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Surrogate hosts: Hunting dogs and recolonizing grey wolves share their endoparasites

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Understanding how closely related wildlife species and their domesticated counterparts exchange or share parasites, or replace each other in parasite life cycles, is of great interest to veterinary and human public health, and wildlife ecology. Grey wolves (*Canis lupus*) host and spread endoparasites that can either directly infect canid conspecifics or their prey serving as intermediate hosts of indirectly transmitted species. The wolf recolonization of Central Europe represents an opportunity to study parasite transmission dynamics between wildlife and domestic species for cases when a definitive host returns after local extinction – a situation equivalent to a 'removal experiment'.

Here we investigate whether the re–appearance of wolves has increased parasite pressure on hunting dogs – a group of companion animals of particular interest as they have a similar diet to wolves and flush wolf habitats when hunting. We compared prevalence (P) and species richness (SR) of helminths and the protozoan *Sarcocystis* to determine whether they were higher in hunting dogs from wolf areas ($n_{dogs} = 49$) than a control area ($n_{dogs} = 29$) without wolves. Of particular interest were *S. grueneri* and *S. taeniata*, known as 'wolf specialists'.

Five helminth and 11 *Sarcocystis* species were identified, of which all helminths and eight *Sarcocystis* species were shared between dogs and wolves. Overall prevalence and species richness of helminths (P:38.5% *vs.* 24.1%; SR_{mean}:0.4 vs. 0.3 species) and *Sarcocystis* (P:63.3% *vs.* 65.5%, SR_{mean}:2.1 *vs.* 1.8 species) did not differ between study sites. However, hunting dogs were significantly more likely to be infected with *S. grueneri* in wolf areas (P:45.2% *vs.* 10.5%; $p = 0.035$). The findings suggest that wolves indirectly increase *S. grueneri* infection risk for hunting dogs since cervids are intermediate hosts and occasionally fed to dogs. Furthermore, a periodic anthelminthic treatment of hunting dogs may be an effective measure to control helminth infections regardless of wolf presence.

1. Introduction

Many pathogens circulate in multi–host systems and do not depend on one single host species. Understanding the epidemiology of multi–host pathogens is critical to the 'One Health' concept as wildlife, domesticated animals and humans may be affected by such pathogens and share and exchange them (Taylor et al., 2001; Aguirre et al., 2002; Haydon et al., 2002; Thompson, 2013). Species or populations that maintain a pathogen and are responsible for its spill–over to a target species of interest are generally defined as "reservoirs" (Haydon et al., 2002; Hatcher and Dunn, 2011). In the context of conserving endangered species (van Kesteren et al., 2015; Millan et al., 2016), and recolonization or reintroduction projects (Almberg et al., 2012), the identification of pathogen reservoirs plays an important role for their success. Although spill–over to wildlife species and its effect on endangered or reintroduced species have received increasing attention, the influence of wildlife on closely related domesticated species has rarely been investigated (Thompson, 2013).

The return of an apex predator such as the grey wolf (*Canis lupus*) to a human–dominated landscape, from which it was absent for a century, is the equivalent of an (unintended) 'removal

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experiment'. Such an event provides an excellent opportunity to study how its close relative, the domestic dog, may be affected by the resurrection of parasite cycles for which returning wolves are definitive hosts. Currently, there 47 recognized wolf packs and 15 scent marking pairs in Germany that belong to the Western part of the Central European lowland wolf population (http://www.wolf-sachsen.de/de/verbreitung-in-deutschland), (Supplementary Fig. 1). According to the German Hunting Association (DJV, 2017b; https://www.jaegermagazin.de/jaeger-praxis/jagdschule/die-jagd-2016-in-zahlen) and the University of Göttingen (Ohr and Zeddies, 2006), the number of dogs in Germany ranges between 4.8 and 5.3 million, of which more than 300,000 are owned by hunters.

Given their similar biology and close relatedness, mesopredators like red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) and the wolf as an apex predator are known to share several helminth species with domestic dogs (Al-Sabi et al., 2013b; Otranto et al., 2015a). All of these carnivores have been recognized as hosts of the protozoan *Sarcocystis* (Barutzki and Schaper, 2011; Stronen et al., 2011; Prakas et al., 2015; Moré et al., 2016). However, it is unclear at present to what extent one canid may act as 'substitute' host for the other and how close their relationship as 'joint' definitive hosts of *Sarcocystis* is (Otranto et al., 2015b). This lack of information is very likely caused by methodological challenges, as there are no morphological techniques to discriminate *Sarcocystis* sporocysts or oocysts shed by definitive hosts (Xiang et al., 2009). With current molecular genetic tools such as metabarcoding, species detection from canid faecal samples has become possible, and recently wolves have been described as hosts for 12 *Sarcocystis* species (Lesniak et al., 2017). Furthermore, epidemiological studies of wolves and their ungulate prey species demonstrated that wolf presence increased the prevalence of sarcocysts in their ungulate prey. Accordingly, the *Sarcocystis* species *S. grueneri* and *S. taeniata*, that were identified as well−adapted to wolves and therefore termed 'wolf specialists', were mostly responsible for this increase (Lesniak et al., under review).

In general, hunting activities have been identified as a risk factor altering parasite infection risk, for instance, by the protozoan *Sarcocystis* (Thompson, 2013). In this context, hunting dogs − domestic dogs trained for hunting ungulates and other game − are of interest for several reasons. They can be considered the most likely source of pathogens or parasites that could be transmitted to wolves, but at the same time are potentially at risk of being exposed to wolf−derived parasites themselves. Transmission could occur when hunting dogs are used for hunting in wolf habitats or when fed with game meat by their owners (ESCCAP, 2010; Otranto et al., 2015b), which usually originates from the same ungulate species that wolves prey on (Wagner et al., 2012). While literature on companion dog parasites is regularly published (Barutzki and Schaper, 2003, 2011), little is known about the parasite fauna of hunting dogs, and it is unlikely that they are identical (Al-Sabi et al., 2013a; Gómez-Morales et al., 2016). The current wolf recolonization of Central Europe is therefore an ideal system to investigate the potential link between a wild apex predator and its domesticated equivalent, since hunting dogs can be examined in the presence and absence of wolves in comparable habitats. However, in such 'field experiments' several (unknown) factors that potentially influence parasite development, survival and transmission cannot be controlled for. This includes, e.g. the microclimate that might affect the survival of parasite stages in the environment (Randolph and Storey, 1999). Another relevant but uncontrollable factor is the anthelminthic treatment of dogs. Depending on a product's target site, it may selectively clear trematodes, cestodes, nematodes or all helminths, but anthelminthics have no effect on protozoa (Martin et al., 1997). Even though the *European Scientific Counsel for Companion Animal Parasites* recommends a monthly anthelminthic treatment for pets belonging to risk groups such as hunting dogs, the dewormification routine of dogs falls to their owners and may therefore be inconsistent and strongly differ among individuals (ESCCAP, 2010).

We hypothesized that wolves transmit endoparasites to hunting dogs. Such transmission might either occur directly from wolves via the environment to hunting dogs (no intermediate host required) or indirectly via intermediate hosts. There are several reasons why transmission effects might differ among parasites. In contrast to protozoan *Sarcocystis* species, transmission of helminth species might be strongly reduced or even absent because dogs usually undergo anthelminthic treatments. In addition, transmission might strongly differ among *Sarcocystis* species and might be particularly strong for species recognized as wolf specialists (Lesniak et al., under review) because of the similar biology of dogs and wolves. Taken these considerations into account, we predicted that (1) the general prevalence and species richness of *Sarcocystis* would be higher in hunting dogs from areas affected by wolf recolonization compared to hunting dogs from the control site, and that (2) particularly *Sarcocystis* species recognized as wolf specialists should show a higher prevalence in hunting dogs from the wolf area. Finally, we also predicted that (3) helminth prevalence and species richness of hunting dogs from wolf areas will not be increased compared to the control area.

2. Material and methods

2.1. Sample collection

Between November 2012 and January 2015, we collected 359 faecal triplicate samples of 78 hunting dogs residing in areas occupied by wolves in the German federal states of Brandenburg and Saxony (50°10′−53°33′ N and 11°14′−15°2′ E; $n_{dogs} = 49$, $n_{samples} = 230$). Hunting dogs were also sampled in a control area in the German federal state of Schleswig−Holstein (53°20′−54°55′ N and 8°36′−11°7′ E; $n_{dogs} = 29$, $n_{samples} = 129$) where no territorial wolves were recognized during the sampling period (Supplementary Fig. 1). Detailed information about the exact collection dates and application of anthelminthics for triplicate samples making up the faecal pools per dog are provided in Supplementary Table S2. If dog owners applied anthelminthics within the intended quarterly sampling schedule, they were asked to collect the triplicate sample beginning with the first faecal dropping after drug application. We intended to avoid a bias towards false negative findings, if samples were collected too late after helminths had been cleared. Additionally, this sampling strategy avoided missing particular parasite taxa, if e.g. a product against only nematodes was applied because we would capture both the flushed species cleared by the drug and the non−affected species due to their ongoing shedding of eggs.

Dog age, breed, function for hunting, information on routine of anthelminthic treatments and feeding habits were supplied by their owners who voluntarily supported the study (Supplementary Table S2). Hence, diet and medical treatments were not controlled by the authors but rely on the voluntary information of participants.

2.2. DNA extraction

Dog faeces were collected on three consecutive days and pooled. DNA was extracted using the NucleoSpin® Soil Kit (Macherey−Nagel, Düren, Germany) according to the manufacturer's protocol. This kit has previously been successfully used for DNA isolation from nematode and cestode eggs from faecal samples (Demeler et al., 2013; Maksimov et al., 2017). For subsequent