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Regional and seasonal effects on the gastrointestinal parasitism of captive forest musk deer

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ABSTRACT

Parasite infections can cause adverse effects on health, survival and welfare of forest musk deer. However, few studies have quantified the parasite infection status and evaluated the parasite temporal dynamics and differences between breeding centers for captive forest musk deer. The purpose of this study was to assess seasonal and regional effects on the parasite prevalence, shedding capacity, diversity, aggregation and infracommunity to establish baseline data on captive forest musk deer. The McMaster technique was applied to count parasite eggs or oocysts in 990 fecal samples collected at three breeding centers located in Qinling Mountains and Tibetan Plateau during spring, summer, and winter. Five gastrointestinal parasite groups were found in musk deer, and *Eimeria* spp. were dominant (mean oocysts per gram = 1273.7 ± 256.3). A positive correlation between *Eimeria* spp. and *Strongyloides* spp. ($r = 0.336$, $p < 0.001$) based on shedding capacity data was found, as well as a negative correlation between *Eimeria* spp. and *Moniezia* spp. ($r = -0.375$, $p = 0.003$). Both seasonal and regional differences in diversity, prevalence, shedding capacity, aggregation and infracommunity were observed for five parasite groups. The low level of aggregation and high shedding capacity of *Eimeria* spp. and *Strongyloides* spp. might reflect the contaminated environment, and indicate that host-parasite relationships are unstable. The high degree of aggregation of *Trichuris* spp., *Ascaris* spp., and *Moniezia* spp. also suggests that some individual hosts had less ability to resist pathogens and greater transmission potential than others. These conclusions suggest that a focus on disease control strategies could improve the health of forest musk deer in captivity.

1. Introduction

Parasite infection poses major threats to the productivity, welfare, and survival of hosts. The spread and outbreak of parasites can reduce host survival and cause declines in population size in some cases (Ebert et al., 2000; Strona, 2015). Because of the poor immune response of the abomasum, ruminants are often more vulnerable to gastrointestinal parasites (Gasbarre, 1997). Many studies have explored the host-parasite relationship of ruminants, such as cattle (Li et al., 2015), sheep (Fthenakis et al., 2015), and deer (Davidson et al., 2014; Hernández and González, 2012), but few have investigated forest musk deer (FMD).

Musk deer are shy ruminants that are distributed throughout the forests and mountains of Asia, and China is a particularly important distribution area (Green, 1986; Yang et al., 2003). The musk secreted by adult males, is a raw material used in the perfume industry and Traditional Chinese Medicine. Steep declines in wild musk deer populations have resulted from over-exploitation and habitat destruction, particularly those of FMD, which has the largest yield and highest quality musk (Sheng and Liu, 2007). The breeding programs for FMD are a national ex situ protection strategy, which began in the 1950's and aims to provide sustainable musk resources. Though the farming of FMD has lasted for several decades, this species is still difficult to domesticate mainly because of its timidity and preference for being solitary (Zhang, 1983). With the progress in knowledge of FMD's management, the space for each captive FMD has increased a lot from little houses, to individual cells with an outdoor yard, and finally the semi-free-range. Nevertheless, the population size of captive FMD remains still low partly because of parasite infection. Based on the anatomy of dead FMD, *Moniezia* spp. and *Eimeria* spp. have a negative effect on

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survival (Liu et al., 2002; Yang et al., 2001b), especially for the young animals. The principal transmission routes for gastrointestinal parasites are soil (Bethony et al., 2006), diet (Vitone et al., 2004), social contact (MacIntosh et al., 2012), and intermediate hosts (Bush et al., 2001; Mbora and McPeek, 2009), whereas the spread of parasites can be affected by season (Viljoen et al., 2011), temperature (Tinsley et al., 2011), and humidity (Altizer et al., 2006; Setchell et al., 2007). A limited number of earlier studies on gastrointestinal parasites in FMD focused on diagnosis of single parasite species (Yang et al., 2001b), anthelmintic treatment (Yang et al., 2001a; Wang et al., 2011), and epidemiological characteristics of parasites in separate host populations (Liu et al., 2002; Wang et al., 2015b), yet comprehensive baseline data remains insufficient. Longitudinal studies of several populations in various geographical regions and details of temporal and spatial variations in parasitic infections would provide a baseline from which potential pathogenic changes could be identified (Bjork et al., 2000).

This study set out to determine the baseline parasite community and identify how this parasitism impacts on the health of captive FMD. Three FMD breeding centers located in southern Qinling Mountains and eastern Tibetan Plateau were chosen as study sites. The McMaster technique was applied to count parasite eggs or oocysts in 990 fecal samples collected at these three breeding centers during spring, summer, and winter of 2014, and the parasite prevalence, diversity, shedding capacity, infracommunity and parasite aggregation were measured to assess how seasonal and regional factors affect five measures of parasitism. The shedding capacity of different parasite groups was investigated to reveal patterns of coinfection.

2. Materials and methods

2.1. Study sites and animals

Qinling Mountains and the Tibetan Plateau are renowned for their rich biodiversity and also provide natural food and suitable climate for FMD, as such, these regions are ideal for breeding centers. The Huangniupu center (Huang) (34°11′N, 106°50′E) is located in Baoji, Shaanxi Province, a region of southern Qinling Mountains with an altitude of 1500 m. The Huoshaodian center (Huo) (33°35′N, 106°49′E) located in Hanzhong, Shaanxi Province, is also on the south slope of the Qinling Mountains with an altitude of 1400 m. The Miyaluo center (Mi) (34°11′N, 106°50′E) is located in Aba, Sichuan Province, a region of eastern Tibetan Plateau with an altitude of 2800 m. All three breeding centers have multiple adjacent enclosures and adopted the following reproductive system. One male and three females were kept in the same enclosure consisting of several individual brick cells ($2 \times 2 \times 1.5\ m^3$) and an outdoor yard ($15 \times 15\ m^2$). The FMD were kept together during the daytime but separated at night, so feces could be collected from individuals.

Musk deer were fed with leaves collected from their natural habitat, twice daily at 7:00 and 18:00. The plants mainly included *Ulmus pumila*, *Picrasma chinensis*, *Anacardiaceae rhus, Morus alba* and *Usnea diffracta.* Supplementary artificial food consisted of soybean flour, wheat bran, corn flour, milk, seasonal vegetables, and fruits. Fresh water was provided *ad libitum*. All musk deer are dewormed twice annually using albendazole tablets, and the treatment is ceased three months prior to the study through to the end of sampling.

2.2. Feces collection

A total of 990 fresh fecal specimens were collected from 330 FMD during spring, summer, and winter 2014, and each individual was sampled once daily and continuously for 3 days (Table 1). The feces from all houses was cleaned out every evening from 18:00 to 20:00 h, which could allow collection of fresh feces from each musk deer the next day at 7:00. Ear tags were used to distinguish each musk deer. All fresh fecal samples were preserved in 10% formalin solution at room temperature. All FMD included in the trial were aged between 3 and 5 years.

Table 1
The number of forest musk deer used for collecting fecal samples from Huangniupu breeding center during summer, Huoshaodian breeding center during summer, Miyaluo breeding center during summer, Miyaluo breeding center during winter, Miyaluo breeding center during spring.

Sampling sites	Sampling time			Total (M:F)
	Summer (M:F)	Winter (M:F)	Spring (All males)	
Huang	A 45(25:20)	–[a]	–[a]	45(25:20)
Huo	B 83(45:38)	–[a]	–[a]	83(45:38)
Mi	C 88(48:40)	D 70(40:30)	E 44	202(132:77)
Total (M:F)	216(118:98)	70(40:30)	44	330(202:128)

M, males; F, females.
[a] No sample collected.

2.3. Sample analysis and data analysis

The mean eggs per gram (EPG) or oocysts per gram (OPG) of parasites in fecal samples were counted using the McMaster technique (Cringoli et al., 2004). The copromicroscopic analysis was conducted within 2 weeks according to our previous study (Hu et al., 2016, Supplementary file 1).

The five measures of parasitism were defined for this study as: i) prevalence, the percentage of infected FMD within a host population; ii) shedding capacity, the number of eggs or oocysts that are shed from parasites per host (whilst the number of eggs or oocysts released cannot directly inform on the number of parasites present, this can give us an indication of the transmission potential for a given host); iii) parasite diversity, which is expressed as parasite groups richness (S), reflecting the number of parasite groups per deer; iv) parasite aggregation, is used to indicate the distribution pattern of parasites within FMD populations. Here we used the corrected moment estimate of k as per the following equation (Sherrard-Smith et al., 2015): $k = (x^2 - s^2/N)/(\sigma^2 - x)$, where x, σ^2 and N represent mean shedding capacity, variance, and sample size, which is an inverse measure of aggregation. Several measures including the corrected moment estimate of k (Gregory and Woolhouse, 1993), Poulin's Index of Discrepancy (Poulin, 1993), Boulinier's J (Boulinier et al., 1996) and Taylor's Power Law (Taylor and Taylor, 1977), had been developed to explore parasite aggregation. The k is the most commonly used measure because it was found to vary least with parasite abundance and sample size. v) infracommunity composition, is a comprehensive measure which is based on the number and shedding capacity of parasite groups in an individual FMD.

The one-sample Kolmogorov-Smirnov test was used to test the normality of the data. The effects of sex, season, and region on the prevalence were investigated using binomial generalized linear models (GLMs), and the negative binomial GLMs were used to model the effects of sex, season and region on shedding capacity. The shedding capacities of other parasites were included as explanatory variables in above GLMs. As for parasite diversity, we used multinomial GLMs to test the effects of factors. The two-sample Kolmogorov-Smirnov (K-S) test was applied to test the variation of distributions (aggregation) among sample groups. The paired-sample Friedman test was used to indicate differences in prevalence, shedding capacity, and aggregation among the parasite groups found in the fecal samples. Meanwhile, the correlation among shedding capacity of parasite groups was conducted using Spearman correlation analysis. All above statistical analyses were performed in SPSS ver. 20.0 software (IBM Corp., Armonk, NY, USA). Furthermore, non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarities of the log (x + 1) transformed shedding capacity was applied to rank the infracommunity, and one-way analysis