Relationship between social factors and gastrointestinal parasite product excretion in a group of non-cooperatively breeding carrion crows

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Abstract

The social environment strongly affects the physiological stress response in group living animals, which in turn can affect the immune system and increase susceptibility to parasites. Here, I investigate relationships between social behaviour and gastrointestinal parasite product excretion in the carrion crow (*Corvus corone*). Crows kept in groups of eight or nine individuals, and higher-ranking individuals excreted less droppings containing coccidian oocysts compared to crows kept in pairs or trios, and lower-ranking individuals, respectively, whereas the strength of affiliative relationships and number of related individuals in the group did not relate to the proportion of droppings containing coccidian oocysts. The present results confirm a relationship between social environment and parasite excretion patterns in carrion crows, but the patterns described in the present study differ from previously published data derived from a group of cooperatively breeding crows. Here, only dominance rank but not strength of affiliative relationships correlated with parasite excretion patterns. This illustrates that differences between the social systems of carrion crows might result in different relationships between the social environment and parasite product excretion patterns.

Key words: social relationships, affiliation, aggression, parasites, coccidian oocysts, nematodes, carrion crows

Introduction

Increased parasite burden is considered a major cost of group living (Alexander 1974). Several mechanisms can potentially play a role in increased transmission and infection rates in group living animals, *e.g.* social proximity and frequency of interactions between individuals can facilitate parasite transmission among group members, social behaviour can significantly affect an individual's physiological stress response (Wascher et al. 2009; Wittig et al. 2015), which can affect the immune system and make individuals more susceptible to parasite infections (Apanius 1998). Similarly, parasitic infections affect an individual's ability to engage in social behaviour (Sheridan et al. 1994; von Holst 1998; Hanley and Stamps 2002; DeVries et al. 2003; Klein 2003). Behavioural effects on an individual's physiology and immune system range from relatively short-term, as in the effect on disease susceptibility (McEwen et al. 1997), to long-term, as in the effects on reproductive outcome (Buchholz 1995; Marzal et al. 2005; Hillegass et al. 2010) and have serious impacts on host mortality (Rousset et al. 1996).

Adverse effects of the social environment on health and susceptibility to parasites may be caused for example by increased competition and aggressive behaviour (Azpiroz et al. 2003; Hawley et al. 2006; Chester et al. 2010), which affects low-ranking individuals especially (Zuk et al. 1998; Ungerfeld and Correa 2007), instability within the social group (Gust et al. 1991; Capitanio and Cole 2015) as well as large group sizes (Cote and Poulin 1995; Loehle 1995). Social interaction patterns might also facilitate pathogen transmission (Drewe 2010; Fouchet et al. 2012). Affiliative behaviour and reliable social allies in turn, have a stress-reducing effect (Sachser et al. 1998; Frigerio et al. 2003; Stöwe et al. 2008; Young et al. 2014) and might positively affect an individual's immune system. Positive effects of a

secure social embedding on health are also well investigated in humans (Cassel 1976; Cobb 1976; Berkman 1995; Theorell et al. 1995; Uchino et al. 1996; Kiecolt-Glaser et al. 2002).

In the present study, I investigate relationships between social behaviour and gastrointestinal parasite burden in the carrion crow (*Corvus corone*). Corvids express high variability in their social organisation depending on life history and ecological factors. Within species the social organisation might vary between different life history stages, seasons or populations. For example, in most European populations carrion crows form socially monogamous pairs during the breeding season and large flocks during the rest of the year (Meide 1984; Glutz von Blotzheim 1985), whereas in northern Spain crows live in stable social groups of up to nine individuals, consisting of the breeding pair and retained offspring as well as immigrants, which are in most cases male individuals (Baglione et al. 2003). Group living in carrion crows is linked to a higher level of cooperation, for example in nestling provisioning (Baglione et al. 2005).

Within their social groups, corvids establish valuable social relationships, characterised by spatial proximity, high levels of tolerance, relatively low frequencies of aggressive interactions and relatively high frequencies of affiliative behaviours (Fraser and Bugnyar 2010a; Heinrich 2011). Within such valuable relationships, individuals support one other in agonistic encounters (Emery, et al. 2007; Fraser and Bugnyar 2012) and share information and resources (Bugnyar et al. 2001; de Kort et al. 2003). Affiliative relationships are preferably formed with kin, but also non-kin individuals, in which case, long-term monogamous pair-bonds may ultimately develop (Loretto et al. 2012). Monogamous pair-bonds in corvids are characterised by complex social interactions, that demand a high level

of cooperation, coordination and affiliation between the paired individuals, which may explain, in turn, the evolution of advanced cognitive skills ('relationship intelligence': Emery et al. 2007; Wascher et al. 2018). Social relationships facilitate individuals in gaining access to resources, *e.g.* food or territories, and individuals in social relationships are more likely to win agonistic encounter through coalitional support (Bugnyar 2013). Outside such valuable social relationships corvids act more competitively (Bugnyar and Heinrich 2005; Bugnyar and Heinrich 2006) and usually establish linear dominance hierarchies (Izawa and Watanabe 2008). In a previous study on a cooperatively breeding population of captive carrion crows, strength of affiliative social relationships, and group size but not dominance rank or sex correlated with excretion of gastrointestinal parasite eggs and oocysts. Individuals with strong affiliative bonds excreted a smaller proportion of samples containing coccidian oocysts and as did individuals living in larger groups (Wascher et al. 2019).

In the present study, firstly, I investigate the relationship between aspects of the social environment of non-cooperatively breeding captive carrion crows, *e.g.* strength of affiliative relationships, dominance hierarchy, and group structure. Secondly, I will compare the results of the present study with the previously published data in Wascher et al. (2019). I expect a positive social environment, *e.g.* strong affiliative relationships to reduce excretion of parasite eggs and oocysts. Individual position in the dominance hierarchy has previously been shown not to correlate with eggs and oocysts excretion patterns. In the present study I expect to confirm these results. Generally, the effect of dominance rank on an individual's stress response and immune system can be predicted based on the relative allostatic load of social status in the respective species (Goymann and Wingfield 2004). Lastly, I investigate the relationship between group size (*i.e.* pairs or trios versus flocks of eight or nine

individuals) and parasite product excretion. Parasite transmission may be expected to be facilitated by an increase in group size, however, in keeping with Wascher et al. (2019), I expect to find a negative relationship between group size and the number of parasite eggs and oocysts excreted by individuals.

Methods

Study subjects and ethics statement

Data for this study were collected in four phases, between 2008-2010 and between 2012-2015 from a population of captive carrion crows housed in large outdoor aviaries at the Konrad Lorenz research station (KLF), Grünau, Upper Austria (47°51'02 N 13°57'44 E). I observed 23 individuals (11 male and 12 female). Aviaries were approximately 20 m^2 and were equipped with wooden perches, natural vegetation and rocks. An enriched diet consisting of fruit, vegetables, bread, meat and milk products was provided on a daily basis. Water was available *ad libitum* for both drinking and bathing. The keeping of these captive birds was authorized under a licence issued to the Cumberland Wildlife park Grünau (AT00009917).

Over the extended data collection period, due to natural processes, there were changes in group formation. Therefore, in some cases, the identities of the study subjects within a particular group were not constant throughout (Table 1). The study subjects were kept in different group formations, designed to emulate natural social organisations: (A) 'Flock': Individuals kept in groups of eight or nine individuals; (B) 'Pair or trio': Individuals kept in male-female pairs; in one case, also in a trio (1 male, 2 female). Keeping conditions of individual birds changed due to age, reproduction, group expansion or the death of individual birds.

Behavioural data

A total of 899 individual focal observations were conducted. Each observation lasted five minutes, and all occurring behaviours were recorded. For this study, I focused on the frequencies of agonistic behaviour (threat, chase flight, and fight) and affiliative behaviours

(allopreen and contact sit). The identity of the interacting individuals was noted, as well as their role (initiator/receiver) and the outcome of the agonistic interaction (winner/loser). The loser of an agonistic interaction was defined as the individual that retreated.

Composite sociality index

For each phase of data collection, I calculated a composite sociality index (CSI) for each crow dyad within a group according to Silk *et al.* (2010). The CSI was constructed from two affiliative behaviours: contact sit and allopreen. The higher the CSI of a dyad, as compared with the frequency of the affiliative interactions observed within that dyad's group, the stronger the affiliative bond between the two individuals in that dyad. Dyads with CSI higher than the average of the entire sample were classified as 'bonded'. I calculated the maximum CSI among all dyads for each individual, which reflected their strongest affiliative relationship within the group.

Elo-rating

I calculated the relative success levels of individuals in agonistic encounters as an Elo-rating in the R package '*aniDom*' (version 0.1.4; Sánchez-Tójar et al. 2018), which allowed me to track dynamic changes in rank over the different phases of data collection. Each individual was rated based on the outcome of each discrete interaction (winner / loser) and the (predicted) probability of that outcome occurring (Neumann et al. 2011).

Parasitological examination

During the entire study period, a total of 559 individual droppings were collected directly after defecation (for a detailed overview see table 1). Individual gastrointestinal parasite

load was determined from droppings. From 2008 until November 2011, I used a modified version of the flotation method (Schnieder et al. 2006). Fresh faeces (0.1 g) were suspended in a 2 ml collection tube with 1 ml saturated saline. Collection tubes were shaken for 10 seconds and afterwards centrifuged for 5 minutes at 3000 rpm. After centrifugation, the collection tubes were filled with saline solution and a cover slip (18 x 18 mm) was positioned onto the tube. The high density of the saline solution causes the parasite eggs and oocysts to float up and be caught on the cover slip (Carta and Carta 2000). After 10 minutes, the cover slip was moved onto an object slide and the parasite eggs and oocysts were identified (by size and shape) and counted. From December 2011 onwards, I used a McMaster counting chamber. The entire faecal sample was weighed, then diluted with 3 ml saturated NaCl solution per 0.1 g of dropping and thoroughly mixed. Afterwards the solution was poured into both compartments of the McMaster counting chambers. After a 10-minutes resting period I counted the number of parasite eggs and oocysts in each compartment and calculated the number of parasite products per 1 ml of dropping.

For inspection, a compound microscope with 100-fold and 400-fold amplification was used. Coccidian oocysts, several nematode species (Capillaria sp., Ascarida sp., Syngamus sp. and Heterakis sp., Trichostrongylus tenius) and cestodes to a varying degree (nematode eggs: 387 zero, 172 positive samples; coccidia oocysts: 316 zero, 243 infected samples; cestodes: 550 zero, 9 positive samples) were identified in the samples. I used presence versus absence of parasite eggs and oocysts for further analysis, which allowed a direct comparison between the two applied methods of droppings examination to be made (flotation versus McMaster). As only nine samples were found to contain cestodes, no further statistical analysis was conducted on this parasite group.

Data analysis

Factors affecting the proportion of droppings containing coccidian oocysts and nematode eggs in crows were analysed using the *glmer* function in R (version 3.5.3; R Core Team 2019) in the *lme4* package (version 1.1-19; Bates et al. 2015). The number of infected samples for each period of data collection was the response term. GLMMs with binomial error distribution and a two-vector response variable comprising the number of infected and non-infected samples for each individual in each phase were calculated. Various model diagnostics were employed to confirm model validity (visual inspection of the distribution of residuals, Q-Q plots, residuals plotted against fitted values), none of which suggested violation of the model's assumptions. To assess multicollinearity between fixed factors, we calculated variance inflation factors (VIFs) using the *vif* function in the package *car* (version 3.0-6; Fox and Weisberg 2011). VIFs for all models were below 1.6, indicating that there was no issue with multicollinearity (Zuur et al. 2009). Strength of affiliative relationships (CSI value), social structure (pair/trio or group), number of related individuals, and Elo-rating were included as explanatory variables. For each model, we fitted individual identity as a random term to control for the potential dependence associated with multiple samples from the same individuals. The statistical significance level was adjusted to $P \leq 0.025$ following Bonferroni, to account for multiple testing of coccidia oocysts and nematode eggs. In addition to the main analysis, I compared the results based on the present dataset (collected from a non-cooperatively breeding population of captive carrion crows) with those based on a previously published data (from a cooperatively breeding population) (Wascher, *et al.* 2019). Between the two populations, I compared the number of social relationships applying a GLMM with Poisson error distribution, as well as CSI value and Elo-rating applying two

general linear models (GLMs) with gaussian error distribution. Individual identity was fitted as a random term in each model.

Results

Social relationships

In this study 30 bonded dyads (out of 213 dyads in total) were observed, of which 19 were male-female dyads (five between related individuals and 14 between unrelated individuals), six were male-male dyads (all between unrelated individuals) and five female-female dyads (all between unrelated individuals). On average (± SD), males and females had 2.125 (± 1.642) and 1.625 (± 1.505) bonded partners, respectively. The mean CSI (± SD) between bonded dyads was 4.122 (± 2.612) for male-female, 2.561 (± 1.154) for female-female and 3.911 (± 2.062) for male-male bonds. Neither the number of social bonds, the CSI value or Elo-rating differed between the cooperatively breeding and non-cooperatively breeding populations of crows (number of social bonds: estimate ± SE = -0.085 ± 0.361, z = -0.235, P = 0.813; CSI: estimate ± SE = -0.582 ± 0.767, z = -0.758, P = 0.448; Elo-rating: estimate ± SE = 0.001 ± 0.001, z = 1.113, P = 0.265).

Occurrence of coccidian oocysts and nematode eggs

Of the total of 559 samples, collected from 23 individuals, 243 samples derived from 18 individuals contained coccidian oocysts (43 % compared to 31 % in the Spanish population of cooperatively breeding crows). Crows kept in groups of eight or nine individuals excreted less samples containing coccidian oocysts compared to crows kept in pairs or trios (estimate ± SE = 0.996 ± 0.285, z = 3.487, P < 0.001, Fig. 1). Elo-rating was significantly related to number of samples excreted containing coccidian oocysts, with higher ranking individuals excreted less samples containing coccidian oocysts compared to lower ranking individuals (estimate ± SE = -0 ± 0, z = -2.236, P = 0.025, Fig. 2). However, the

number of samples containing coccidian oocysts was not related to CSI and number of related individuals in the group (Table 2a).

Overall, 172 samples, collected from 14 individuals contained nematode eggs (31 % compared to 9 % in the Spanish population of cooperatively breeding crows). None of the social factors investigated significantly affected excretion patterns of nematode eggs (Table 2b).

Discussion

In this, individuals from a population of non-cooperatively breeding carrion crows excreted less samples containing coccidian oocysts when kept in larger groups (8 or 9 individuals) compared to those individuals kept in smaller groups (2 or 3 individuals). Further, lower-ranking individuals excreted more samples containing parasite oocysts compared to higher-ranking individuals. A similar relationship between group-size and parasite excretion patterns was found in a population of cooperatively breeding carrion crows (Wascher et al. 2019). However, other patterns in the present study contrast with those of this previous study. In a group of cooperatively breeding crows, the proportion of samples containing coccidian oocysts was negatively correlated with the strength of affiliative social relationships, but dominance hierarchy did not affect parasite excretion patterns (Wascher et al. 2019).

Carrion crows live in a range of different social system, the structure of which is influenced by different life-history stages and environmental factors. For example, crows can occur in large non-breeder flocks, territorial pairs and cooperative family groups (Meide

1984). Different social systems impose a different range of social challenges, *e.g.* repeated interactions with kin in stable family groups, versus high fission-fusion dynamics and social interactions with numerous conspecifics including kin, affiliates and non-affiliates in a non-breeding flock, versus cooperative interactions exclusively with the pair-partner and competitive interactions against others in stable pair-bonds. The results of this study suggest that different social systems may lead to different parasite excretion patterns in carrion crows. This indicates that the social environment affects individuals in different social systems differently. Differences in parasite excretion patterns are likely to be mediated by the physiological stress response, which, in turn, may compromise the immune system and lead to increased parasite burden (Muehlenbein and Watts 2010).

According to a model presented by (Goymann and Wingfield 2004) the level of allostatic load and consecutively glucocorticoid concentrations differ between dominant and subordinate individuals, depending on characteristics of the social system, such as costs of rank acquisition and maintenance. This results in different species to differ regarding the levels of stress hormones in relation to rank (Goymann and Wingfield 2004). The present study suggests that variation in the social system within a species can result in differences in parasite product excretion patterns, which likely result from differences in stress level and related to this, differences in the ability of the immune system to fight off parasites. Here, in a population of non-cooperatively breeding carrion crows, subordinate individuals excreted more samples containing coccidian oocysts, most likely due to elevated stress levels. In contrast, no such relationship was found in a population of cooperatively-breeding carrion crows (Wascher et al. 2019). In a way this is surprising, as dominance has been described as a mechanism to supress reproduction in subordinate individuals in some cooperatively

breeding mammalian species (Young et al. 2006; Creel et al. 1992). On the other hand, avian cooperatively breeding societies have often been described as highly tolerant, especially towards related individuals (Baglione et al. 2003; Dickinson et al. 2009).

Increased exposure to parasites and disease transmission is considered as one of the major disadvantages of group living. Group size is usually positively related to parasite burden, which is seen as a major selective force in group living animals (Cote and Poulin 1995). Similar to a previous study in captive cooperatively breeding carrion crows, individuals in the present study excreted less samples containing coccidian oocysts when living in larger groups. This illustrates that not only the number of individuals in a group and related to this the probability of pathogen transmission has to be considered, but also social interaction patterns, which are likely to mediate an individual's physiological stress response which might suppress or enhance an individual's immune system. In cooperatively breeding azure-winged magpie (*Cyanopica cyanus*) group size positively affected the immune response of nestlings (Valencia et al. 2006).

In contrast to Wascher et al. (2019), strengths of affiliative social relationships did not correlate with the proportion of samples containing coccidian oocysts. Neither the strength nor the number of affiliative relationships differed significantly between the subject populations in these studies. Therefore, it does not seem that populations show a general, behavioural difference in affiliative relationships within groups. In cooperatively breeding crows, individuals with strong affiliative relationships excreted less samples containing coccidian oocysts (Wascher, et al. 2019), which could reflect a stress reducing and immune system enhancing function of affiliative social relationships. Strong relationships between

pair-partners, between kin, and also between non-paired and unrelated individuals play a significant role in the social life of corvids, and have been hypothesised to be an important driver in the evolution of cognition (Emery, et al. 2007).

Social relationships in common ravens have been shown to facilitate access to resources (Braun and Bugnyar 2012) and cooperation (Fraser and Bugnyar 2012). Following conflicts, common ravens reconcile and consolidate valuable relationships, therefore mitigating potential costs of disrupted relationships (Fraser and Bugnyar 2010b; Fraser and Bugnyar 2011). Based on these previous findings, it can be assumed that strong affiliative relationships hold significant benefits for corvids. Therefore, I expected to find, but was unable to confirm in the present study, that the nature of an individual's social relationships would affect their health and physiology. This is especially interesting as dominance rank was negatively correlated with the proportion of samples excreted containing coccidian oocysts. Aggressive social interactions have previously been shown to be among the most potent stressor (Wascher et al. 2008) and position in the hierarchy is related to stress in a number of species (Creel 2001). Social relationships have been suggested to mitigate the effects of aggression onto the physiological stress response (Sachser et al. 1998) and therefore I would have expected to find an effect of strength of affiliative relationships on parasite product excretion in carrion crows.

Here, the excretion of coccidian oocysts but not excretion of nematode eggs was found to be related to social factors. These results replicated those of Wascher et al. (2019) and may be related to differences between these parasite species in their life-cycles. Coccidian oocysts have a shorter prepatent period compared to nematode eggs and are therefore

potentially more sensitive to short-term changes in stress levels and immune system. This is supported by a study in carrion crows, showing coccidian oocysts but not nematode eggs to significantly increase in the first week after a major stressor (Spreafico et al. 2012) and in greylag geese excretion of coccidian oocysts but not nematode eggs are significantly increased in the first week after social isolation (Ludwig et al. 2017).

In summary, more droppings containing coccidian oocysts were excreted by individuals kept in pairs and trios compared to groups of eight or nine individuals. Strength of affiliative social relationships did not correlate with parasite product excretion; however subordinate individuals excreted more samples containing coccidian oocysts compared to dominant individuals. The present results illustrate differences in the social system in carrion crows also resulting in different relationships between social environment and parasite product excretion.

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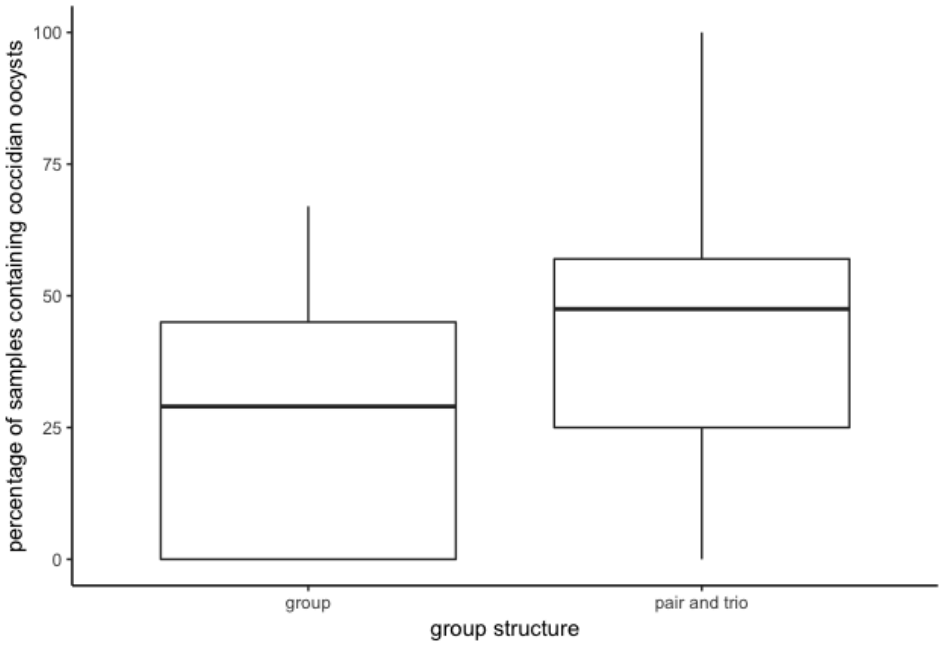
Table 1: List of all focal individuals and information about population, sex (F = female, M = male), age (year of hatching; if not known, individuals are classified as adult), group (group composition: family, flock, pair, trio), phases of data taking during which the individual was recorded, number of faecal samples and behavioural focal observations collected, maximum composite sociality index (CSI) and Elo-rating.

	Sex	Age	Group	Phase	Faecal samples	Observations	Maximum CSI	Elo-rating
Baerchen	M	2008	Group	G09	10	31	5.156	-515.459
			Pair	G10	7	34	4	-166.849
				G12	40	24	0	-166.849
Franz	M	2007	Group	G09	8	48	1.152	-636.627
			Pair	G10	4	30	0	-636.627
				G12	34	23	2.181	-636.627
Fritz	M	2008	Group	G09	2	9	4.769	133.72
Gabi	F	2007	Group	G08	6	39	0	-41.797
				G09	14	28	2.863	192.788
			Pair	G10	4	27	0	139.761
			Trio	G12	43	24	0	133.466
			Solo	G12	3	0	0	133.466
Gertrude	F	2011	Pair	G12	33	24	4.363	-215.052
Hitchcock	M	2007	Group	G08	2	48	9.538	113.269
				G09	4	22	8.95	260.420
Hugo	M	1989	Group	G08	2	33	11.923	-96.304
				G09	14	33	1.536	-187.089
Klaus	M	2009	Group	G08	13	10	0.384	350.719
				G09	5	14	0	403.747
			Trio	G12	33	25	1.09	329.27
Mate	M	2008	Group	G09	2	45	5.913	-243.863
Olaf	F	adult	Pair	G12	2	4	0	0
Peter	F	2007	Group	G08	3	52	14.307	226.598
				G09	10	56	6.297	-128.351
			Pair	G10	6	33	0	-476.962
				G12	48	24	0	-476.962
Pinky	F	2008	Group	G08	7	10	7.153	383.915
Resa	F	2009	Trio	G12	35	24	0	80.771
Ronja	F	2008	Group	G08	2	8	2.384	207.35
Ruediger	M	adult	Pair	G12	2	4	0	0
Sissi	F	2008	Group	G08	2	9	0.973	221.942
Toeffel	F	2008	Group	G08	3	20	4.769	-138.099
				G09	8	37	22.92	-39.464
			Pair	G12	38	23	2.181	-39.464
Walter	M	2011	Pair	G12	37	24	1.885	0

Table 2. Results of the generalized mixed linear model investigating factors affecting patterns of coccidian oocyst and nematode egg excretion. Models (a) and (c) investigate effects of group structure, number of related individuals, strength of affiliative relationships (CSI) and dominance hierarchy (Elo-rating). Models (b) and (d) investigate effects of frequency of affiliative and agonistic interactions (won and lost). Significant values ($p \leq 0.05$) are highlighted in bold.

	Parameters	Estimate ± SE	*z*	*p*
(a) Coccidia	**Intercept**	**-1.221 ± 0.291**	**-4.194**	**<0.001**
	Group structure	**0.996 ± 0.285**	**3.487**	**<0.001**
	Nr related	-1.024 ± 0.6	-1.705	0.088
	CSI	0.059 ± 0.035	1.69	0.091
	Elorating	**-0 ± 0**	**-2.236**	**0.025**
(b) Nematodes	**Intercept**	**-1.389 ± 0.45**	**-3.084**	**<0.001**
	Group structure	-0.157 ± 0.328	0.478	0.632
	Nr related	-1.046 ± 0.509	-1.22	0.222
	CSI	0.005 ± 0.064	0.131	0.895
	Elorating	0 ± 0	-0.026	0.978

Figure 1.
Percentage of samples containing coccidian oocysts in carrion crow droppings in relation to the group structure. Box plots show the median and the interquartile range from the 25th to the 75th percentiles.



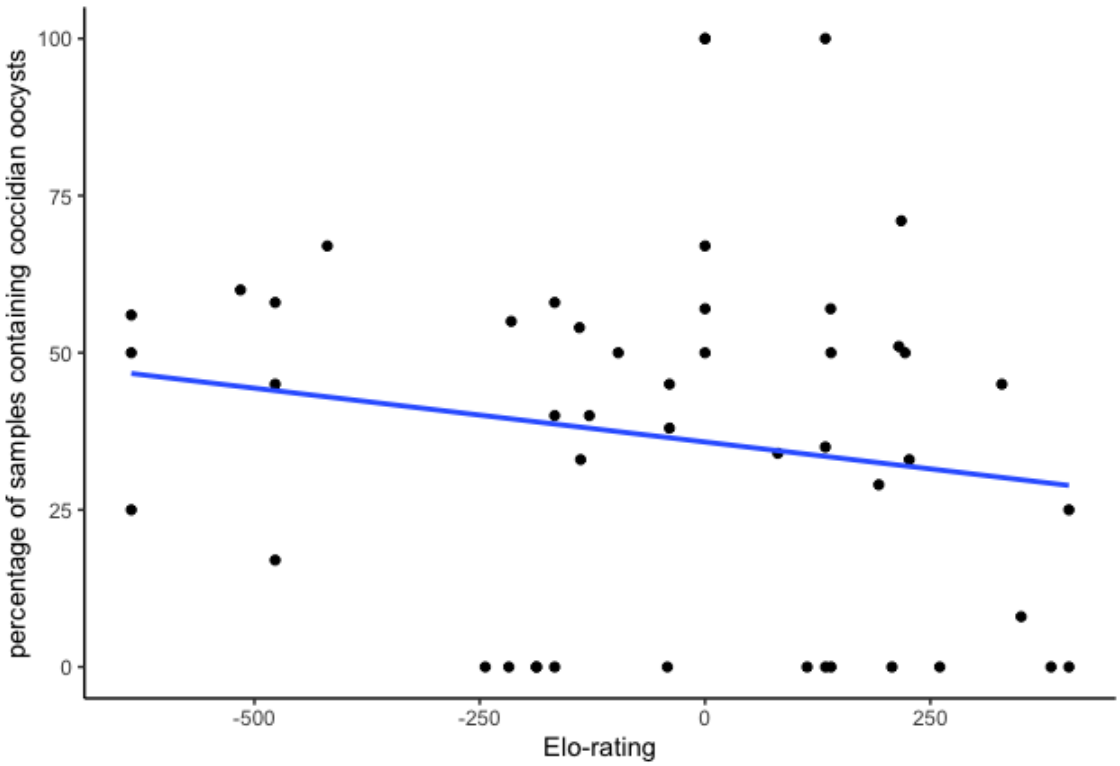


Figure 2.
Percentage of samples containing coccidian oocysts in carrion crow droppings in relation to individual Elo-ratings.