parasite burden (Fig. 5). We did not detect significant differences in the acute phase protein Serum Amyloid A (mg L^{-1}) levels between clusters ($p > 0.05$) or in any of the other protein electrophoresis fractions (albumin (g L^{-1}), alpha 1 (g L^{-1}), alpha 2 (g L^{-1}), beta (g L^{-1}), and gamma (g L^{-1}: for red deer)).

4. Discussion

This study provides the first overview of endoparasite infection, and the impacts on body condition, in two large deer species in Denmark. We found four particularly important results in this context.

First, when testing each endoparasite taxa separately, we frequently found no apparent association between infection and deer condition and, moreover, a positive effect of the zoonotic protozoan *T. gondii* in red deer. These findings follow the traditional view that the relationship between native, common parasites and deer is one of coexistence rather than harmful impact (Sugár, 1997). However, null effects can occur when parasites fail to reach pathogenic threshold loads (Sánchez et al., 2018). This was likely the case in our study as excretion levels were low for most of the gastrointestinal parasites detected. The underlying reason for the weakly positive correlation between *T. gondii* on body

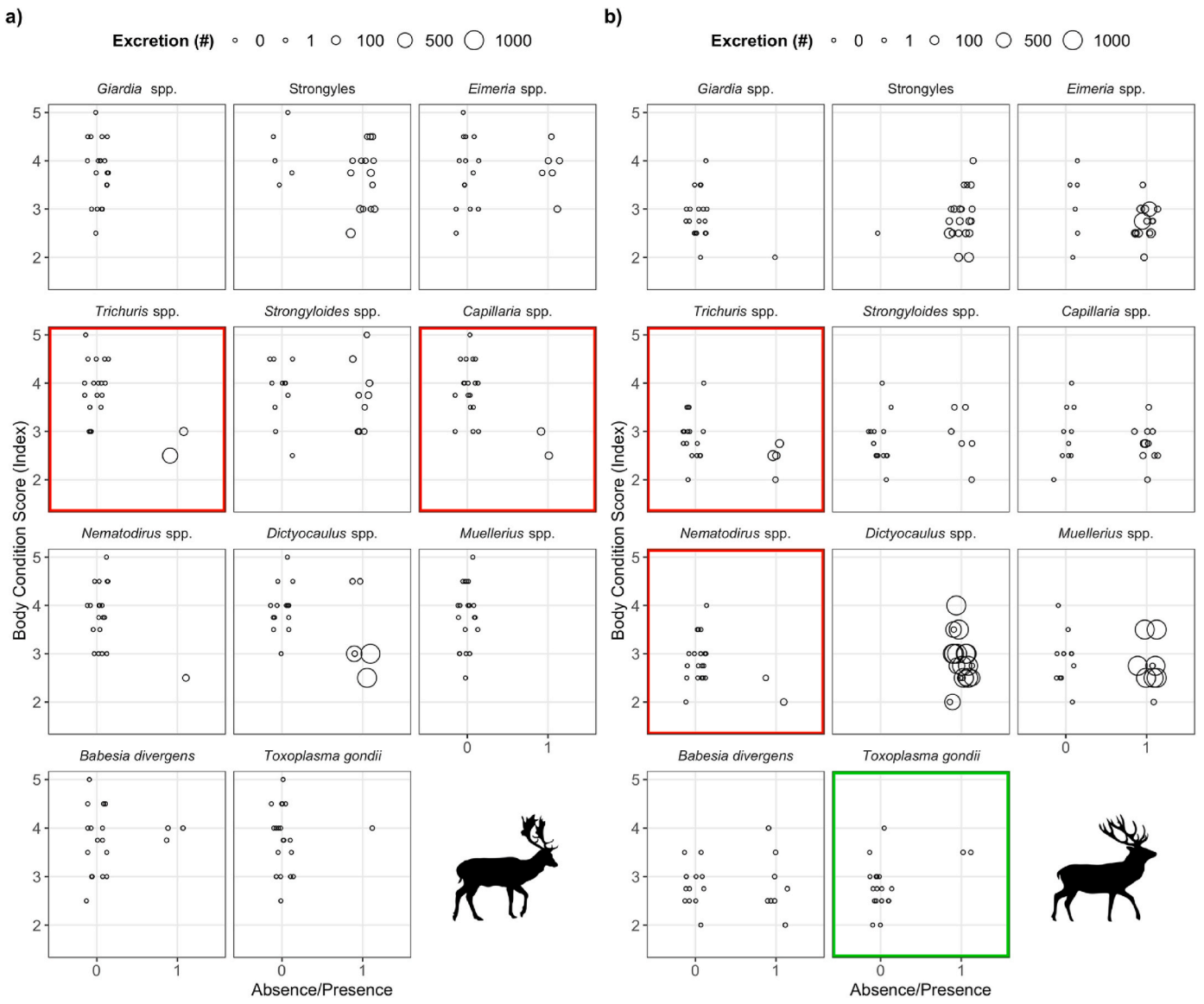

Fig. 3. Body condition scores (BCS) of individual a) fallow deer (*Dama dama*) and b) red deer (*Cervus elaphus*) in relation to the absence (0) and presence (1) of 11 endoparasite taxa. Panels outlined in red indicate a significantly ($p < 0.05$) lower BCS with presence of that endoparasite taxon, while panels outlined in green indicate a trend ($p < 0.1$) for higher BCS with presence of that endoparasite taxon. Size of presence data points is scaled relative to the number of larvae, eggs or (oo) cyst excreted per gram faeces except for *Babesia divergens* and *Toxoplasma gondii*, which were either present (1) or absent (0). Note that all individuals were also screened for presence of *Cryptosporidium* spp. and two species of liver flukes (Table 1), but none were detected and thus not plotted here. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

condition in red deer is unclear and deserves further attention, especially as sero-prevalence is high in wild cervids in Denmark and represents a risk factor for transmission to humans via consumption of raw or undercooked deer meat (Stensgaard et al., 2022).

Second, when considering the sum of endoparasite taxa present in individual hosts, we found strong negative effects on body condition, a relationship that was apparent in both deer species. These results highlight that adopting a multiparasite approach is important when assessing parasite-condition relationships (Oliver-Guimerá et al., 2017; Vaumourin et al., 2015) as it incorporates potential cumulative costs and synergistic interactions between all detected parasites (Carrau et al., 2021).

Third, we found reduced total protein and iron concentrations in the serum of both deer species with increased parasitic load and reduced body condition (k-means cluster analyses). As we did not detect any inflammatory reactions in serum samples or observed internal bleeding in deer during the sampling process, loss of iron is unlikely due to tissue damage. Instead these findings may suggest that multiparasite burden can lead to reduced intake or maldigestion of forage or malabsorption of nutrients, which can occur even at low-level infestation rates (Gunn and Irvine, 2003). Thus, the use of serum chemistry assays appears to be a valuable diagnostic tool to detect subtle and sub-clinical impacts of parasitism on deer health and nutrition and should be applied more systematically in wildlife studies.

Fourth, the negative relationships between parasite burden and deer condition reported here appeared independent of the condition metric used, which is a known source of uncertainty in the study of parasite-condition relationships (Sánchez et al., 2018; Wilder et al., 2016). We applied two metrics of deer condition: one qualitative measure that assesses body fat and muscle mass at multiple locations (BCS) and one quantitative metric based on mass-length ratios (MLR). Application of several condition metrics in the same study is advised as it can highlight the need for further and more detailed investigations when inconsistent results are found. In our case, however, the results were similar and thus

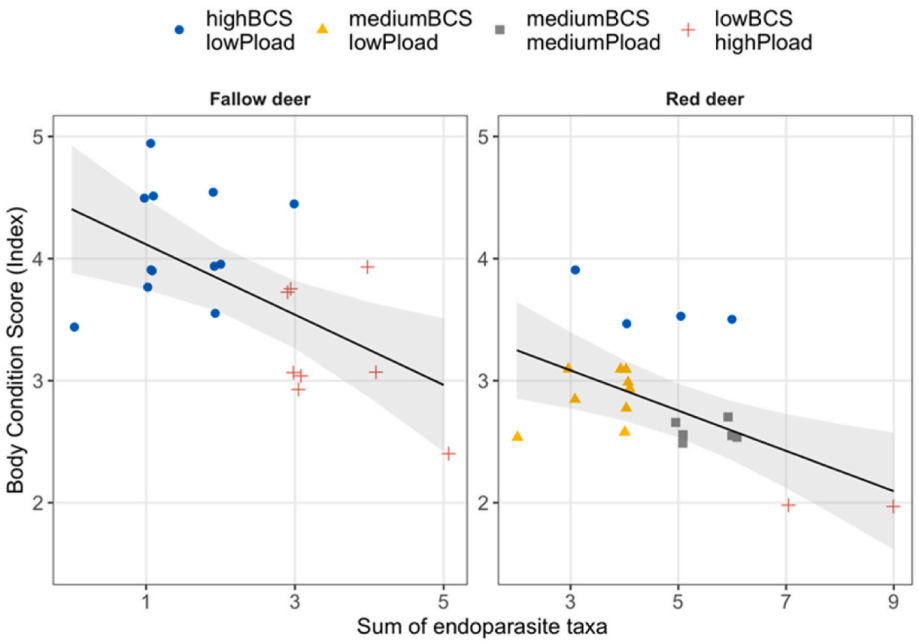

Fig. 4. Body condition scores (BCS) of fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) individuals in relation to the sum of endoparasite taxa detected. Black solid lines show the mean predicted relation for each deer species and grey areas are the 95% confidence intervals. Colours and shapes of points indicate the classification of individuals into groups (two for fallow deer and four for red deer) based on their (dis)similarity in total endoparasite load (Pload) and BCS as determined by k-means cluster analyses (see text for details). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

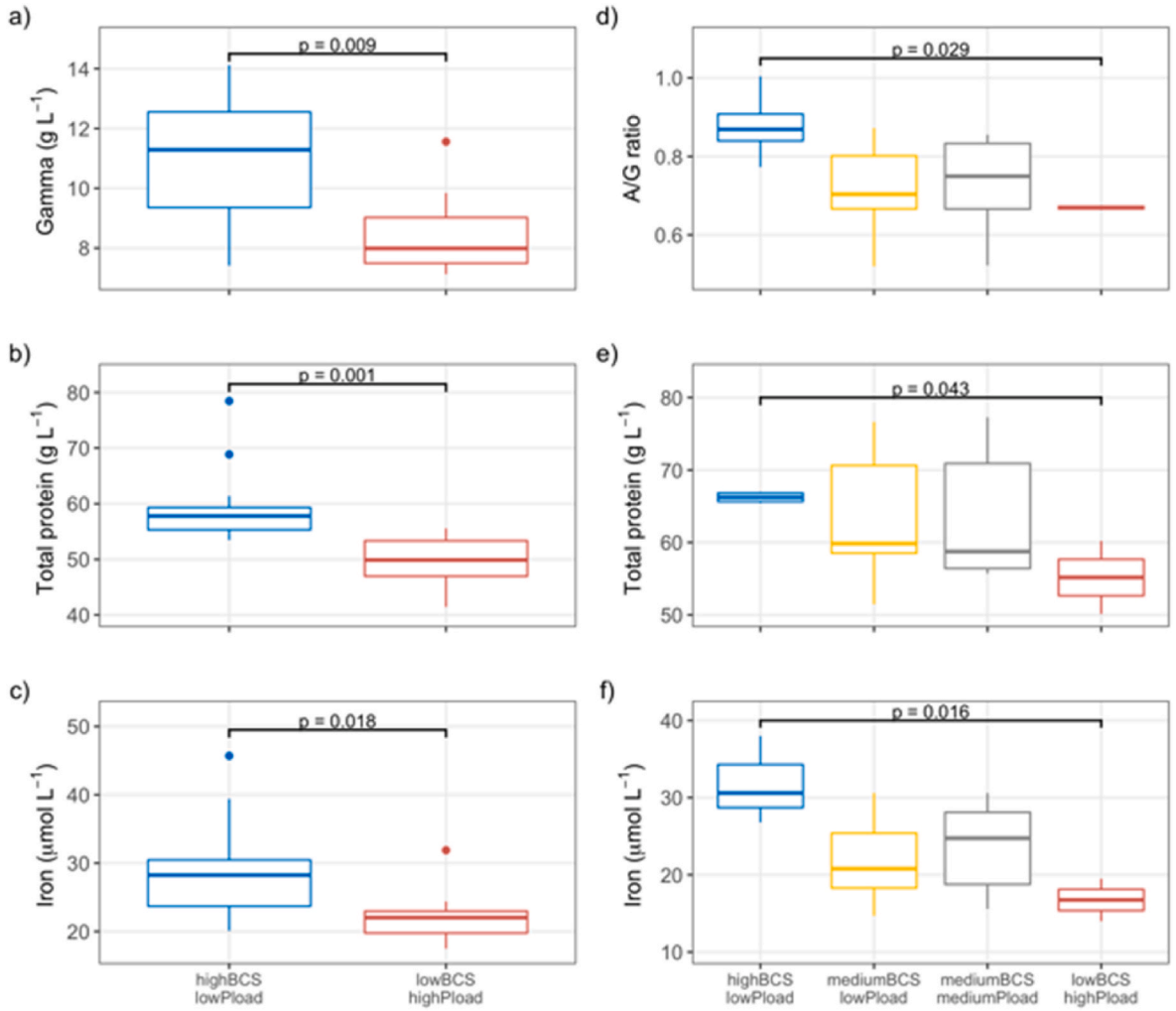

Fig. 5. Differences in serum chemistry between groups of individuals with varying body condition scores (BCS) and endoparasite load (Pload) for fallow deer (*Dama dama*; a,b,c) and red deer (*Cervus elaphus*; d,e,f). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

appeared robust to the condition metric applied.

Although multiparasitism is considered common in wildlife (Petney and Andrews, 1998), most studies focus on single parasite-host interactions (Carrau et al., 2021; but see: Oliver-Guimerá et al., 2017). It has been suggested that we need to move past the one parasite paradigm and adopt a multiparasite perspective (Vaumourin et al., 2015). We would argue that using both approaches in parallel is preferred as they provide complimentary insights into host-parasite interactions. Indeed, analyses of single parasite effects on host condition can quickly identify parasite taxa with the greatest cost to the host. In our study, the whipworm *Trichuris* spp. had a negative impact on the body condition of both deer species. By combining multiparasite-condition relationships with serum chemistry analyses, we were able to detect changes in nutritional status of deer host as a function of total parasitic load. This finding would have been missed in a traditional single parasite-host analyses.

Differences in parasite load and body condition are often detected between sex and age classes (Oliver-Guimerá et al., 2017) as previously described for red deer (Irvine et al., 2006; Vicente et al., 2007) and other ungulates (Filip-Hutsch et al., 2020; Turner and Getz, 2010). Although we did not find strong age or sex-related differences in BCS in our data (Table S5 in Supporting Information), we did detect an age class effect in fallow deer parasite load with juvenile fallow deer having a higher sum of endoparasite taxa than adults and sub-adults (Fig. S3 in Supporting Information). This finding is to be expected given that age-dependent parasitism in wild vertebrates is common, with higher infection intensities typically observed in the young and then declining with age (Turner and Getz, 2010; Wilson et al., 2004). Juvenile red deer also had higher sum of endoparasite taxa than adults and sub-adults, but this difference was not statistically significant (Fig. S3 in Supporting Information). Instead, female red deer had a substantially higher sum of endoparasite taxa than male red deer (Fig. S3 in Supporting Information), which contrasts with the general notion of higher parasite burdens in males than females in polygynous mammals (Moore and Wilson, 2002). The sum of endoparasite taxa also differed between study sites for both deer species (Fig. S3 in Supporting Information). The underlying drivers of these age-, sex- and site-related differences in parasite load and body condition remain to be investigated further but may be linked to variation in local deer densities or management practises such as culling effort and supplementary feeding, both of which are known to positively relate to parasite load in deer (Hines et al., 2007; Vicente et al., 2007). In our case, parasite load in fallow deer was higher at Dyrehaven than at Gyldensteen strand. Dyrehaven is a fenced estate where supplementary feeding of deer is ongoing and where densities are suspected to be higher than at Gyldensteen strand, a non-fenced area where deer roam freely without supplementary feeding. Similarly, red deer at Høstemark had the highest parasite load compared to the other two sites, which is an area with the highest red deer densities of all sites and where supplementary feeding is also ongoing. Given the moderate sample size of 20 fallow deer and 21 red deer in our study it is challenging to provide robust inference of the underlying drivers of regional variation in parasite load and, as such, we recommend future studies to evaluate the importance of these parameters explicitly. Finally, a common caveat in parasite-wildlife studies, including ours, is that hosts are screened for a non-exhaustive number of endoparasites. While we focused our efforts to endoparasites known to occur in deer from Denmark (Guildal, 1966; Stensgaard et al., 2022), it is likely that some parasite taxa went undetected. To increase the detection probability of gastrointestinal parasites carried by deer hosts, we foresee great value in the application of metabarcoding with (next-generation) DNA sequencing as recently applied to deer in France (Beaumelle et al., 2021) and moose in Poland (Świsłocka et al., 2020). Despite these caveats, we detected strongly negative parasite-condition relationships in two large deer species with implications for nutritional status even though none of the deer sampled were in poor body condition (BCS <2) or showed clinical signs of disease.

5. Conclusion

Results of this study clearly revealed negative relationships between body condition and the sum of endoparasite taxa carried by individual hosts and, moreover, that increased parasite load can reduce total protein and iron concentrations in serum. Therefore, we conclude that multiparasitism-condition relationships are evident in red deer and fallow deer populations in Denmark and that serum chemistry assays are a valuable diagnostic tool to detect subtle and sub-clinical health impacts of parasitism, even at low-level infestation. Building on these findings, future studies should adopt a hypothesis-driven research approach and corresponding sampling design, specifically to quantify the impacts of different deer management strategies on parasite-condition relationships and population health. Recent advances in parasite detection techniques will be crucial in this to incorporate the potential effects of a wider range of endoparasites.

Data availability statement

Data available from Zenodo Digital Repository, https://doi.org/10.5281/zenodo.7856572.

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Author contributions

FMvB, HHP, MLF and SVH designed the study. FMvB, HHP and MLF performed the sample collection. HHP performed all parasitic analyses and AKHK performed all serum chemistry analyses. FMvB performed all statistical analyses and generated the figures with input from NMS. SVH created the artwork/graphical abstract. All authors participated in the interpretation of results. FMvB led the writing of the manuscript with input of all authors.

Ethics approval and consent to participate

This study did not require official or institutional ethical approval as none of the deer were killed specifically for the purpose of this study. Instead, all deer were part of the annual regulation in the respective estates and local game keepers consented to participate in the study. Certified hunters shot the deer during the official hunting season in Denmark and all samples were collected post-mortem.

Declaration of competing interest

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.05.002.

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