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ABSTRACT

Among the various costs of gregarious behaviors, proximity highly contributes to facilitating parasite transmission. However, the intensity of parasite infection varies between individuals of a social group. It is then of high interest to identify the causes of such inequality for parasite management. In this study, we investigated the impact of individual social behaviors on parasite distribution in the largest European group of captive plains zebras (*Equus quagga boehmi*) and the consequences of anthelmintic treatment. For six months, 22 plains zebras were kept in a safari enclosure and observed for 10 minutes each by focal sampling for a total of 44 days over three months. Strongyle and coccidia individual infections were estimated monthly with a commercial OVATEC® Plus fecal analysis kit provided to veterinarians for fecal egg/oocyst count. Then, we used GLMM models with age, sex and time until or since anthelmintic treatment as fixed factors and with social factors (degree of connection and dominance) as predictors of individual variation in fecal parasite eggs and oocyst shedding.

The social structure observed in captive plains zebras is comparable to that of wild plains zebras and horses. Before treatment, females were more infected than males. Then, we observed an increase in coccidia infection after anthelmintic treatment, and approximately 20% of zebras carried 70% of the total strongyle infection. Dominance, and degree were strong predictors of an individual's strongyle infection intensity after anthelmintic treatment. Further investigation is needed to identify new treatment strategies based on social behaviors and to improve anthelmintics administration in large groups of equids.

KEYWORDS

coccidia; fecal egg count; hierarchy; social network; strongyle; *Equus quagga*

ABBREVIATIONS

Réserve Africaine de Sigean (RAS)

Egg Per Gram (epg)
Oocyst Per Gram (opg)
Fecal Egg Count (FEC) Fecal Oocyst Count (FOC)

1 INTRODUCTION

While gregariousness includes benefits (Lehtonen and Jaatinen, 2016; Olson et al., 2015; Taraborelli et al., 2012; Webster et al., 2004), it also has negative consequences, such as intraspecific competition for food or reproductive sites (Krause and Ruxton, 2002), and pathogen transmission between group members which increases with proximity (Hawley et al., 2011; Torfs et al., 2021). Furthermore, social species are excellent systems to examine a pathogen spread (Aubert and Richard, 2008; Rimbach et al., 2015; Webber et al., 2016), especially because social groups are small systems and made easier to study than pathogen transmission at the population level.

Research with social mammals suggests that social behaviors affect pathogen transmission within groups (Rimbach et al., 2015; VanderWaal and Ezenwa, 2016) and individual infection levels (Altizer et al., 2003; Ezenwa, 2002; Halvorsen, 1986). Indeed, parasite infection intensity and prevalence, such as gastrointestinal parasites, varies with group size and can either increase or decrease, mostly depending on the parasite's transmission path (Coté and Poulin, 1995). However, the mechanisms linking social behaviors and parasitism are still poorly known, and the need to identify the causes of individual infection variation through social behaviors was recently highlighted (Briard and Ezenwa, 2021). For this purpose, social network analyses highly contribute to understanding parasite infection dynamics within a group (Silk et al., 2019; White et al., 2017; Williams et al., 2017). Indeed, many studies explain pathogen transmission through social networks, mostly with three parameters: *degree*, *eigenvector centrality* and *betweenness centrality*. The *degree* corresponds to the number of connections received or given by one individual (Newman, 2010; Whitehead, 2008; Williams et al., 2017); *eigenvector centrality* corresponds to how well-connected, both directly and indirectly, an individual is (Freeman, 1978; Newman, 2010; Whitehead, 2008); and *betweenness centrality* corresponds to the frequency an individual occurs on the shortest path between two other individuals in the network (Krause et al., 2015; Newman, 2010; Torfs et al., 2021). The parameter best explaining parasite transmission highly depends on the method of transmission used by the parasite and on the

social organization of the host (Briard and Ezenwa, 2021). On the other hand, parasite infection can modify social networks because of the avoidance of infected individuals (Müller-Klein et al., 2019). Moreover, an individual parasite infection can be affected by its own behaviors, as parasite avoidance behaviors were observed in herbivorous species (Michel, 1955). For example, horses choose their foraging zone at least one meter from potentially contaminated environments, such as dung patches (Fleurance et al., 2007).

Gastrointestinal parasites are a common equid pathology (Francisco et al., 2009) and can be lethal (Giles et al., 1985; Love et al., 1999; Murphy et al., 1997). Strongylidae nematodes (Francisco et al., 2009) and *Coccidia* (Lathuilliere, 2018) are observed in captive conditions. Both present with a simple life cycle with only one host during the endogenous phase, making good subject models to study parasite transmission through host contact (Arneberg, 2002). The main source of infection is the ingestion of the infective form (Bowman and Georgi, 2014). In equid groups, approximately 20% of individuals shed 80% of the total group's strongyle egg shedding. This 20/80 rule is relevant for horses (Scala et al., 2020; Stratford et al., 2011) as well as for wild zebras (Fugazzola and Stancampiano, 2012; Turner and Getz, 2010). The individual parasite infection intensity of horses is linked with the host's age or sex (Kornaś et al., 2010; Scala et al., 2020). Indeed, adults are more infected by strongyle than foals, and females are more infected than stallions (Kornaś et al., 2015; Nielsen et al., 2018; Scala et al., 2020). The cause of this last statement might be that pregnancy and lactation could reduce the immune activity of females and thus be favorable to larval development (Connan, 1976).

Likewise, the strongyle prevalence of wild plains zebra populations is approximately 98%, which is comparable to that of horses, and approximately 20% of zebras shed 80% of the total group's egg shedding (Fugazzola and Stancampiano, 2012; Turner and Getz, 2010). Dominant wild zebras are less infected by strongyles than subordinates (Fugazzola and Stancampiano, 2012). However, if horses can be affected by both strongyle and coccidia parasites (Cizauskas et al., 2015; Nielsen et al., 2019), coccidia infection has yet to be reported in wild zebra populations (Lathuilliere, 2018; Turner

and Getz, 2010) and was only once reported in captive plains zebra (Dubey and Bauer, 2018). However, the relationship between life history traits (age and sex) and individual parasite infection levels has not yet been investigated in zebras.

Plains zebras originate from the southern and eastern African savannas and are the most widespread and represented wild equid in the world. However, they are categorized as Near Threatened in the IUCN Red List since 2016 with significant population decline (King and Moehlman, 2016). They live in two kinds of groups: very unstable bachelor groups composed of males only and harems composed of one breeding stallion, 2-3 mares and their foals (Hack et al., 2002; Klingel, 1969; Mills and McDonnell, 2005). Harem composition is known to be stable (Mills and McDonnell, 2005; Vitet et al., 2020). They are ruled by a linear hierarchy stable as well, with the older mare being the most dominant female in most cases (Andersen, 1992; Pluháček et al., 2006; Rubenstein and Hack, 2004; Simpson et al., 2012).

Previous studies on predictors of plains zebra parasite infection have only been conducted in wild systems (Fugazzola and Stancampiano, 2012; Seeber et al., 2020). Seeber et al.'s (2020) study only focuses on the life history stage of zebras (age and sex). Moreover, dominance was assessed only for the most dominant individual in Fugazzola and Stancampiano's study despite dominance in equids being mostly linear (Klingel, 1969; Sigurjónsdóttir et al., 2003; van Dierendonck et al., 1995, 2004), and they based the identification of dominant females on leadership, although it has been demonstrated that leadership is not reliable for this purpose (Krueger et al., 2014). To our knowledge, no researcher has published and studied social network in any way as a predictor of plains zebra parasite infection.

In the current study, we estimated the individual parasite infection in the largest European group of captive plains zebras before and after anthelmintic treatment and conducted behavioral observations to characterize hierarchy and social structure. We assumed that the confined living conditions induced a homogeneously contaminated environment. Therefore, we tested the hypothesis that life history

(age and sex), dominance and social network metrics (degree and strength) are predictors of individual parasite infection in captive plains zebra groups.

2 MATERIALS AND METHODS

2.1 Animal environment

Experiments were conducted at the Réserve Africaine de Sigean (RAS) France (43°03'45.5"N 2°57'00.9"E). The RAS holds 22 plains zebras (later called zebras) in a 6.5 hectares safari enclosure, with five other species (six wildebeest (*Connochaetes taurinus*), four watussi oxen (*Bos taurus*), four ostriches (*Struthio camelus*), two common elands (*Taurotragus oryx*) and one male white rhinoceros (*Ceratotherium simum*)). All zebras were born in the RAS except for the two stallions who were imported from another European park to limit inbreeding. The group composition changed during the study period due to the death of one individual, the departure of two others in the first month of observation and four births during the last month of observation. None of these individuals were included in the current study. Thus, the study group was composed of 12 mares (from 10 years old. to 18 y.o.), one of whom was not lactating, 2 stallions (5 y.o. and 10 y.o.), and 8 foals (from 6 months to 4 y.o.).

The animals had free access to five shelters. They are fed hay and grass distributed *ad libitum* and parasite-free pellets once a day year-round. The hay and grasses were considered parasite-free, as they were cultivated in dedicated plots inaccessible to herbivores. Water is available *ad libitum* at the drinkers next to the shelters or directly at the stream constituting one of the borders of the enclosure. The RAS is open every day year for visits, and visitors can drive through the safari enclosure with their own vehicle but are restricted to the road.

2.2 Behavioral observations

Zebras were observed from 25 March to 7 July 2019 by a single observer from a car. Zebras live in a safari park and are habituated to cars crossing the enclosure. The observer was able to individually identify zebras based on their stripe patterns after 2 weeks of daily observations. Those two weeks also constituted the habituation period for animals to the continuous presence of the car and the observer. Observations were always conducted during sessions of two hours starting either at 09:00 h and 13:00 h or at 11:00 h and 15:00 h every other day. Observation days were divided into 22 time slots of focal sampling (Altmann, 1974) of 10 minutes: 11 time slots during the morning session and 11 time slots during the afternoon session. Each individual was observed daily for one focal sampling and always at different time slots. Two similar interactions noted at 5-second intervals were considered to have occurred twice (Vervaecke et al., 2007). Data were collected using a voice recorder. All behaviors available in ethograms of previous equid studies (Christensen et al., 2002; McDonnell and Haviland, 1995; Ransom and Kaczensky, 2016) and including at least 2 protagonists were considered social behaviors and were recorded. Behaviors were classified into three categories: affiliation, dominance and submission. The Encyclopedia of Animal Cognition and Behavior defines affiliation behaviors as “Friendly and peaceful interactions exchanged among individuals with the function of developing, maintaining, or strengthening social bonds” (Jasso del Toro and Nekaris, 2019). Thus, we included social grooming, rubbing, physical contacts without intentions and suckling in the category Affiliation. Both categories of dominance and submission included agonistic behaviors, but in one case, the individual observed was victorious, while in the other case, the individual observed was defeated. Thus, the dominance category included biting, stomping, threatening, chasing, pushing, displacing, and defecating over the feces of another zebra. Moreover, the Submission category included snapping, avoiding and back kicking another zebra. A description of each behavior is presented in Table 1. All behaviors were considered directed (an individual carrying out the behavior toward another) except for head-to-head contact and body-to-body contact. To build a social network, we used Affiliation behaviors (Clauset et al., 2004). As the number of zebras present in the study (22) largely exceeded the average number of individuals present in harems

(one stallion and 2-3 mares with their foals), we determined potential subgroups from the social network (Feh, 2005). Then, we identified three metrics from social behavior networks that are best representative of host contacts: degree, strength and eigenvector centrality. In the study, the first is the number of partners, the second is the number of interactions, and the third is a dependent variable of the previous two. We then focused on degree and strength to perform a finer-scale analysis of the effect of social interactions on parasite transmission. Individual dominance was calculated with David's score, which is recommended by previous studies (de Vries, 2009; Hemelrijk et al., 2005), by using behaviors from Dominance and Submission categories (Feh, 1988; Hemelrijk et al., 2005; Ransom and Cade, 2009). The David's score calculation is based on the proportion of "gains" by individual i in interactions with individual j , abbreviated as follows (P_{ij}), and the proportion of "defeats" of individual i in its agonistic interactions with individuals j , abbreviated as follows (P_{ji}). Gains correspond to events when i is an actor and j is a receiver during a dominance behavior or when j is an actor and i is a receiver during a submission behavior. In contrast, defeats correspond to events when i is an actor and j is a receiver during a submission behavior or when j is an actor and i is a receiver during a dominance behavior. Thus, the proportion of gains is calculated as $P_{ij} = \alpha_{ij}/n_{ij}$, (α_{ij}) being the abbreviation of the number of gains of i against j , and (n_{ij}) being the abbreviation of the total number of agonistic interactions between i and j . The proportion of defeats is calculated as $P_{ji} = 1 - P_{ij}$. David's score method uses the following formula (David, 1987; de Vries, 1998):

$$Dominance = w + w_2 - l - l_2$$

where w represents the sum of the P_{ij} values of i , w_2 represents the sum of the w values of the individuals with whom i interacted, l represents the sum of the P_{ji} values of i and l_2 represents the sum of the l of the individuals with whom i interacted.

2.3 Evaluation of individual parasite infection

Individual parasite infection intensity was evaluated by fecal egg count (FEC) and fecal oocyst count (FOC) (Matthews, 2014; Nápravníková et al., 2019; Nielsen, 2022) from February to July 2019. Our sample size follows the methods of previous studies, corresponding to approximately one FEC and FOC per month and per individual (Aviruppola et al., 2016; Kuzmina et al., 2006). However, in total, we analyzed 106 fecal samples instead of the 132 samples corresponding to 22 individuals sampled across 6 months. Indeed, some samples were missed, as the sampling was opportunistic to avoid disturbing zebras. Also, fecal samples were always collected after behavioral observations to limit interferences. They were collected 3 minutes after defecation and then placed in a refrigerator before being analyzed within the next 24 hours for optimal freshness of the feces (Ballweber et al., 2014; Crawley et al., 2016; Jagła et al., 2013). Parasite infection was evaluated by a single operator at the veterinary clinic of the RAS using a commercial OVATEC® Plus fecal analysis kit (Zoetis, USA). Approximately 1 g of homogenized fecal sample was placed in the device's filter. Next, the insert was immersed in saturated zinc sulfate solution and rotated manually for 2 minutes inside the container to homogenize the solution. The container was then filled with zinc sulfate saturated solution until a meniscus was obtained. A lamella was placed on the top of the meniscus for 20 minutes and then gently removed from the top carrying zinc sulfate solution drops containing parasite eggs to be next laid flat on a microscope slide for observation. Identification was based on egg shapes for strongyles and oocyst shapes for coccidia (Figure 1). Eggs of all different species of strongyles are similar; no attempt to distinguish species was made. Both eggs and oocysts were counted by the same operator. As we used 1 g of fecal sample, the count corresponded to the number of eggs per gram (epg) and oocysts per gram of feces (opg).

Zebras were not treated with anthelmintics within 6 months prior to the beginning of the study and were treated in May (three months from the beginning of the study) as a routine treatment. Consequently, sample collection corresponds to three samples before treatment and three samples after treatment per individual. The anthelmintics used are mainly efficient against strongyles (7.5 mg/kg fenbendazole per os, mixed with pellets distributed by the zookeepers), targeting adult

nematodes. Finally, all other species present in the enclosure were also treated, as anthelmintics were provided via daily distributed pellets in free access to every animal in the enclosure.

2.4 Data analysis

All statistical analyses were carried out with RStudio software (R Core Team, 2023; RStudio Team, 2023). The significance was determined at the alpha threshold of 0.05. Data will be presented with the mean and the standard error. The social network was built using the R package *Igraph* (Clauset et al., 2004), which allowed us to calculate the degree of connection and to determine the presence of subgroups. Individual dominance was defined using David's score method calculated with the R package *dominance* based on a matrix of winning and losing interactions (Sueur et al., 2011). Individual parasite infection was evaluated by the number of eggs observed in each individual fecal analysis for both types of parasites. Comparisons between strongyle and coccidia FECs were made with a Spearman rank correlation, and differences in strongyle parasite infection between subgroups were analyzed with the Wilcoxon signed-rank test. Finally, we used linear models to analyze the correlation between age and dominance and dominance and degree, as it is known to be correlated in the literature (Andersen, 1992; Pluháček et al., 2006; Rubenstein and Hack, 2004; Simpson et al., 2012). No random factor or fixed factor was included. The normality was analyzed with the Shapiro–Wilk normality test in R, and homoscedasticity was tested with the *var.test* function.

For statistical analysis, associations between predictors (degree, strength, and dominance) and individual parasite infections were analyzed separately before and after anthelmintic treatment. Then, time was calculated, such as before treatment, which represents the number of days until the next treatment, and after treatment, which represents the number of days elapsed since the last treatment. We used generalized linear mixed models from the package *glmmTMB* (Brooks et al., 2017), with FECs and FOCs as the response variables. As our data showed overdispersion (variance twice as high as the mean), the model was fitted to a negative binomial distribution (*nbinom2*). All models included the time since/until treatment (continuous, days), age (continuous), subgroup (three levels, 1/2/3) and

sex (two levels, female/male) as fixed factors. Furthermore, all models allowed a random variation accounting for repeated measures from the same individual: (1 | ID). We designed three different models with one predictor each: degree (continuous), strength (continuous) and dominance (continuous, normalized David's score). Models were checked for goodness of fit with the *DHARMA* package using residual diagnostic controls (Hartig, 2022). We used the interaction between the predictor and the fixed factors when it was significantly better fitted than the model without interaction after running a likelihood ratio test with the *ANOVA* function from the *car* package. Variance explained by the entire model (pseudo R^2) was estimated with the *r.squared* function from the *MuMIn* package, with the trigamma-estimate method. As mentioned in Lynsdale, 2022, all fixed factors were tested using the likelihood test ratio, comparing starting models to replicates without each singular term in turn, and only the significant ones were kept (Lynsdale et al., 2022). In addition, a quantile deviation and a significant combined adjusted quantile test were detected in the model exploring the effect of degree and dominance on FOCs before treatment. Thus, we included the quadratic form of time until treatment as a fixed factor (Hartig, 2022). Finally, we predicted that FEC should start from 0epg for all zebras immediately after treatment; thus, random variation in slope depending on the time since the last treatment across individuals has been included, which is expressed as (0+time since the treatment | ID) (Bolker, 2023).

3 RESULTS

3.1 Social network

In total, we recorded the behaviors of 22 individuals daily for 44 days spread over 3 months of observation, cumulating 5 750 social behavior events. Each zebra was observed for a total of 7 hours and 20 minutes. We identified three subgroups within the enclosure, composed of eleven zebras for subgroup 1, five zebras for subgroup 2 and six zebras for subgroup 3 (modularity = 0.61). Each stallion was associated with one of the two largest subgroups. Subgroup 1 consisted of six mares and four foals (2 males and 2 females), subgroup 2 consisted of three mares and two foals (females), and

subgroup 3 consisted of three mares and two foals (females). In between subgroup interactions represent only 7% of all interactions observed (407 in between subgroup interactions: 143 behaviors between subgroups 1 and 2, 219 between subgroups 1 and 3, 45 interactions between subgroups 2 and 3), and most of them are agonistic behaviors (164 affiliation behaviors, 147 domination behaviors, 60 submission behaviors). Finally, 75% of in-between subgroup interactions included at least one male (204 between two males from different subgroups, 101 between one male and one female from different subgroups).

The David's score varied from 7.78 to 14.55 (mean = 10.53 ± 1.70). Age and dominance are correlated; thus, older zebras are more dominant (linear regression: $F_{1,46} = 14.34$, $R^2 = 0.24$, $P < 0.01$), although both stallions were dominant in their respective subgroups independently of their age. Finally, dominance and degree are independent (linear regression: $F_{1,46} = 0.54$, $R^2 = 0.01$, $P > 0.5$).

3.2 Individual parasite infection

Eggs of strongyles and oocysts of coccidia were observed at least once in each zebra. Among the 106 analyzed samples, 55 samples were collected before treatment, and 53 samples were collected after treatment. Before treatment, a maximum of 42epg for strongyles (mean = 8.32 ± 9.5 epg) and 13opg for coccidia (mean = 2.02 ± 3.37 opg) were recorded. After treatment, a maximum of 8epg for strongyles (mean = 1.06 ± 2 epg) and 32opg for coccidia (mean = 5.58 ± 6.56 opg) were recorded. Anthelmintic treatment significantly decreased strongyle FECs (Mann–Whitney U test: $U = 246$, $N_1 = N_2 = 22$, $P < 0.001$; Figure 3). This was expected, as the anthelmintic used is known to be efficient against adult nematodes. However, after treatment, coccidia FOCs increased (Mann–Whitney U test: $U = 27.5$, $N_1 = N_2 = 22$, $P < 0.001$; Figure 3).

3.3 Predictors of parasite infection

Before and after treatment, there was no significant difference in mean parasite infection between subgroups for either strongyle or coccidia (Wilcoxon signed-rank test: $W > 50$, $P > 0.05$). While males

excreted fewer eggs than females before (male mean = $4.5 \pm 4\text{epg}$; female mean = $9.23 \pm 10.3\text{epg}$) and after treatment (male mean = $0.88 \pm 2.1\text{epg}$; female mean = $1.1 \pm 2.01\text{epg}$), the difference between sexes was not significant ($X^2 > 1$, $P > 0.05$). Age was not correlated with parasite infection intensity, either before or after treatment (linear regression: $F_{1,20} < 5$, $R^2 < 0.2$, $P > 0.5$). None of the models run before treatment with FECs as the response variable showed a significant association with any of the predictors (degree, strength and dominance). None of the models run before treatment with FOCs as response variable showed a significant association with any predictors as well, but the effect of the quadratic form of the time until treatment was significant (Table 2).

As predicted, all zebras showed 0epg at their first strongyle FEC after treatment (Figure 3). The interaction between predictors and the time since the last treatment was significant for two models with FECs after treatment as the response variable: dominance*time since last treatment ($X^2 = 8.19$, $P < 0.01$) and degree*time since last treatment ($X^2 = 8.83$, $P < 0.01$). Thus, both showed that more dominant zebras are infected more slowly than their subordinates over time (model estimate = 0.03 ± 0.01 , $X^2 = 8.58$, $P < 0.01$), and a higher degree increases the speed of infection over time (model estimate = -0.03 ± 0.01 , $X^2 = 5.67$, $P < 0.05$). In contrast, FOCs after treatment were not predicted by strength in our model (model estimate = -0.006 ± 0.009 , $X^2 = 0.47$, $P = 0.36$). None of the predictors significantly affected coccidia FECs, despite a significant effect of time until/since treatment on FECs in each of the model runs (Table 2).

4 DISCUSSION

4.1 Social structure of captive plains zebra

In this study, we observed the social structure of the largest group of captive plains zebras in Europe. We determined three different subgroups in the enclosure, two of which included one stallion and 3-6 adult females with their respective foals. One subgroup (number 3) comprises females and their

foals only. Identification of the foal's father was not possible, as both stallions present in the enclosure were sexually active. Both group structures are comparable to observations in wild plains zebra populations (Simpson et al., 2012). In addition, observations showed that older females are more dominant within their respective subgroups, which also corroborates previously described results in wild populations (Lloyd and Rasa, 1989) and captive populations (Andersen, 1992; Pluháček et al., 2006).

4.2 Fecal analysis: an indicator of parasite infection intensity

Methods for parasite infection estimation create a debate as to which is the best, whereas the best universal method does not exist, and consequently, information about any deviation is needed (Ballweber et al., 2014). Using the OVATEC® Plus kit, we were able to identify differences between individual parasite infection intensities (Egwang and Slocombe, 1981), although it is known for its lack of sensitivity and is not recommended to evaluate precise FECs for anthelmintic treatment decision purposes (Lester and Matthews, 2014). However, the results from this method give a good approximation of reality, and the number of eggs observed with this method is proportionate to the real number of eggs present in one gram of feces (Egwang and Slocombe, 1981). Thus, we suspect an underestimation of fecal egg shedding; for example, we counted a maximum of 42 eggs and 32 oocysts per gram of feces before treatment in this study, while it was approximately 250-1000epg in wild zebra populations (Fugazzola and Stancampiano, 2012; Seeber et al., 2020) and in captive zebra populations (Epe et al., 2001; Lia et al., 2010; Palomero et al., 2018) with the FLOTAC™ or McMaster method. However, our study was conducted with the same experimenter all along to limit “the personal factor” (Levine et al., 1960) and with the same tools, and samples were homogenized, which had a positive effect on accuracy (Went et al., 2018). Finally, this method respects the reality of veterinarians' worldwide zoos, who commonly use the OVATEC® Plus kit because of its low price per sample and its quickness of application. We hypothesize that we would detect egg reappearance sooner after treatment with a more accurate method for FECs, but the increase or

decrease in parasite infection is still well represented. Finally, we might have been able to highlight weaker factors of parasite infection variation, in addition to the ones observed here.

4.3 Anthelmintics impacts on strongyle and coccidia infections

Anthelmintic treatment was efficient and significantly decreased strongyle egg shedding, and as all individuals presented a zero FEC count immediately after treatment, the possibility of parasite resistance was avoided. Furthermore, after treatment, the models showed an increase in FECs over time and a decrease in FOCs over time. Similar results have been reported in previous studies of nontargeted parasites after treatment with anthelmintics used with wild mice (Ferrari et al., 2009; Knowles et al., 2013; Pedersen and Antonovics, 2013), but to our knowledge, it has never been reported before in equids. The most plausible reason to explain the increase in FOCs is a competitive interaction between strongyles and coccidia, sharing the same infection site within the host (Dallas et al., 2019; Pedersen and Fenton, 2007). Anthelmintic treatment kills adult strongyles, creating less competition and a more favorable environment for coccidia development. This is supported by the fact that time since treatment was the only factor predictive of coccidia FECs after treatment, unlike degree, strength or dominance. It is also supported by the fact that coccidias rapidly decreased as the strongyle infection increased after treatment, as described by Knowles in 2013 in wild mice (Knowles et al., 2013). Under wild conditions, no coccidia oocysts have been observed in plains zebra feces, although they were described in other wild species living in proximity (Fugazzola and Stancampiano, 2012; Turner and Getz, 2010). However, coccidia is a common parasite in captive equids, including plains zebra (Dubey and Bauer, 2018), and is commonly observed in our study site in RAS. The difference in coccidia prevalence between captive and wild plains zebras must be the subject of future studies, with possible applications to parasite management in captive wildlife: Is coccidia infection a general matter for captive wildlife equids? What are the coccidia species that infect captive plains zebras? Do captive plains zebras share mechanisms of infection with horse populations known to be infected by coccidia? Moreover, the increase in coccidia oocyst shedding in captive plains zebras

observed after treatment in our study raises the question of what the possible consequences of anthelmintics are administered to captive wildlife. Further studies could contribute to a better understanding of anthelmintic use aftermaths on nontargeted parasite species.

4.4 Predictors of parasite infection

Considering that parasitic nematodes are mostly host species specific (Titcomb et al., 2022), grazing by multiple species can have a positive impact on individual parasitism due to a dilution effect (Mahieu, 2013; Rocha et al., 2008). As an example, individual parasite infection decreases and the time of effectiveness of anthelmintics increases when horses and bovines share the same enclosure (Forteau et al., 2020). In the current study, zebras share their enclosure with three species of ruminants and one species of bird; to our knowledge, none of them share parasite species with equids. Furthermore, the white rhinoceros present in part of the enclosure was not infected by either strongyle or coccidia (unavailable data collected by the veterinarians of the zoo), and direct interspecies contact between zebra and any of the four other species was not observed. Consequently, we hypothesized that if cross-species transmission between the zebras and the other species present within the enclosure is possible, we considered it negligible.

The prevalence of strongyle eggs in collected feces was 95% before treatment and 52% three months after treatment. Then, 20% of zebras, the lowest in the hierarchy, shed 70% of all strongyle eggs of the group observed during the last three months of the study. Our results, after treatment, are consistent with a previous study on captive horses that showed that 20% of individuals carried 85% of all strongyle eggs (Brazik et al., 2006; Scala et al., 2020; Stratford et al., 2011) and follow the widely known 20:80 rule also observed in wild zebra (Fugazzola and Stancampiano, 2012; Turner and Getz, 2010). This implies that targeted treatment could be applied in plains zebra populations living in safari enclosures, but it is crucial to precisely identify the 20% of individuals to be treated (Woolhouse et al., 1997). In our study, females shed more strongyle eggs than males, although the difference was not significant. Despite no effect of parturition on parasite infection intensity has been

demonstrated with equids (Steuer et al., 2022), lactation is known to induce immune relaxation in mares (Connan, 1976). As 11 of the 12 adult females studied were lactating, the global parasite infection intensity of females could be higher than that of males, as hypothesized in a previous study (Debeffe et al., 2016). In our study, parasite infection intensity did not depend on host age. However, previous studies showed that age as a quadratic factor explained the parasite infection distribution within a group, with older and younger individuals being more infected than middle-aged individuals (Lynsdale et al., 2020). Studies on wild zebras obtained divergent results, with adults once being more infected than foals (Seeber et al., 2019), and once being less infected than younger individuals (Fugazzola and Stancampiano, 2012). Seeber explained the difference by adult plains zebras spending more time grazing than foals (Seeber et al., 2019). In addition, mares present inhibited larvae of strongyle untargeted by anthelmintics that reactivate and migrate to the mammary gland around the time of parturition. In contrast, foals might also become infected with larvae present in the milk of the mother (Lyons and Tolliver, 2015; Mirck, 1977; Thamsborg et al., 1998; Tyrnenopoulou et al., 2021). However, we have no clue about the importance of this transmission compared to the infection risk of free larvae stages present in the environment, and we do not know if more infected mares transmit more larvae through their milk. Parasite infection in young animals can have a crucial impact on population viability, and variation in infection intensity among juvenile hosts is still poorly understood. A study quantifying gastrointestinal nematode infection of wild juvenile birds revealed that almost all chicks were infected (Granroth-Wilding et al., 2016). Thus, understanding the main routes of contamination and the prevalence of maternal vertical transmission is necessary to limit parasite dynamics in the infected captive population.

Parasite infection intensity depends on many social factors and their interactions (Craft, 2015; Ezenwa, 2004). Our study highlights two predictive factors of parasite infections in a captive plains zebra group after treatment with anthelmintics, all in relation to time since the administration of the treatment: degree of connection among the social network and individual dominance. Social networks seem to be a valuable indicator of parasite dynamics. Indeed, strongyle FECs of individuals with a

higher degree of connection within the group increase faster. The degree of connection is the most studied social network parameter to explain pathogen transmission (Briard and Ezenwa, 2021). A previous study reported such a correlation with direct cycle gastrointestinal parasite transmission in a chipmunk population (Gear et al., 2013). Contacts were approximated as sharing the same foraging area, and chipmunks sharing an area with a higher number of chipmunks were more infected. Thus, studying the space distribution of zebra in the enclosure would contribute to a better understanding of the link between these variables. However, in the current study, we designed a social network with affiliation behaviors. Among them, some include the possibility of ingesting larvae present in the environment. As an example, grooming consists of scratching the fur of another zebra with the teeth, and it has been demonstrated in mice that free-stage larvae of gastrointestinal parasites are present on the fur of the host, thus contaminating the other mice grooming the infested fur (Hernandez and Sukhdeo, 1995). Thus, multiplying the number of partners could increase the probability of ingesting an infectant stage larvae. The fact that the occurrence of social interactions between individuals is not a predictor of parasite infection intensity at the individual scale in our study makes it interesting to design a contact network with more specific behaviors that could contribute to parasite transmission (White et al., 2017). These behaviors remain to be defined (Briard and Ezenwa, 2021), but our study provides some clues for exploring the predictive success for parasite infection intensity of such contact networks with equids. In addition, the degree could be impacted by the subgroup size as the number of partners available for interaction might increase with the subgroup size. Despite there was no significant differences in infection intensity between subgroups in our study, larger groups show a higher prevalence of gastrointestinal parasites (Ezenwa and Worsley-Tonks, 2018; Patterson and Ruckstuhl, 2013), as much as pathogens in general (Nunn et al., 2015), and parasite infection of strongyles is also known to increase with population density with wild Soay sheep (Hayward et al., 2022). However, we found no difference in parasite infection intensity among the three subgroups studied. Finally, young individuals tend to have connections with a higher number of individuals within their respective groups but also with other groups as they change of groups when they grow

(Klingel, 1969). This can lead to higher parasite infection risk (Podgórski et al., 2018), although it has never been explored in equids and our study did not bring evidence of a relation between age and degree.

Our results revealed that strongyle FECs increase faster as the individual is lower in the hierarchy. This suggests that a low hierarchy rank either increases the risk of infection or decreases the access to anthelmintics distributed in free access in the enclosure. Both hypotheses are based on the possibility that subordinates forage in lower quality food areas than dominants. Indeed, foraging in low parasite infection risk areas could have an effect on the parasite infection intensity of wild zebra (Tombak et al., 2022), and as with horses (Krüger and Flauger, 2008), a behavioral study with captive zebra showed that dominants would have better access to distributed parasite-free food (Schilder and Boer, 1987). In the experimental conditions of our study, anthelmintics were mixed with daily distributed pellets in free access to all animals in the enclosure, suggesting that dominants would have more access to anthelmintics in addition to parasite-free food. Thus, they would better be treated, in addition to having less risk of contamination through foraging, explaining parasite infection distribution after treatment among the group. As recommended before with ivermectin administration in captive zebras (Lia et al., 2010), anthelmintics should be administered individually to assure that each zebra receives the right dose for proper treatment. Moreover, such a relation between dominance and parasite infection intensity is reported with wild populations of zebras in the same proportion of 20:80 as was observed in this study (Fugazzola and Stancampiano, 2012). This suggests that the mechanisms behind parasite contamination in captive conditions might be similar to those applied in wildlife. It is now essential to precisely evaluate resource access individually in captive plains zebra populations and take social behaviors into account to improve equid parasite management in captivity. Thus, it could reduce reinfestation risk after treatment and improve the distribution of drugs through the group.

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Animal welfare statement

The present study included plains zebra (*Equus quagga bohemii*) held captive in a southern French zoo: the Réserve Africaine de Sigean. All zebras were born in captivity. They share a 6ha safari enclosure with other species (six wildebeest (*Connochaetes taurinus*), four watusi oxen (*Bos taurus*), four ostriches (*Struthio camelus*) and one male white rhinoceros (*Ceratotherium simum*). Animals are accustomed to living with visitors driving on a definite path.

The present study is based on fecal analysis and behavioral observations. Both methods are not invasive. Behavioral observations were effectuated from a car with binoculars in the enclosure, never closer than 10 meters from animals. It took care of the animal's reaction, and if the animals were walking away from the car, observers immediately stopped the vehicle. Anthelmintic treatment is part of regular zoo veterinary protocols. No tag was used during the experiment, as the observer used zebra's stripes to individually identify them.

Our experimentations meet the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences and the ethical guidelines provided by the International Society for Applied Ethology (CIOMS and ICLAS, 2012; Sherwin et al., 2003).

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