The Distribution of Gastrointestinal Parasites in Two Populations of Common Mole-Rats (*Cryptomys hottentotus hottentotus*)

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ABSTRACT: The spread of parasites through a host population is based on the variation in behavior and immune function between individuals and is rarely uniform. We studied the gastrointestinal parasites of common mole-rats (*Cryptomys hottentotus hottentotus*, Lesson 1826) from 2 sites and assessed the levels of infection based on host sex, breeding status and season. Only nematode species were found; *Neoheligmonella* sp. and *Mammalakis macrospiculum* (Ortlepp, 1939), and a single specimen of *Trichuris* sp., all of which have direct life cycles. Parasite burden and species richness was greater in the mesic habitat. The abundance of *Neoheligmonella* sp. differed significantly between seasons and the season of peak abundance differed between sites, perhaps due to differences in host densities between sites. In addition, parasite burden did not differ between the sexes but breeding animals had higher infections of *Neoheligmonella* sp. and *M. macrospiculum* than non-breeding animals. This and previous studies thus suggest that the subterranean environment is beneficial in reducing parasite diversity, although the restrictions on movement may lead to certain individuals suffering higher parasite burdens.

The probability of a host individual becoming infected with a parasite depends on both the likelihood that it will encounter the parasite and the ability of the individual to fight off the infection (Poulin, 2007). Due to behavioral differences between potential hosts and variation in immune function, parasite infections are seldom evenly spread throughout a host population (Wilson et al., 2002; Poulin, 2007). For example male vertebrates often have greater parasite burdens, either due to increased body size associated with sexual dimorphism, increased activity patterns and/or home range or reduced immune function as a result of androgens such as testosterone (Schalk and Forbes, 1997; Klein, 2000; Scantlebury et al., 2010). Temporal variation in parasite burden is also common. Free-living stages of parasites, subject to changes in the environment, may increase or decrease in abundance depending on environmental conditions (Altizer et al., 2006). Host behavior and immune function is also known to change seasonally, often leading to peak parasite loads in the breeding season (Christe et al., 2007; Møller et al., 2013). In subterranean hosts, the restrictions of the environment appear to impact on how parasite infections are spread due to limitations on host dispersal (Hafner et al., 2000; Rossin et al., 2010; Archer et al., 2016). Most studies on the parasite fauna of subterranean rodents indicate that parasite diversity is low as a consequence of the restrictive environment of the burrow (Hafner et al., 2000; Rossin and Malizia, 2002; Rossin et al., 2010; Lutermann and Bennett, 2012; Lutermann et al., 2015).

The common mole-rat (*Cryptomys hottentotus hottentotus*, Lesson 1826) is a member of the African mole-rat family (Bathyergidae), which are herbivorous, subterranean rodents endemic to sub-Saharan Africa (Bennett and Faulkes, 2000). Colonies of *C. h. hottentotus* contain up to 14 individuals comprising a breeding female, one or two breeding males and non-breeding 'helpers' (Spinks et al., 1999). It has a relatively widespread distribution compared to many other mole-rat species, distributed on the western side of South Africa from the south-west coast all the way to the Namibian border (Bennett and Faulkes, 2000). While it has been well studied in terms of its reproduction, foraging behavior and social dynamics, little work has been carried out on the parasites associated with *C. h. hottentotus* (de Graaff, 1964, 1981, Archer et al., 2014, 2016)

and there is virtually no information on their gastrointestinal parasites. Even earlier non-quantitative work done is not reliable due to more recent taxonomic changes in the genus *Cryptomys* Gray 1864 (Van Daele et al., 2007). However, studies have been carried out on 2 closely related species, the highveld (*C. h. pretoriae*, Roberts 1913) and Natal mole-rats (*C. h. natalensis*, Roberts 1915) providing some preliminary findings (Viljoen et al., 2011; Lutermann et al., 2013). The aim of the current study was to describe the endoparasite communities in *C. h. hottentotus* and evaluate the effect of season, host sex and breeding status on their spatial and temporal distribution. Previous studies on *Cryptomys* spp. have shown that cestode infections peaked during the dry season, which was attributed to the greater restrictions on host movement facilitating reinfection (Viljoen et al., 2011; Lutermann et al., 2013). The latter study also showed that in breeding individuals, males may carry higher parasite loads (Lutermann et al., 2013). Based on the above, we predict that the species richness sustained by *C. h. hottentotus* will be low, that parasite infection will peak in the dry season and that males will carry higher abundances of parasites.

Collections of mole-rats and the associated endoparasites were made from two farms in different habitats along the western coast of South Africa. The 'arid' site, was approximately 25 km outside of Kamieskroon, Northern Cape (30.13°S, 17.57°E) The 'mesic' site was near Darling, Western Cape (33.25°S, 18.25°E) Both of these sites are situated within the winter rainfall region of South Africa. *Cryptomys h. hottentotus* were collected on 2 separate occasions within each season (February – March, i.e., 'wet' season and June – August, i.e., 'dry') between February 2011 and August 2014. Individuals were captured using modified Hickman live traps baited with sweet potato (for detailed capture methods refer to Archer et al., 2014). Between dawn and dusk traps were checked every 2-3 hr until activity at the capture sites had ceased for more than 48 hr indicating that the entire colony had been captured. This criterion was applied to ensure that only complete colonies were sampled and a maximum of 2 wk elapsed before the entire colony was captured. Animals were housed with other members of their colony in plastic crates (41 x 28 x 25 cm minimum size) containing a minimum of 3 cm of soil and clip-lids with air holes and fed on sweet potato.

Each individual was overdosed with the anesthetic halothane, prior to being sexed and weighed. Breeding status was determined according to Spinks et al., (1999). The gastrointestinal (GI) tract was removed via dissection and stored in 70% ethanol. When processed, the GI tract was separated into 3 sections; stomach, small intestine and large intestine. Each section was opened, the contents were flushed out and then searched through for parasites using a binocular dissection microscope. The lining of the GI tract was also inspected. Endoparasites collected were stored in 70% ethanol until ready to be identified and sexed. Specimens were cleared and temporarily mounted in lactophenol and identified with the use of a compound light microscope. Reference specimens for all nematode species retrieved were deposited in the National Collection of Animal Helminths, ARC-Onderstepoort Veterinary Institute, South Africa (accession no. S/2016/5-15).

The overall prevalence and abundance for each parasite species across both populations was calculated, as well as per host individual (see Bush et al., 1997). Generalized linear mixed effects models (GLMMs) were carried out on the prevalence of each species. GLMMs were also carried out on the abundance of a given species where the mean abundance was greater than 2 (see below). Where parasites were present in both habitats, the site and season, as well as the interaction between site and season, were used as predictive variables. Otherwise season alone was used. Host sex and breeding status were included in all models only as main effects. The colony identity (an indication of which social group the individual came from) was included as a random effect. Prevalence data were analyzed using a binomial distribution with a logit link function and abundance data required a negative binomial distribution with a log link function. Where interaction terms were significant, a separate set of models were run to compare the different levels of the pairwise interactions to identify significant differences. Significant results from models are provided as the Z-value with the P-value. Models were run in R 3.2.3 (R Core Team, 2015) using

the glmmADMB and lme4 packages (Bates et al., 2015; Fournier et al., 2012; Skaug et al., 2016). We tested for biases in sex ratios of adult worms using binomial tests. Prevalence results are given as percentages, while abundance is reported as mean ±SE.

Data were collected from 245 *C. h. hottentotus*. From the arid site 127 animals were collected (38 and 89 from the dry and wet season, respectively) and 118 were from the mesic site (52 from the dry and 66 from the wet season). Only nematodes with direct life cycles were retrieved. A total of 2,621 nematodes were collected, mostly from 2 species (Table I). *Neoheligmonella* sp. was the most prevalent and abundant nematode species and found at both sites. In contrast, *Mammalakis macrospiculum* (Ortlepp, 1939) was only present in the mesic habitat, together with *Trichuris* sp., which was represented by a single specimen collected from the small intestine of its host. *Neoheligmonella* sp. (adult and L4 stage) were primarily found in the small intestine, with some L3 stages collected from the stomach (Table I). Adults and juveniles of *M. macrospiculum*, on the other hand occurred almost exclusively in the large intestine. Among adult worms both nematode species had a female bias which was significant for *Neoheligmonella* sp. ($P < 0.0001$) but not *M. macrospiculum* ($P = 0.064$, Table I).

The prevalence of *Neoheligmonella* sp. did not differ significantly between sites, seasons or between different sexes of the host (Table II). In contrast, the number of breeding *C. h. hottentotus* infected (67.7%) was significantly higher than non-breeding animals (33.1%; Table II). The abundance of *Neoheligmonella* sp. was significantly greater in the mesic (14.3 ±2.5) than the arid site (6.2 ±1.4; Table II). Additionally we found a significant difference in abundance of *Neoheligmonella* sp. between seasons though, unlike prevalence, overall mean abundance was greater in the dry (11.6 ± 2.9) than wet season (9.2 ±1.5, Table II). The interaction between site and season was significant (Table II; Fig. 1). Further analysis showed that there was no significant difference in *Neoheligmonella* sp. abundance between seasons in the wet site ($P = 0.45$). In contrast, the abundance of *Neoheligmonella* sp. was significantly lower in the dry season compared to the wet season in the arid site ($Z = 2.06$, $P = 0.04$, Fig. 1). It was also significantly lower than in both seasons in the mesic site ($P \leq 0.01$, Fig. 1). No other pairwise interactions were significant (GLM: $P \geq 0.09$). *Neoheligmonella* sp. abundance was significantly higher in breeding (21.3 ± 4.0) compared to non-breeding hosts (5.2 ±1.1; $Z = 3.62$, $P < 0.01$). Host sex did not have a significant effect on *Neoheligmonella* sp. abundance (Table II).

The prevalence of *M. macrospiculum* did not vary significantly with the seasons, nor was it influenced by host sex (Table II). However, the prevalence of this nematode was greater in breeding (79.4%) than in non-breeding animals (46.7%, Table II).

As expected, the species richness of gastrointestinal parasites in *C. h. hottentotus* was generally low with only 3 nematode species, one of which, *Trichuris* sp., was found in a single host individual only and may have been accidental. As in other subterranean mammals, low species richness of macroparasites is common in bathyergids (Viljoen et al., 2011; Lutermann and Bennett, 2012; Lutermann et al., 2013, 2015; Archer et al., 2014). *Mammalakis macrospiculum* has previously only been reported from *Bathyergus suillu*s (Schreber 1782), which occurs sympatrically with our study species in the mesic habitat and this may account for the host sharing (Inglis, 1991; Bennett and Faulkes, 2000; Lutermann and Bennett, 2012). Specimens of *Neoheligmonella* sp. could not be assigned to any of the hitherto described species of this genus (see Digiani and Durette-Desset, 2013), and might be a parasite typical of bathyergid hosts; confirmation of this would, however, necessitate further studies. Differences in the climatic conditions in the two sites may account for the absence of *Mammalakis macrospiculum* in the arid host population (Moore and Wilson, 2002).

Greater abundances of *Neoheligmonella* sp. were found in the mesic site compared to the arid site. Increased levels of moisture in the soil could be beneficial to the survival of the parasite itself as most trichostrongyloid L1 and L2 stages are free-living (Morand et al., 2006). Alternatively, greater population densities of *C. h. hottentotus* and increased movement between colonies in mesic areas could account for the greater infection of hosts at this site (Archer et al., 2016). A combination of greater host densities and increased frequency of inter-colony

interaction creating better opportunities for horizontal transfer could explain both the greater prevalence and abundance of *Neoheligmonella* sp. found in the mesic site (Archer et al., 2016).

However, the observation that the overall abundance of *Neoheligmonella* sp. was greater in the dry season despite greater prevalence in the wet season does not seem to support this hypothesis. Energetic constraints due to increased restrictions on foraging as well as higher re-infection rates within a colony as a result of smaller home ranges in the dry season may increase both host susceptibility and exposure to this worm and this could account for the observed pattern (Rossin et al., 2010; Viljoen et al., 2011).

Probably as a result of the shared burrow system sex-biases in nematode burden were absent in our study. In contrast, we found effects of the breeding status similar to what has been reported for the closely related Natal mole-rat (Lutermann et al., 2013). The larger body size of breeders in both sexes, as well as their reproductive investment and greater involvement in intra-colony encounters for breeding purposes may all have contributed to their elevated nematode burden (Schalk and Forbes, 1997; Bishop et al., 2007; Christe et al., 2007). Alternatively, if, like in other mole-rat species, breeders live longer than non-breeding animals, breeders may have accumulated more nematodes throughout their lifetime (Schmidt et al., 2013).

This furthermore suggests that breeding animals in these populations are likely to be the primary spreaders of nematode infection in the study species.

In conclusion, the species diversity of parasites in subterranean rodents appears to be consistently low. Differences in nematode burden between sites and seasons could be attributable to either direct effects of climatic conditions on the free-living stages of nematodes or indirect effects by their effects on the movement patterns of hosts. Host breeding status but not sex furthermore predicted nematode burden suggesting that body size, reproductive investment and/or dispersal behavior may increase exposure and/or susceptibility to nematode infection in social bathyergids.

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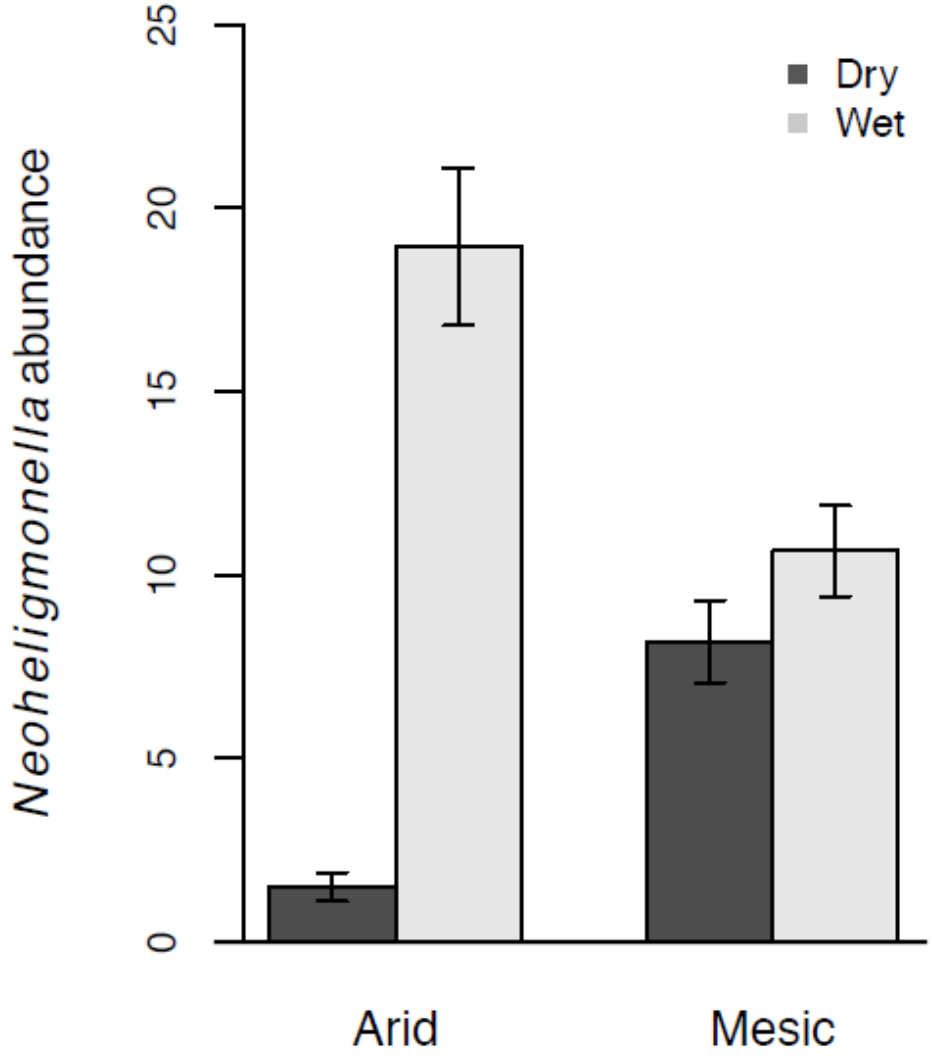

Figure 1. The seasonal variation in *Neoheligmonella* sp. abundance within a mesic and an arid site.

Table I: Nematode parasites found to be infecting *Cryptomys h. hottentotus* from the Northern and Western Cape Provinces, South Africa.

Taxon	Site	Infection site	Total count	Prevalence (%)	Abundance (mean ± SE)	Sex ratio (%)
Heligmonellidae						
Neoheligmonella sp.	arid/mesic	small intestine (L3 in stomach)	2473	44.90	10.09 ± 1.41	40.18♂, 47.52♀, 12.30 larvae
Kiwinematidae						
Mammalakis macrospiculum	mesic	large intestine	147	11.84	0.60 ± 0.16	40.44♂, 55.15♀, 4.41 larvae
Trichuridae						
Trichuris sp.	mesic	small intestine	1	0.41	0.17 ± 0.88	-

Table II: Results of the GLMMs evaluating the effects of site, season, host sex and breeding status on the prevalence and abundance of *Neoheligmonella* sp. and *Mammalakis macrospiculum*. Significant P-values indicated with *.

	***Neoheligmonella* sp.**		***M. macrospiculum***	
Variable	Prevalence	Abundance	Prevalence	Abundance
Site	Z = 1.95, P = 0.05	Z = 3.31, P < 0.01*	-	-
Season	Z = 1.81, P = 0.07	Z = 2.06, P = 0.04*	Z = 1.07, P = 0.29	-
Site x Season	Z = 0.75, P = 0.45	Z = 2.06, P = 0.04*	-	-
Sex	Z = 0.76, P = 0.45	Z = 1.89, P = 0.06	Z = 0.61, P = 0.54	-
Breeding status	Z = 4.17, P < 0.01*	Z = 5.01, P < 0.01*	Z = 2.23, P = 0.03*	-