

## ***Gasterophilus* (Diptera, Gasterophilidae) infestation of equids in the Kalamaili Nature Reserve, China**

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**Abstract** – We investigated infections with *Gasterophilus* spp. in three equids within the Kalamaili Nature Reserve (northern China). We conducted necropsies on 6 Przewalski's horses (*Equus ferus przewalskii*) and 6 Mongolian wild asses (*Equus hemionus*) and administered ivermectin to 10 overwintering domestic horses to expel parasites during winter periods. All 22 equids studied (100%) were infested with *Gasterophilus* spp. and a total of 17,225 larvae were collected. These included six species: *G. haemorrhoidalis*, *G. inermis*, *G. intestinalis*, *G. nasalis*, *G. nigricornis*, and *G. pecorum*. The mean intensity of *Gasterophilus* spp. larvae was 1904 in Przewalski's horses, 780 in Mongolian wild asses, and 113 in domestic horses. *Gasterophilus pecorum* was the most abundant species in all three equids. Przewalski's horses, a reintroduced species, had a significantly higher intensity of *Gasterophilus* spp. than the Mongolian wild ass, indicating greater susceptibility to parasites in its ancestral home.

**Key words:** *Gasterophilus*, Equids, Przewalski's horses, Epidemiology, Differential analysis.

**Résumé – Infestation des équidés par *Gasterophilus* (Diptera, Gasterophilidae) dans la réserve naturelle de Kalamaili, Chine.** Nous avons étudié les infections de *Gasterophilus* spp. chez trois équidés dans la réserve naturelle de Kalamaili (Chine du Nord). Nous avons effectué des autopsies sur 6 chevaux de Przewalski (*Equus ferus przewalskii*) et 6 ânes sauvages de Mongolie (*Equus hemionus*) et administré de l'ivermectine à 10 chevaux domestiques hivernants pour expulser leurs parasites pendant les périodes d'hiver. Tous les 22 équidés étudiés (100 %) étaient infestés par *Gasterophilus* spp. et un total de 17,225 larves ont été recueillies. Elles comprenaient six espèces : *G. haemorrhoidalis*, *G. inermis*, *G. intestinalis*, *G. nasalis*, *G. nigricornis* et *G. pecorum*. L'intensité moyenne des larves de *Gasterophilus* spp. était 1904 chez les chevaux de Przewalski, 780 chez les ânes sauvages de Mongolie et 113 chez les chevaux domestiques. *Gasterophilus pecorum* était l'espèce la plus abondante chez les trois équidés. Les chevaux de Przewalski, une espèce réintroduite, avait une intensité significativement plus élevée de *Gasterophilus* spp. que les ânes sauvages de Mongolie, ce qui indique une plus grande sensibilité aux parasites dans leur aire ancestrale.

### Introduction

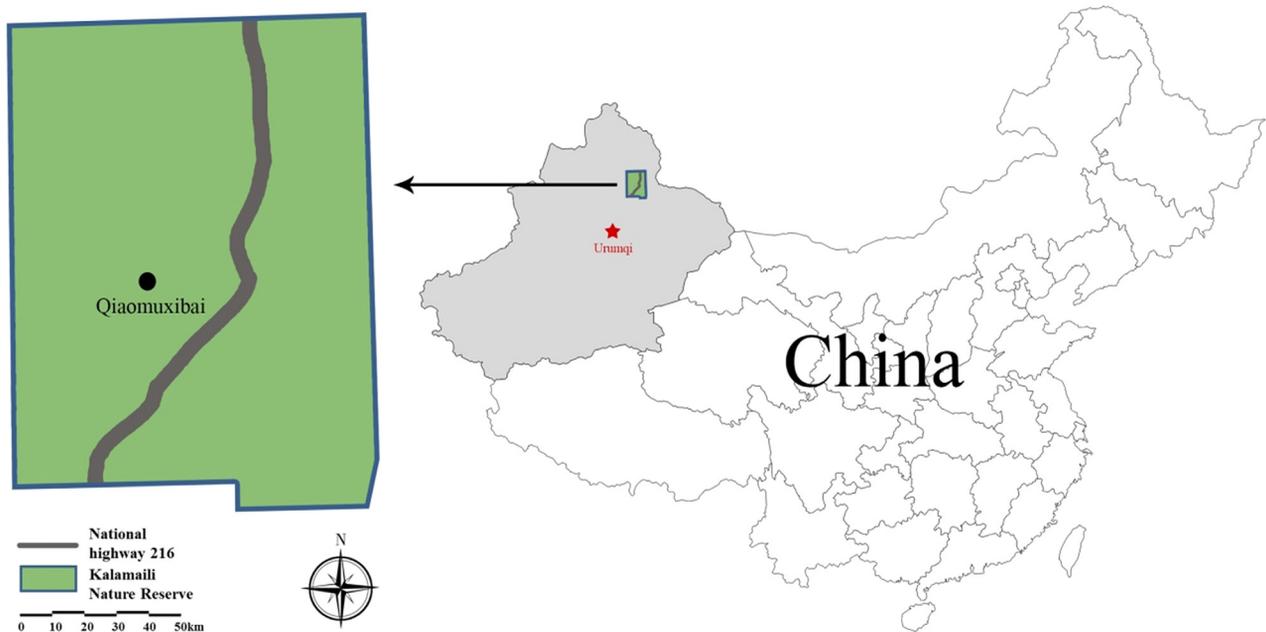
The Kalamaili Nature Reserve (KNR) (latitude: 44°36'–46°00' N, longitude: 88°30'–90°03' E, altitude: 600–1464 m) is located in the desert steppe of Xinjiang, China. Nomadic Kazakh populations in KNR traditionally migrate 200 km northward to summer pastures every spring and return in autumn [20]. Three equid species live in the KNR: the Przewalski's horse, the Mongolian wild ass, and the overwintering domestic horse [3].

More than 150 species of internal parasites infect horses [5]. *Gasterophilus* spp. are obligate parasites that infest the gastrointestinal tracts of equids, affecting the horses' health

by absorbing nutrients and secreting toxins [15, 19]. They may cause host death when the infestation is severe [4]. *Gasterophilus* spp. consists of nine species distributed worldwide [21]. In China, six of them are present, namely *G. haemorrhoidalis*, *G. inermis*, *G. intestinalis*, *G. nasalis*, *G. nigricornis*, and *G. pecorum* [6, 18]. All of them have been reported in wild populations of Przewalski's horses in KNR, China [9].

The present study was carried out on Przewalski's horses and Mongolian wild asses that died accidentally and with preserved corpses and feces of domestic horses following antiparasitic treatment during the winter. The aim of the study was to investigate the epidemiological features of *Gasterophilus* spp. in the three equid species.

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**Figure 1.** Location of the Kalamaili Nature Reserve, China.

## Materials and methods

### Study area

The KNR is located in the southeast corner of the north-east Junggar Basin, Xinjiang (Fig. 1). It is dry and cold in winter and hot during the summer. Mean annual precipitation is 159 mm, and mean annual evaporation 2090 mm, which is characteristic of a typical temperate continental arid climate [2].

### Larvae collection

Six Przewalski's horses and six Mongolian wild asses that died accidentally during the winter from 2010 to 2015 were preserved and necropsied. All *Gasterophilus* spp. larvae were collected from the digestive tracts.

A total number of 10 domestic horses (with no repeat samples) were randomly selected and treated once with ivermectin at the conventional dose of 0.2 mg/kg orally during the winter from 2013 to 2015. Following ivermectin treatment, we collected *Gasterophilus* spp. larvae that were eliminated in all feces of each horse three times a day until there were no larvae for three consecutive days. The domestic horses had received no other anti-*Gasterophilus* drug treatment prior to the survey.

Larvae were stored in ethanol (100%), washed with phosphate-buffered saline (PBS) buffer or saline solution (0.9% NaCl), counted, and identified using the morphological keys in Zumpt [21].

### Statistical analysis

The infestation prevalence, intensity, and abundance intensity were estimated according to Margolis and Schad [11].

Differences among the mean intensities of *Gasterophilus* spp. in the three equids were tested by the Kruskal-Wallis test. The statistical analysis and graphics were performed using SPSS version 20.0. A significant difference was assumed when  $p \leq 0.05$ .

## Results

### Prevalence of *Gasterophilus* spp. larvae

*Gasterophilus* spp. larvae were found within all 22 individuals of all three equid species. These included six species: *G. haemorrhoidalis*, *G. inermis*, *G. intestinalis*, *G. nasalis*, *G. nigricornis*, and *G. pecorum*.

*Gasterophilus pecorum* was the most common species (100%) in the Przewalski's horse and the Mongolian wild ass. *G. pecorum*, *G. nasalis*, and *G. nigricornis* were found in every domestic horse (Table 1).

### Intensity infestation by *Gasterophilus* spp. larvae

A total of 17,225 larvae were collected from the three equid species. There were significant differences in the intensities of *Gasterophilus* spp. ( $p = 0.000$ ), *G. haemorrhoidalis* ( $p = 0.047$ ), *G. nasalis* ( $p = 0.017$ ), and *G. pecorum* ( $p = 0.000$ ) in the three equids. The Przewalski's horse had the highest mean intensity of *Gasterophilus* spp. (1904) followed by the Mongolian wild ass (780) and the domestic horse (113) ( $p = 0.000$ ) (Table 2).

### Abundance intensity of *Gasterophilus* spp. larvae

*G. pecorum* was the most abundant species of *Gasterophilus* spp. in the three equids. The abundance intensity

**Table 1.** Prevalence of *Gasterophilus* spp. larvae in three equids in Kalamaili Nature Reserve, China.

<i>Gasterophilus</i> spp.	Equidae		
	<i>Equus ferus przewalskii</i>	<i>Equus hemionus</i>	<i>Equus caballus</i>
	Positive number (%)	Positive number (%)	Positive number (%)
<i>G. pecorum</i>	6 (100)	6 (100)	10 (100)
<i>G. nasalis</i>	3 (50)	4 (66.67)	10 (100)
<i>G. nigricornis</i>	2 (33.33)	4 (66.67)	10 (100)
<i>G. intestinalis</i>	2 (33.33)	1 (16.67)	7 (70)
<i>G. haemorrhoidalis</i>	3 (50)	6 (100)	7 (70)
<i>G. inermis</i>	0 (0)	1 (16.67)	3 (30)
Total	6	6	10

of *G. pecorum* was high, especially in the Przewalski's horse (1875.33) and the Mongolian wild ass (749.33). In the Mongolian wild ass, other species were *G. nigricornis* (17.83), *G. haemorrhoidalis* (9.67), *G. nasalis* (2.67), *G. inermis* (0.33), and *G. intestinalis* (0.17). In the domestic horse, the abundance intensity of *G. nasalis* (30.40) was higher than in the other two equids (Table 3).

## Discussion

All three equids were infected with *Gasterophilus* spp. larvae. This may reflect a wide distribution of *Gasterophilus* spp. in KNR. The high prevalence of *Gasterophilus* spp. larvae (100%) in the three equids is comparable to that reported for horses in Kazakhstan (100%) [8]. In contrast, this prevalence is much higher than reported in Sanliurfa, Turkey (9.82%) [7].

The mean intensities of *Gasterophilus* spp. larvae in Przewalski's horses (1904) and Mongolian wild asses (780) were higher than some reports from donkeys and horses in Asia [7, 12]. The lack of anti-parasitic treatment in wild equids in KNR may be the main reason for this situation, but it may also be due to the different climate and environment. However, the domestic horse had the lowest intensity of *Gasterophilus* spp. compared to the other two equids. This difference was partly attributed to the difference of habitats between the domestic horse and the other two equids. The summer pastures were mountain meadow with a lower temperature. The vegetation and water resources were more abundant than in KNR. These different environmental conditions may affect the activity of *Gasterophilus* spp. On the whole, our results indicate that the equids in KNR are severely affected by *Gasterophilus* spp. and the winter is not the main infection period for *Gasterophilus* spp. here.

*G. pecorum* was the most abundant species of *Gasterophilus* spp. in the three equids, which differs from studies in other regions of the world where it was reported that *G. nasalis* and *G. intestinalis* are the abundant species [8, 13, 14, 16]. Our results suggest that *G. pecorum* is more adaptable to the local environment in KNR. *G. pecorum* was the only *Gasterophilus* spp. species that oviposits on grass [21]. The association with this unique behavior and the desert steppe ecosystem may help explain the situation.

**Table 2.** Intensity of *Gasterophilus* spp. larvae in three equids in Kalamaili Nature Reserve, China.

<i>Gasterophilus</i> spp.	Equidae		
	<i>Equus ferus przewalskii</i>	<i>Equus hemionus</i>	<i>Equus caballus</i>
	No.	No.	No.
<i>G. pecorum</i>	11,252	4493	621
<i>G. nasalis</i>	91	16	304
<i>G. nigricornis</i>	45	107	126
<i>G. intestinalis</i>	6	1	38
<i>G. haemorrhoidalis</i>	28	58	30
<i>G. inermis</i>	0	2	7
Total	11,422	4677	1126
Mean ± SD	1904 ± 536	780 ± 248	113 ± 54

**Table 3.** Abundance intensity of *Gasterophilus* spp. larvae in three equids in Kalamaili Nature Reserve, China.

<i>Gasterophilus</i> spp.	Equidae		
	<i>Equus ferus przewalskii</i>	<i>Equus hemionus</i>	<i>Equus caballus</i>
	No.	No.	No.
<i>G. pecorum</i>	1875.33	749.33	62.10
<i>G. nasalis</i>	15.17	2.67	30.40
<i>G. nigricornis</i>	7.50	17.83	12.60
<i>G. intestinalis</i>	1.00	0.17	3.80
<i>G. haemorrhoidalis</i>	4.67	9.67	3.00
<i>G. inermis</i>	0.00	0.33	0.70

Water availability restricts the activity area of wild animals in a territory such as KNR which has low precipitation, high evaporation, and limited surface runoff. A previous study showed that the oviposition sites of *G. pecorum* are often near a water source [10]. This suggests that the water locations may be the important "epidemic" areas of *G. pecorum*. Przewalski's horses seem to drink daily [17]. For wild asses, it is often assumed that they can "regularly do without water" [1]. Frequent drinking at water sources may increase the risk of *G. pecorum* infection. Thus, the equids in arid desert grasslands have a higher intensity of *Gasterophilus* spp., and the intensity in Przewalski's horses is higher than in Mongolian wild asses.

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