Analysis on the relationship between winter precipitation and the annual variation of horse stomach fly community in arid desert steppe, Northwest China (2007–2019)

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Abstract

Gasterophilus spp. have been found to be widespread in reintroduced Przewalski’s horses in the Kalamaili Nature Reserve (Northwest China). However, data on the annual variation in Gasterophilus infections are lacking. To analyze the epidemiological features and determine the cause of the annual variation in Gasterophilus infections, we treated 110 Przewalski’s horses with ivermectin and collected Gasterophilus larvae from fecal samples each winter from 2007 to 2019. All 110 Przewalski’s horses studied were found to be infected by Gasterophilus spp., and a total of 141,379 larvae were collected. Six species of Gasterophilus were identified with the following prevalence: G. pecorum (100%), G. nasalis (96.36%), G. nigricornis (94.55%), G. haemorrhoidalis (56.36%), G. intestinalis (59.09%), and G. inermis (3.64%). The mean infection intensity of Gasterophilus spp. larvae in Przewalski’s horses was 1285 ± 653. G. pecorum (92.96% ± 6.71%) was the most abundant species. The intensity of Gasterophilus spp. (r = –0.561, P < 0.046) was significantly correlated with winter precipitation. Our findings confirmed that, in the Kalamaili Nature Reserve, gasterophilosis is a severe parasitic disease in Przewalski’s horses. Winter precipitation at the beginning of the year can indirectly affect the intensity and composition of Gasterophilus spp. in Przewalski’s horses at the end of the year. Therefore, the water-related ecological regulation should be carried out to help reduce the parasite infection of Przewalski’s horses.

Key words: annual infection, arid desert steppe, Gasterophilus, Przewalski’s horses, winter precipitation
INTRODUCTION

Przewalski’s horses (Equus przewalskii) originate from the Hovd Basin of Western Mongolia and the eastern part of the Junggar Basin in China. The wild population became extinct in 1969, and the existing populations are the descendants of individuals captured 100 years ago (Mohr 1971). In order to restore wild populations of Przewalski’s horses, The Foundation Reserves for the Przewalski’s Horse (FRPH) was founded in 1980, and has sought to reintroduce Przewalski’s horses in their native habitat (Wit et al. 2012).

In 1985, the Przewalski’s horse was reintroduced in China. In 2001, 27 Przewalski’s horses were released in the Kalamaili Nature Reserve (KNR), and the wild population has grown to around 230 in 2019. The KNR is also home to other ungulates, including the Mongolian wild ass (Equus hemionus), goitered gazelle (Gazella subgutturosa), and Argali sheep (Ovis ammon), and it provides winter pastures for populations of the nomadic Kazakhs (Wang et al. 2014). Currently, 3 equid species inhabit the reserve: the Przewalski’s horse, the Mongolian wild ass, and overwintering domestic horses (E. caballus) (Huang et al. 2016).

Gasterophilus myiasis is a common disease of equine animals. The larvae of Gasterophilus species develop in the digestive tracts of equids, and the adults lay eggs on horses or grass. The generation time of these parasites is 1 year (Zumpt 1965). In China, 6 of the 9 known Gasterophilus species are present: G. haemorrhoidalis, G. inermis, G. intestinalis, G. nasalis, G. nigricornis, and G. pecorum (Xue & Zhao 1998). These species have all been reported to infect wild populations of Przewalski’s horse in the KNR (Liu et al. 2016). Although the KNR reserve department drives all released Przewalski’s horses back to fenced enclosures each winter, along with providing feed and administering ivermectin to ensure their health and reduce the impact of Gasterophilus spp. infection, the intensity of Gasterophilus spp. in the Przewalski’s horse remains significantly higher than in the other 2 equids in the KNR (Huang et al. 2016). Previous studies have found that the source of Gasterophilus spp. in Przewalski’s horses is the sympatric Mongolian wild asses and the domestic horses (Wang et al. 2014).

The KNR is located in arid desert steppe, with low precipitation, large evaporation, and no surface runoff, which made water source become an important factor restricting the range of wildlife activities in the reserve (Huang et al. 2016). Water is the source of life and has tremendous effects on animals. The shortage of water resources intensified the overlap of the activity space of Przewalski’s horses and Mongolian wild asses around the water source (Huang et al. 2017). Frozen water sources and accumulated snow in winter will melt by the end of winter and the beginning of spring, which will affect the water volume and distribution of the water source, thus indirectly affect the activity range and overlap of equine animals in spring. Simultaneously, the annual spring migration of herders and their horses from the south (winter pasture) to the north (summer pasture) was also affected by water resources (Wang et al. 2014). In addition, there was a relatively fixed phenology and regularity in the occurrence of Gasterophilus spp. every year, and it mainly occurred in spring in the KNR (Wang et al. 2015). Therefore, we speculate that the winter precipitation will affect the parasite infection intensity of Przewalski’s horses. The aim of the present study was to determine the factors that caused the variation in the epidemiological features of Gasterophilus spp. infection and to examine the effects of winter precipitation on the annual variation of infection in the released Przewalski’s horses.

MATERIALS AND METHODS

Study area

The KNR (latitude: 44°36′–46°00′N, longitude: 88°30′–90°03′E, altitude: 600–1464 m) covers an area of approximately 17 000 km². The vegetation in the KNR is sparse, mostly consisting of shrubs (Anabasis brevifolia, Ceratoides la ten) and herbage (Stipa caucasia). The area is characterized by a temperate continental arid climate, with a mean annual precipitation of 159 mm and a mean annual evaporation of 2090 mm and extremely little surface water (Zhang et al. 2015; Zang et al. 2017).

Study samples

Due to the annual freezing of water sources and shortage of plants in winter, the KNR reserve department drives all the released Przewalski’s horses back to fenced enclosures of Qiaomuxibai (45°14.18′N, 89°02.75′E) (Fig. 1) in November and release them back into the wild in March of the following year, and our study samples were derived from the Przewalski’s horses that were fenced each winter. However, some of the reintroduced populations have gradually adapted to the desert steppe and could follow the traces of the local indigenous species, the Mongolian wild ass, to explore a broader area to survive the harsh winter. Therefore, not all of the Przewalski’s horses can be cycled back to fenced enclosures every winter. In addition, although the released
population was managed and cared for every winter, they were still wild animals in the process of rewilding, thus, kept high vigilance around humans, making it nearly impossible to study and had to be released. With all the reasons mentioned above, we carried out the experiment, whenever possible, with around 10 horses per year, and the horses selected varying in sex and age. According to the characteristics of wild horses, their ages were categorized into: <2 years (foal and yearling), 2–4 years (subadult), and >4 years old (adult).

**Collection of Gasterophilus spp. larvae**

The high efficacy of ivermectin in controlling parasite infection has been established in horses (Bello 1996). In this study, Przewalski’s horses were selected randomly during each winter (December to February) from 2007 to 2019. Individuals were kept in separate fenced enclosures and treated once with orally administered ivermectin (Beijing Wangfeng Farming Pharmaceutical Co., Ltd., Veterinary Drug) at the conventional dose of 0.2 mg/kg (Costa et al. 1998). The age and sex of each individual was recorded. After ivermectin treatment, all feces in each fenced area were collected separately at a frequency of 3 times daily (1000, 1400, 1800). The collection of feces continued to the fifth day (Dawson 2003) or until there were no larvae in feces for 3 consecutive days.

Tweezers were used to break up the horse feces and to separate the larvae from the feces. Larvae were stored in ethanol (75%), washed with phosphate-buffered saline (PBS) or saline solution (0.9% NaCl), counted, and identified according to the identification method of the stomach bot fly larvae (Zumpt 1965; Li et al. 2018).

**Diversity**

Shannon–Wiener diversity index ($H'$) and Pielou evenness index ($J$) were used to analyze the diversity of stomach bot flies in different years (Shannon 1948; Pielou 1966), and the relevant formulas are as follows:

$$H' = - \sum Pi \ln Pi \quad (i = 1, 2, 3, \ldots, S)$$

$$J = H' / H'_{\text{max}}$$

In Equation (1), $Pi$ is the ratio of the infection intensity of species $i$ to the total intensity of *Gasterophilus* spp.; $S$ is the number of species; $H'_{\text{max}} = \ln S$.

**Meteorological data collection**

Winter precipitation data (December 1 to March 1 of the following year) for the relevant years were collected from the nearest observation point (45°22'N, 90°32'E) (CIMISS 2020). For example, the winter precipitation in 2007 is the accumulation of December 2006 plus January and February 2007, and 2019 is the accumulation of December 2018 and January plus February 2019.
Table 1 The prevalence of *Gasterophilus* spp. larvae in Przewalski's horses in the Kalamaili Nature Reserve

| Gasterophilus spp. | Sex          | Age       | No. | %         | No. | %         | No. | %         | No. | %         | No. | %         | No. | %         |
|------------------|--------------|-----------|-----|-----------|-----|-----------|-----|-----------|-----|-----------|-----|-----------|-----|-----------|
| G. pecorum       | Male         | <2 year   | 53  | 100       | 7   | 100       | 79  | 100       | 110 | 100       |     |           |     |           |
|                  | Female       | 2–4 year  | 57  | 100       | 24  | 100       | 77  | 97.47     | 106 | 96.36     |     |           |     |           |
|                  |              | >4 year   |     |           | 79  | 100       | 76  | 96.20     | 104 | 94.55     |     |           |     |           |
|                  |              | Total     |     |           | 110 | 100       | 104 | 100       | 208 | 100       |     |           |     |           |
| G. nasalis       | Male         | <2 year   | 50  | 94.34     | 4   | 57.14     | 77  | 97.47     | 106 | 96.36     |     |           |     |           |
|                  | Female       | 2–4 year  | 55  | 96.49     | 24  | 100       | 77  | 97.47     | 106 | 96.36     |     |           |     |           |
|                  |              | >4 year   |     |           | 77  | 97.47     | 76  | 96.20     | 104 | 94.55     |     |           |     |           |
|                  |              | Total     |     |           | 106 | 96.36     | 104 | 94.55     | 210 | 97.05     |     |           |     |           |
| G. nigricornis   | Male         | <2 year   | 51  | 96.23     | 4   | 57.14     | 76  | 96.20     | 104 | 94.55     |     |           |     |           |
|                  | Female       | 2–4 year  | 53  | 92.98     | 24  | 100       | 76  | 96.20     | 104 | 94.55     |     |           |     |           |
|                  |              | >4 year   |     |           | 76  | 96.20     | 76  | 96.20     | 152 | 95.42     |     |           |     |           |
|                  |              | Total     |     |           | 104 | 94.55     | 152 | 95.42     | 256 | 99.97     |     |           |     |           |
| G. intestinalis  | Male         | <2 year   | 28  | 52.83     | 5   | 71.43     | 16  | 66.67     | 44  | 55.70     |     |           |     |           |
|                  | Female       | 2–4 year  | 37  | 64.91     | 24  | 100       | 77  | 97.47     | 106 | 96.36     |     |           |     |           |
|                  |              | >4 year   |     |           | 77  | 97.47     | 76  | 96.20     | 104 | 94.55     |     |           |     |           |
|                  |              | Total     |     |           | 106 | 96.36     | 106 | 96.36     | 212 | 100       |     |           |     |           |
| G. haemorrhoidalis| Male       | <2 year   | 26  | 49.06     | 4   | 57.14     | 18  | 75.00     | 40  | 50.63     |     |           |     |           |
|                  | Female       | 2–4 year  | 36  | 63.16     | 18  | 75.00     | 40  | 50.63     | 80  | 50.00     |     |           |     |           |
|                  |              | >4 year   |     |           | 36  | 63.16     | 36  | 63.16     | 72  | 42.96     |     |           |     |           |
|                  |              | Total     |     |           | 82  | 60.71     | 72  | 42.96     | 154 | 100       |     |           |     |           |
| G. inermis       | Male         | <2 year   | 2   | 3.77      | 1   | 14.29     | 3   | 3.80      | 4   | 3.64      |     |           |     |           |
|                  | Female       | 2–4 year  | 2   | 3.51      | 0   | 0         | 3   | 3.80      | 4   | 3.64      |     |           |     |           |
|                  |              | >4 year   |     |           | 2   | 3.77      | 2   | 3.51      | 4   | 3.64      |     |           |     |           |
|                  |              | Total     |     |           | 4   | 3.64      | 4   | 3.64      | 8   | 3.64      |     |           |     |           |

Statistical analysis

The prevalence, intensity, and abundance of infection were estimated according to the definitions provided by Margolis and Schad (1982). The relationships between the intensity of *Gasterophilus* spp. and winter precipitation were explored using Spearman’s rank correlation. Tukey–Kramer tests were conducted to identify if the infection intensity of different ages and the proportion of *Gasterophilus* species significantly changed. The independent t-test was used to examine the sex difference of infection intensity. The statistical analysis was performed using SPSS 20.0 and the figures were drawn by SigmaPlot 12.5. Significant differences were assumed when $P \leq 0.05$.

RESULTS

Prevalence of *Gasterophilus* spp. larvae

Over the 13 years of this study, the overall prevalence of infection with *Gasterophilus* spp. larvae was 100% among the 110 examined Przewalski’s horses. The collected *Gasterophilus* spp. larvae prevalences were as follows: *G. pecorum* was the most common species (found in 100% of horses) in the Przewalski’s horse, followed by *G. nasalis* (96.49%), *G. nigricornis* (94.55%), *G. intestinalis* (59.09%), *G. haemorrhoidalis* (3.46%), and *G. inermis* (range: 0–0.13%), which was only found in 2008, 2013, and 2016 ($F = 11626.697, \text{df} = 5654, P < 0.001$) (Table 2).

The majority of the infected Przewalski’s horses (78.18%) harbored between 500 and 2000 larvae, and 13.64% had more than 2000 larvae, only 8.18% had less than 500 larvae (Table 3). The average intensity of males (1310.34 ± 636.11) was similar to that of females (1261.95 ± 673.01) ($t = 0.387, \text{df} = 108, P = 0.700$). There were no significant differences in infection intensity among horses of different ages ($F = 0.054, \text{df} = 2107, P = 0.948$) (Table 3).

Diversity

With the exceptions of 2007, 2008, and 2010, the diversity of horse stomach bot flies in different years showed...
Table 2 The intensity and composition of *Gasterophilus* spp. larvae in Przewalski’s horses in the Kalamaili Nature Reserve

| Year | Przewalski’s horses (No.) | *Gasterophilus* spp. (No.) | G. pecorum | G. nigricornis | G. nasalis | G. haemorrhoidalis | G. intestinalis | G. inermis | % Mean ± SD | % Mean ± SD | % Mean ± SD | % Mean ± SD |
|------|---------------------------|----------------------------|------------|---------------|------------|-------------------|----------------|------------|-------------|-------------|-------------|-------------|
|      |                           | Total                      | Range      | Mean ± SD     | 84.47 ± 3.38 | 6.99 ± 2.67 | 6.95 ± 2.32 | 1.42 ± 0.44 | 0.18 ± 0.13 | 0.02 ± 0.04 | 0.04 ± 0.02 | 0.02 ± 0.00 |
| 2007 | 4                         | 4184                       | 817–1324   | 1046.00 ± 234.14 | 84.47 ± 3.38 | 6.99 ± 2.67 | 6.95 ± 2.32 | 1.42 ± 0.44 | 0.18 ± 0.13 | 0.02 ± 0.04 | 0.04 ± 0.02 |
| 2008 | 4                         | 3369                       | 592–1303   | 842.25 ± 329.34 | 82.57 ± 7.73 | 13.74 ± 7.21 | 1.31 ± 1.02 | 2.27 ± 0.80 | 0.09 ± 0.07 | 0.02 ± 0.04 | 0.02 ± 0.00 |
| 2009 | 10                        | 17108                      | 633–2491   | 1710.80 ± 636.12 | 91.88 ± 3.97 | 2.82 ± 2.11 | 3.68 ± 1.83 | 1.54 ± 1.23 | 0.08 ± 0.15 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2010 | 10                        | 7674                       | 300–1592   | 767.40 ± 433.27 | 79.45 ± 8.62 | 12.73 ± 8.42 | 7.33 ± 4.16 | 0.27 ± 0.23 | 0.22 ± 0.36 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2011 | 10                        | 7176                       | 173–1247   | 717.60 ± 364.19 | 94.67 ± 2.99 | 0.98 ± 1.19 | 3.06 ± 2.00 | 1.18 ± 0.66 | 0.11 ± 0.12 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2012 | 10                        | 13291                      | 558–2093   | 1329.10 ± 496.95 | 95.15 ± 2.81 | 1.27 ± 1.39 | 1.44 ± 0.80 | 1.75 ± 1.11 | 0.39 ± 0.36 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2013 | 9                         | 11514                      | 630–1732   | 1279.33 ± 366.51 | 96.38 ± 1.61 | 0.48 ± 0.34 | 1.55 ± 1.10 | 1.41 ± 1.35 | 0.16 ± 0.18 | 0.02 ± 0.05 | 0.00 ± 0.00 |
| 2014 | 5                         | 10271                      | 1404–3571  | 2054.20 ± 877.25 | 98.52 ± 1.46 | 0.59 ± 0.78 | 0.77 ± 0.79 | 0.04 ± 0.10 | 0.08 ± 0.11 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2015 | 9                         | 11506                      | 689–2425   | 1278.44 ± 638.80 | 96.85 ± 3.09 | 1.56 ± 1.16 | 1.53 ± 2.03 | 0.04 ± 0.12 | 0.03 ± 0.08 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2016 | 7                         | 8186                       | 1007–1441  | 1169.43 ± 145.25 | 96.35 ± 4.13 | 2.49 ± 3.97 | 0.83 ± 0.78 | 0.07 ± 0.10 | 0.25 ± 0.40 | 0.01 ± 0.03 | 0.00 ± 0.00 |
| 2017 | 9                         | 7846                       | 357–1371   | 871.78 ± 327.05 | 94.88 ± 2.68 | 2.05 ± 1.84 | 2.37 ± 1.36 | 0.00 ± 0.00 | 0.70 ± 0.84 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2018 | 9                         | 14354                      | 947–2779   | 1594.89 ± 628.25 | 95.65 ± 1.80 | 2.33 ± 1.30 | 1.89 ± 1.44 | 0.05 ± 0.09 | 0.07 ± 0.12 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 2019 | 14                        | 24900                      | 724–3066   | 1778.57 ± 787.77 | 94.65 ± 3.90 | 2.95 ± 3.65 | 2.21 ± 2.00 | 0.00 ± 0.00 | 0.20 ± 0.28 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Total| 110                       | 141379                     | 173–3571   | 1285.26 ± 652.94 | 92.96 ± 6.71 | 3.46 ± 5.11 | 2.68 ± 2.66 | 0.69 ± 1.00 | 0.21 ± 0.36 | 0.00 ± 0.00 | 0.00 ± 0.00 |
Figure 2 Infection intensity and diversity of *Gasterophilus* spp. larvae in Przewalski’s horses from 2007 to 2019. (a) Infection intensity of *Gasterophilus* spp.; (b) diversity index.

Table 3 Number and percentage of *Gasterophilus* spp. in Przewalski’s horses grouped according to size of infection, sex, age

| Size of infection | Examined | Infection intensity |
|-------------------|----------|---------------------|
|                   | No.      | %       | (Mean ± SD)         |
| 0–500             | 9        | 8.18    | 365.56 ± 97.48      |
| 501–1000          | 29       | 26.36   | 760.41 ± 138.51     |
| 1001–1500         | 42       | 38.18   | 1241.21 ± 148.94    |
| 1501–2000         | 15       | 13.64   | 1720.40 ± 172.38    |
| 2001–2500         | 9        | 8.18    | 2276.22 ± 138.72    |
| 2501–3000         | 4        | 3.64    | 2744.25 ± 188.51    |
| 3001–3500         | 1        | 0.91    | 3066                |
| 3501–4000         | 1        | 0.91    | 3571                |
| Sex               |          |         |                     |
| Male              | 53       | 48.18   | 1310.34 ± 636.11    |
| Female            | 57       | 51.82   | 1261.95 ± 673.01    |
| Age               |          |         |                     |
| <2 years          | 7        | 6.36    | 1313.29 ± 420.61    |
| 2–4 years         | 24       | 21.82   | 1247.25 ± 756.99    |
| >4 years          | 79       | 71.82   | 1294.33 ± 642.34    |
that the Shannon–Wiener diversity index ($H'$) was lower than 0.40, especially in 2014 ($H' = 0.06$) (Fig. 2b). Similarly, the Pielou evenness index ($J'$) also showed similar characteristics.

### Correlation analysis between winter precipitation and Gasterophilus spp. intensities

During the period of investigation, winter precipitation was highest in 2010 (Fig. 3). There were significant negative correlations between winter precipitation and the intensities of Gasterophilus spp. ($r = -0.561, n = 13, P < 0.046$), meaning that years with higher rainfall induced a lower Gasterophilus spp. population and vice-versa. The increasing or decreasing tendencies of the intensities of Gasterophilus spp. and G. pecorum in relation to winter precipitation were demonstrated using a linear model (Fig. 4). Gasterophilus spp.: $y = -29.063x + 2057.620$ ($R^2 = 0.1540, P < 0.001$); G. pecorum: $y = -29.134x + 1979.048$ ($R^2 = 0.1637, P < 0.001$).

### DISCUSSION

The findings of the present survey revealed 100% prevalence and high intensity (1285) of Gasterophilus spp. infection in Przewalski’s horses, which was
considerably higher than that among equids in most parts of the world (Pandey et al. 1992; Agneessens et al. 1998; Studzińska & Wojcieszak 2009; Getachew et al. 2011; Ibrayev et al. 2015). We administered ivermectin to the Przewalski’s horses almost every year, and these results indicated the importance of this parasite and that cross-infection of Gasterophilus spp. was prevalent among the 3 types of equid inhabiting the KNR. In addition, there were no correlations between the infection intensity of Gasterophilus spp. larvae and gender and age of Przewalski’s horses in this study.

In almost every year of the study, G. pecorum accounted for more than 90% of the total infection. This may be due to its unique behaviors. Although the adults of Gasterophilus spp. survive for less than 1 week, the eggs of G. pecorum can survive for several months under natural conditions (Zumpt 1965). G. pecorum is unique among Gasterophilus spp. in that it oviposits on grass (Chereshnev 1951). The number of eggs of G. pecorum (1300–2425) is higher than that of G. intestinalis (397–770), G. nasalis (489–518), G. nigricornis (330–350), and G. haemorrhoidalis (134–167) (Zumpt 1965). Chereshnev (1951) reported that the female G. pecorum oviposited 10–15 eggs on each plant, and one female can infect a large area of pasture. The high fecundity and oviposition on grass may thus increase infection probability and extend the time for infection. These features could make G. pecorum more efficient at infecting Przewalski’s horses in sparsely vegetated areas.

The species composition of Gasterophilus may be affected by the host, the parasite, and/or environmental and management factors (Otranto et al. 2005). Furthermore, intraspecific effects may have a significant impact on parasite population regulation (Moller 2005). In general, the proportion of G. pecorum increased, whereas that of some other species decreased over time in the KNR. Annual ivermectin treatments and hostile interactions between Gasterophilus spp. due to interspecific competition may be factors that contributed to these trends.

The diversity of community reflects the characteristics of community structure (Watte & Sukumar 1995; Bechtel et al. 2015). Although there is an abundance of species of Gasterophilus spp. in the KNR, the dominant species, G. pecorum, accounts for a large proportion, resulting in a low diversity in this area. In recent years (2011–2019), the diversity indexes in the KNR have been in a low state ($H < 0.30, J < 0.20$), which were lower than that of other regions in the world, such as South Africa ($H = 0.82, J = 0.75$) (Kreeze et al. 1989), Southern Italy ($H = 1.10, J = 0.68$) (Otranto et al. 2005), and Eastern Turkey ($H = 0.72, J = 0.66$) (Özdağ et al. 2010). Each natural system is composed of many factors that can affect diversity (Frank 1993). The biological and environmental factors are conducive to the adaptation of G. pecorum to desert steppe, resulting in a large number of G. pecorum in the KNR. The stable selection stimulated the prevalence of a specific species and reduced community diversity.

In the KNR, the main source of G. pecorum in Przewalski’s horses is the Mongolian wild ass (Wang et al. 2014). The KNR is located in a desert steppe, water resources are the most important factor limiting the range of wild equines. Therefore, Przewalski’s horses and Mongolian wild asses had close contact in the KNR. Przewalski’s horses appear to drink daily (Scheibe et al. 1998), and the oviposition sites of G. pecorum are often located near water sources (Liu et al. 2015). Lugauer found that established Przewalski’s horses had longer daily travel distances than newly released individuals (Lugauer 2010). The released Przewalski’s horses have not been completely restored to the wild; therefore, they are less sensitive to unnatural interference than Mongolian wild asses. Based on the fact that the cross-spread of G. pecorum were between the 2 equine species, the interspecific adaptation difference in equines may explain the higher prevalence and density of G. pecorum population in this region, as well as the higher number in Przewalski’s horses.

Meteorological factors may affect the population of Gasterophilus spp. (Sequeira et al. 2001), and in this regard, Pilo et al. (2015) found that the egg-laying activity of Gasterophilus spp. was correlated with minimum air temperature and was reduced by rainfall in November and December. In this study, the infection intensity of G. pecorum was negatively related to winter precipitation. The availability of water is an important factor determining the distribution and habitat use of Przewalski’s horses and Mongolian wild asses (Kaczensky et al. 2008). The variability in host distribution may alter parasite occurrence (Pickles et al. 2013). In the KNR, the shortage of water increases the interaction between Przewalski’s horses and Mongolian wild asses (Huang et al. 2017), and the main infection period of Gasterophilus spp. is in spring (Wang et al. 2015). A higher winter precipitation may decrease the likelihood of sharing water sources and thereby reduce the niche overlap between Przewalski’s horses and Mongolian wild asses, thus resulting in lower infection intensities of G. pecorum in Przewalski’s horses.

Ecological regulation can be adopted to reduce the parasite infection of Przewalski’s horses. Artificial water sources can be built in spring to reduce the aggregation of equines during the infection period of stomach bot flies, and help to reduce the cross-spread of bot flies among equines. Considering the distribution of existing
natural water sources in this region, new artificial water sources should be scattered, so as to avoid aggravating cross-infection between animals. Especially in the years with less winter precipitation, control and management measures should be prepared in advance to reduce the cross-infection of 2 wild equines during the transmission season of stomach bot flies and avoid acute occurrences of myiasis in equines.

CONCLUSIONS

In this study, we confirmed that infection by Gasterophilus spp. in released Przewalski’s horses is widespread in the KNR. We detected the occurrence of 6 species of Gasterophilus and a high intensity of infection in Przewalski’s horses, and accordingly suggest that the KNR reserve department works to improve awareness of this major parasite. G. pecorum, the most abundant species, needs further investigation, allowing for better understanding of its infection cycle and to develop better control measures. In addition, winter precipitation can indirectly affect the intensity and composition of Gasterophilus spp. throughout the following year. Given that the local Mongolian wild asses and domestic horses appear to play important roles in transmitting Gasterophilus spp. infection to Przewalski’s horses, it is necessary to take appropriate measures to reduce the cross-infection between these equines.

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CONFLICT OF INTEREST

The authors declare no conflicts of interests.

ETHICS STATEMENT

The study was performed in accordance with the relevant guidelines and regulations regarding animal welfare. All experimental protocols were approved by the Ethnic and Animal Welfare Committee, Beijing Forestry University (Beijing, China).

REFERENCES

Agneessens J, Engelen S, Debever P, Ver Cruyssse J (1998). Gasterophilus intestinalis infections in horses in Belgium. Veterinary Parasitology 77, 199–204.

Buchtel MJ, Teglas MB, Murphy PJ (2015). Parasite prevalence and community diversity in sympatric and allopatric populations of two woodrat species (Sigmomontinae: Neotoma) in central California. Journal of Wildlife Diseases 51, 419–30.

Bello TR (1996). Alternative antiparasitic treatment of horses with pyrantel pamoate suspension and ivermectin oral solution compared with horses treated only with ivermectin oral solution. Journal of Equine Veterinary Science 16, 106–10.

Cheresheh NA (1951). Biologic properties of the gadfly Gasterophilus pecorum Fabr. (Diptera: Gasterophilidae). Doklady Akademii Nauk SSSR 77, 765–8 (in Russian).

CIMISS (China Integrated Meteorological Information Service System) (2020). Monthly precipitation database. [Cited 15 Nov 2020]. Available from URL: http://data.cma.cn

Costa AJ, Barbosa OF, Moraes FR et al. (1998). Comparative efficacy evaluation of moxidectin gel and ivermectin paste against internal parasites of equines in Brazil. Veterinary Parasitology 80, 29–36.

Dawson K (2003). A non-lethal method for assessment of efficacy of antiparasitics against parasites in horses such as Anoplocephala perfoliata and Gasterophilus intestinalis. Veterinary Parasitology 115, 67–70.

Frank SA (1993). Evolution of host-parasite diversity. Evolution 47, 1721–32.

Getachew AM, Innocent G, Crawford AF, Reid SW, Love S (2011). Gasterophilosis: A major cause of rectal prolapse in working donkeys in Ethiopia. Tropical Animal Health and Production 44, 757–62.

Huang HQ, Zhang BR, Chu HJ, Zhang D, Li K (2016). Gasterophilus (Diptera, Gasterophilidae) infection of equids in the Kalamaili Nature Reserve, China. Parasite 23, 36.

Huang HQ, Zhang D, Li K et al. (2017). Distribution of Gasterophilus (Diptera: Gasterophilidae) myiasis foci in arid desert steppe: A case study of Kalamaili Mountain Ungulate Nature Reserve. Scientia Silvae Sinicae 53, 142–9. (In Chinese with English abstract.)

Ibrayev B, Lider L, Bauer C (2015). Gasterophilus spp. infections in horses from northern and central Kazakhstan. Veterinary Parasitology 207, 94–8.
Kaczensky P, Ganbaatar O, Wehrden HV, Walzer C (2008). Resource selection by sympatric wild equids in the Mongolian Gobi. *Journal Applied Ecology* **45**, 1762–9.

Krecek RC, Reinecke RK, Horak IG (1989). Internal parasites of horses on mixed grassveld and bushveld in Transval, Republic of South Africa. *Veterinary Parasitology* **34**, 135–43.

Li XY, Chen YO, Wang QK, Li K, Pape T, Zhang D, Krecek RC, Reinecke RK, Horak IG (1989). Internal parasites of horses in the Mongolian Gobi. *Journal Applied Ecology* **45**, 1762–9.

Liu SH, Hu DF, Li K (2015). Oviposition site selection by *Gasterophilus pecorum* (Diptera: Gasterophilidae) in its habitat in Kalamaili Nature Reserve, Xinjiang, China. *Parasite* **22**, 34.

Liu SH, Li K, Hu DF (2016). The incidence and species composition of *Gasterophilus* (Diptera, Gasterophilidae) causing equine myiasis in northern Xinjiang, China. *Veterinary Parasitology* **217**, 36–8.

Lugauer B (2010). Differences of movement pattern between Asiatic wild ass (*Equus hemionus*) and Przewalski’s horse (*Equus ferus przewalskii*) (M.S. thesis). University of Vienna, Vienna, pp. 15–6.

Margolis L, Schad GA (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *Journal of Parasitology* **68**, 131–3.

Mohr E (1971). *The Asiatic Wild Horse*, 2nd edn. J.A. Allen & Co. Ltd, London.

Moller AP (2005). Parasitism and the regulation of host populations. In: Thomas F, Renaud F, Guégan J, eds. *Parasitism and Ecosystems*. Oxford University Press, Oxford, pp. 43–53.

Otranto D, Milillo P, Capelli G, Colwell DD (2005). Species composition of *Gasterophilus* spp. (Diptera, Oestridae) causing equine gastric myiasis in southern Italy: parasite biodiversity and risks for extinction. *Veterinary Parasitology* **133**, 111–8.

Özdal N, Bicek K, Orung O, Deger S (2010). Presence of *Gasterophilus* species in horses in Van region. *Yüzünçü Yıl Üniversitesi Veteriner Fakültesi Dergisi* **21**, 87–90.

Pandey VS, Ouhelli H, Verhulst A (1992). Epidemiological observations on *Gasterophilus intestinalis* and *Gasterophilus nasalis* in donkeys from Morocco. *Veterinary Parasitology* **41**, 285–92.

Pickles RS, Thornton D, Feldman R, Marques A, Murray DL (2013). Predicting shifts in parasite distribution with climate change: A multitrrophic level approach. *Global Change Biology* **19**, 2645–54.

Pielou ECJ (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* **13**, 131–44.

Pilo C, Altea A, Scala A (2015). Gasterophilosis in horses in Sardinia (Italy): Effect of meteorological variables on adult egg-laying activity and presence of larvae in the digestive tract, and update of species. *Parasitology Research* **114**, 1693–702.

Scheibe KM, Eichhorn K, Kalz B, Streich WJ, Scheibe A (1998). Water consumption and watering behavior of Przewalski horses (*Equus ferus przewalskii*) in a semireserve. *Zoo Biology* **17**, 181–92.

Sequeira JL, Tostes RA, Oliveira-Sequeira TCG (2001). Prevalence and macro- and microscopic lesions produced by *Gasterophilus nasalis* (Diptera: Oestridae) in the Botucatu region, SP, Brazil. *Veterinary Parasitology* **102**, 261–6.

Shannon CE (1948). A mathematical theory of communication. *Bell System Technical Journal* **27**, 623–56.

Sudzinska MB, Wojcieszak K (2009). *Gasterophilus* sp. botfly larvae in horses from the south-eastern part of Poland. *Bulletin of the Veterinary Institute in Pulawy* **53**, 651–5.

Wang KH, Zhang D, Li K et al. (2015). Developmental threshold temperature and effective accumulated temperature for pupae of *Gasterophilus pecorum*. *Chinese Journal of Vector Biology and Control* **26**, 572–5. (In Chinese with English abstract.)

Wang WT, Hu DF, Li K et al. (2012). The Przewalski’s horse and its reintroduction in the steppe of Hustai National Park, Mongolia. In: Werger MJA, van Staalduinen MA, eds. *Eurasian Steppe*. *Ecological Problems and Livelihoods in a Changing World*. Springer, Dordrecht, pp. 357–88. https://doi.org/10.1007/978-94-007-3886-7_13

Xue WQ, Zhao J M (1998). *Flies in China*, Vol. II. Liaoning Science and Technology Press, Shenyang. (In Chinese.)
Zang S, Cao Q, Alimujiang K, Liu SH, Zhang YJ, Hu DF (2017). Food patch particularity and foraging strategy of reintroduced Przewalski’s horse in North Xinjiang, China. *Turkish Journal of Zoology* **41**, 924–30.

Zhang YJ, Cao Q, Leimgruber P et al. (2015). Water use patterns of sympatric Przewalski’s horse and Khulan: interspecific comparison reveals niche differences. *PLoS ONE* **10**, e0132094.

Zumpt F (1965). Morphology, biology and pathogenesis of myiasis-producing flies in systematic order. In: Zumpt F, ed. *Myiasis in Man and Animals in the Old World: A Textbook for Physicians, Veterinarians and Zoologists*. Butterworths, London, pp. 110–28.

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