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PREVALENCE INVESTIGATION OF GASTROINTESTINAL NEMATODES IN SONID BACTRIAN CAMELS

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ABSTRACT

To investigate the prevalence, intensity and predominant species of gastrointestinal nematode infections in Sonid Bactrian camels, Inner Mongolia, a survey on the prevalence of gastrointestinal nematodes was conducted on 307 naturally grazed Bactrian camels from 5 regions in Inner Mongolia using faecal egg, larvae and adult nematode identification methods. The results showed that gastrointestinal nematodes were quite severe in Sonid Bactrian camels, with an overall infection rate of 87.6% (269/307) and an average infection intensity of 1,981 (EPG). Among them, the infection rates of Bactrian camels were generally higher in Abaga Banner, West Uzumqin Banner, Durbed Banner, Sonid East Banner and Sonid West Banner, which were 100% (15/15), 100% (15/15), 98.6% (71/72), 98.1% (101/103) and 65.6% (67/102), respectively with infection intensities ranging from 50 to 10,350 (EPG). *Trichostrongylus* spp. was the predominant species, followed by *Ostertagia ostertagi*. This result was validated through PCR testing of DNA extracted from 46 faecal samples collected from Sonid Bactrian camels, confirming the findings. Therefore, the present study provides preliminary epidemiological survey data on the major nematode infections prevalent in Sonid Bactrian camel herds in Inner Mongolia, contributing to the understanding of gastrointestinal nematodiasis in Sonid Bactrian camels.

Key words: Bactrian camels, epidemiological investigation, gastrointestinal nematodes, PCR

The gastrointestinal nematodes of Bactrian camels mainly include species such as *Haemonchus contortus, Trichostrongylus* spp., *Ostertagia ostertagi*, *Nematodirus* spp. and *Marshallagia* spp. Studies have reported that in Algeria, the infection rate of gastrointestinal parasites in dromedaries is 48.26%, with parasites from 12 genera identified, among which the infection by nematodes of the *Strongyloides* spp. is the most severe, while those of the *Cooperia* spp. were the least prevalent (Bouragba *et al,* 2020). Researchers conducted gastrointestinal nematode examinations on 144 dromedaries in Iran, identifying 26 species of nematodes with an infection rate of 86.3%, primarily by *Haemonchus contortus* and *Trichostrongylus colubriformis* (Anvari-Tafti *et al,* 2013). The population of Bactrian camels is relatively small, accounting for less than 10% of the total old world camelids population, most studies on gastrointestinal nematodes in camels have focused on dromedaries. Sonid Bactrian camels are mainly distributed in Inner Mongolia Autonomous Region, including Xilin Gol League, Ulanqab City and eastern Hulunbuir, with a focus on Sonid West Banner, Sonid East Banner, Abaga Banner and West Uzumqin Banner in Xilin Gol city (He, 2003). The modern Bactrian camel breeding industry is gradually shifting from simple labour purposes to comprehensive utilisation such as milk, meat and fur, with the breeding population showing an increasing trend year by year. However, nematode infections, mainly affecting Sonid Bactrian camels, are prevalent, significantly impacting the productivity of camel herds and hindering the healthy development of the local camel breeding industry. Moreover, the majority of herders have insufficient understanding of the harmfulness economic losses caused by gastrointestinal nematodes, leading to a lack of scientific and targeted approaches in the prevention and control of nematode diseases and a prevalence of indiscriminate drug administration, resulting in a significant lag in parasitic disease prevention and control efforts (Eyeledege, 2018).

In this study, a systematic investigation of gastrointestinal nematodes in Sonid Bactrian camels in 5 different pastoral counties in Inner Mongolia was conducted, aiming to understand the dominant species, epidemiological status and infection intensity of gastrointestinal nematodes in Sonid Bactrian camels.

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Materials and Methods

Ethics Statement

Animal procedures were performed in accordance with the National Standard Guideline for Ethical Review of Animal Welfare (GB/T 35892-2018) and approved by the Animal Care and Use Committee of Inner Mongolia Agricultural University.

Collection of Sample

From January 2022 to December 2023, a total of 307 Bactrian camel fresh faecal samples were collected in Durbed Banner, Sonid West Banner, Sonid East Banner, Abaga Banner and West Uzumqin Banner of Inner Mongolia Autonomous Region, China, with 72, 102, 103, 15 and 15 samples, respectively. Samples were collected by individuals wearing disposable gloves, who directly collected faeces from the rectum or the centre of faecal balls not in contact with the ground (10g-50g). The samples were then placed in self-sealing bags, labeled with the Bactrian camel's basic information and transported back to the laboratory under refrigeration for examination. Faecal samples that could not be examined immediately were stored in a 4°C refrigerator to prevent hatching of eggs that could affect the interpretation of the results.

Identification of nematode eggs

The identification of gastrointestinal nematode species is based on the morphological structure and characteristic features of different eggs, referring to the *Colour Atlas of Morphological Classification of Parasites of Domestic Animals in China*. Additionally, the identification of larvae structures cultured from faeces and the confirmation of adult worms obtained through dissecting deceased camels was done.

Faecal eggs quantitative examination

Faecal egg counts were undertaken using the modified McMaster technique, as described in Veterinary Clinical Parasitology (Anne and Gary, 2012).

PCR identification

Forty-six faecal samples of Bactrian camels were collected from Sonid West Banner, categorised into 5 groups (I, II, III, IV and V) based on the collection locations, with EPG (eggs per gram) values ≥500. PCR detection was conducted on faecal DNA samples extracted from each group using specifically designed primers for 8 common gastrointestinal nematodes of Bactrian camels (some specific primers can identify down to the species level, while other specific primers can only identify down to the genus level), negative control without DNA template.

DNA extraction was performed using the Stool DNA Kit D4015-01 from OMEGA Bio-Tek (USA), following the manufacturer's instructions and the extracted DNA was stored at -20°C. Specific primers (Appendix) for eight gastrointestinal nematodes

Appendix. Sequences of primers specific for 8 gastrointestinal nematodes of the bactrian camel.

Nematode species	Primer	Primer sequence (5′-3′)	Target fragment(bp)	Annealing temperature
Haemonchus contortus (Zou *et al*, 2023)	Upstream primer	ATTGTTCGTCAAATGGCA	270	53°C
	Downstream primer	AGTTTCTTTTCCTCCGCT		
Nematodirus spp. (Jia, 2014)	Upstream primer	GTAGGTGAACCTGCGGAAGGATCATT	800	45°C
	Downstream primer	TTAGTTTCTTTTCCTCCGCT		
*Trichuris ovi*s (Zhang *et al*, 2013)	Upstream primer	TTTGATATCTTTTTACCTTACCATT	900	55°C
	Downstream primer	AGGGCTTATTGCTATGTGGTTA		
Parabronema skrjabini (Zheng, 2015)	Upstream primer	TTTACAAGAGGGATACGCC	551	50°C
	Downstream primer	GGTATCACAAACTTATCGGG		
Strongyloides spp.	Upstream primer	CACCTCTTCAGGGACAT	433	47°C
	Downstream primer	TTTTGGAGCATTTGGAT		
Ostertagia ostertagi (Qu, 2013)	Upstream primer	CGCTTAGAGTGGTAAAATTTTGAAC	342	57°C
	Downstream primer	TTAGTTTCTTTTCCTCCGCTAAATG		
Chabertia spp. (Zhao *et al*, 2013)	Upstream primer	TTTTTTGGGCATCCTGAGGTTTAT	450	55°C
	Downstream primer	TAAAGAAAGAACATAATGAAAATG		
Trichostrongyle spp. (Li, 2020)	Upstream primer	TTGTCGAAACCAACACATGG	173	58°C
	Downstream primer	GGGAACTTCGCATGAACAAT		

synthesised from literature were utilised for PCR amplification (Table 1).

After PCR amplification, 5 µL of the product was taken for 1% agarose gel electrophoresis. The voltage was set to 110 V and electrophoresis was conducted for 30 minutes. Subsequently, the gel was placed into a UV gel imaging system for result observation and photographing for documentation.

Result

The infection status of gastrointestinal nematodes of Sonid Bactrian camels

According to Table 2, the overall infection rate of gastrointestinal nematodes in 307 faecal samples of Bactrian camels from Sonid is 87.6% (269/307), with 269 samples testing positive. The infection rates of gastrointestinal nematodes in Durbed Banner, Sonid West Banner, Sonid East Banner, Abaga Banner and West Uzumqin Banner were 98.6% (71/72), 65.6% (67/102), 98.1% (101/103), 100% (15/15) and 100% (15/15), respectively.

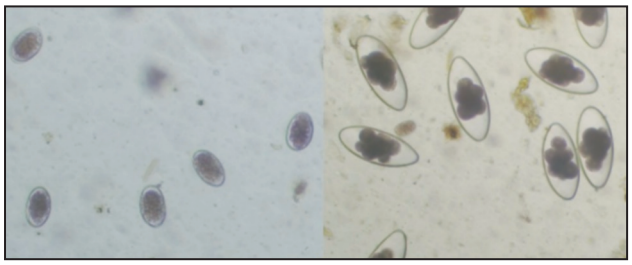

Fig 1. Eggs of *Trichostrongylus* spp. and *Nematodirus* spp. (100X).

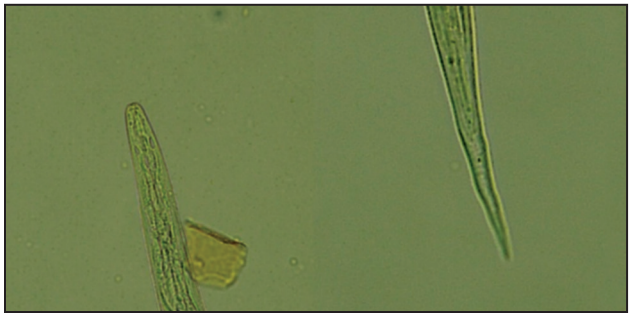

Fig 2. Third-stage (L3) larvae of *Trichostrongylus* spp. (400X).

Table 1. The PCR reaction system by special primers (25 µL)

Components	Volume
Upstream primer	1 µL
Downstream primer	1 µL
Premix Taq	12.5 µL
Template DNA	2 µL
dH_2O	8.5 µL
Total volume	25 µL

The highest EPG of gastrointestinal nematodes in Durbed Banner, Sonid West Banner, Sonid East Banner, Abaga Banner and West Uzumqin Banner

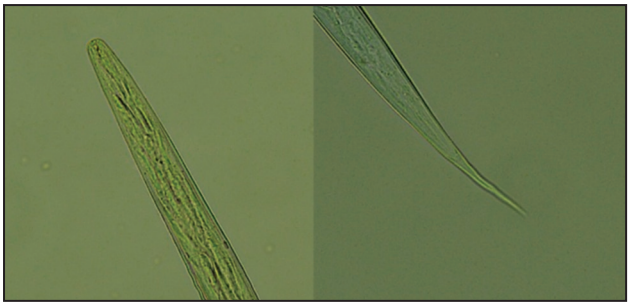

Fig 3. Third-stage (L3) larvae of *Ostertagia ostertagi* (400X).

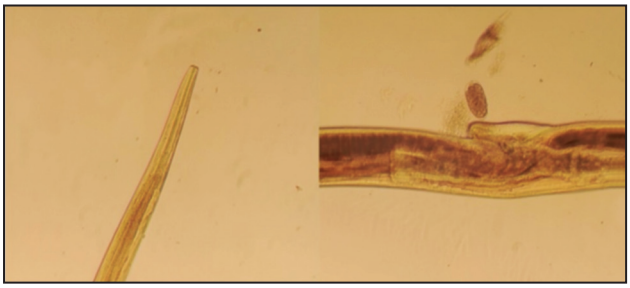

Fig 4. Female anterior end and vulva of *Trichostrongylus* spp. (100X).

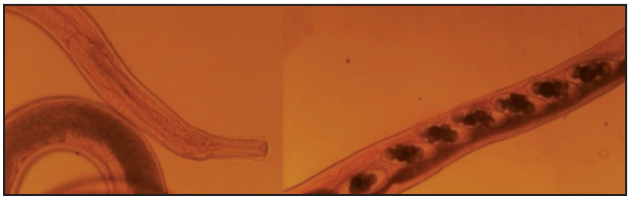

Fig 5. Female anterior end and reproductive tract of *Nematodirus* spp. (100X)

Table 2. Nematode infection in the digestive tract of Sonid Bactrian camels of Inner Mongolia.

Areas	No. Samples	No. Positives	Rate of infection (%)	Infection intensity (EPG)	Mean intensity of infection (EPG)
Durbed Banner	72	71	98.6	50-10,350	1,810
Sonid West Banner	102	67	65.6	50-1,800	396
Sonid East Banner	103	101	98.1	50-7,850	1,409
Abaga Banner	15	15	100	1,000-6,050	3,246
West Uzumqin Banner	15	15	100	250-5,750	3,043
Total	307	269	87.6	50-10,350	1,981

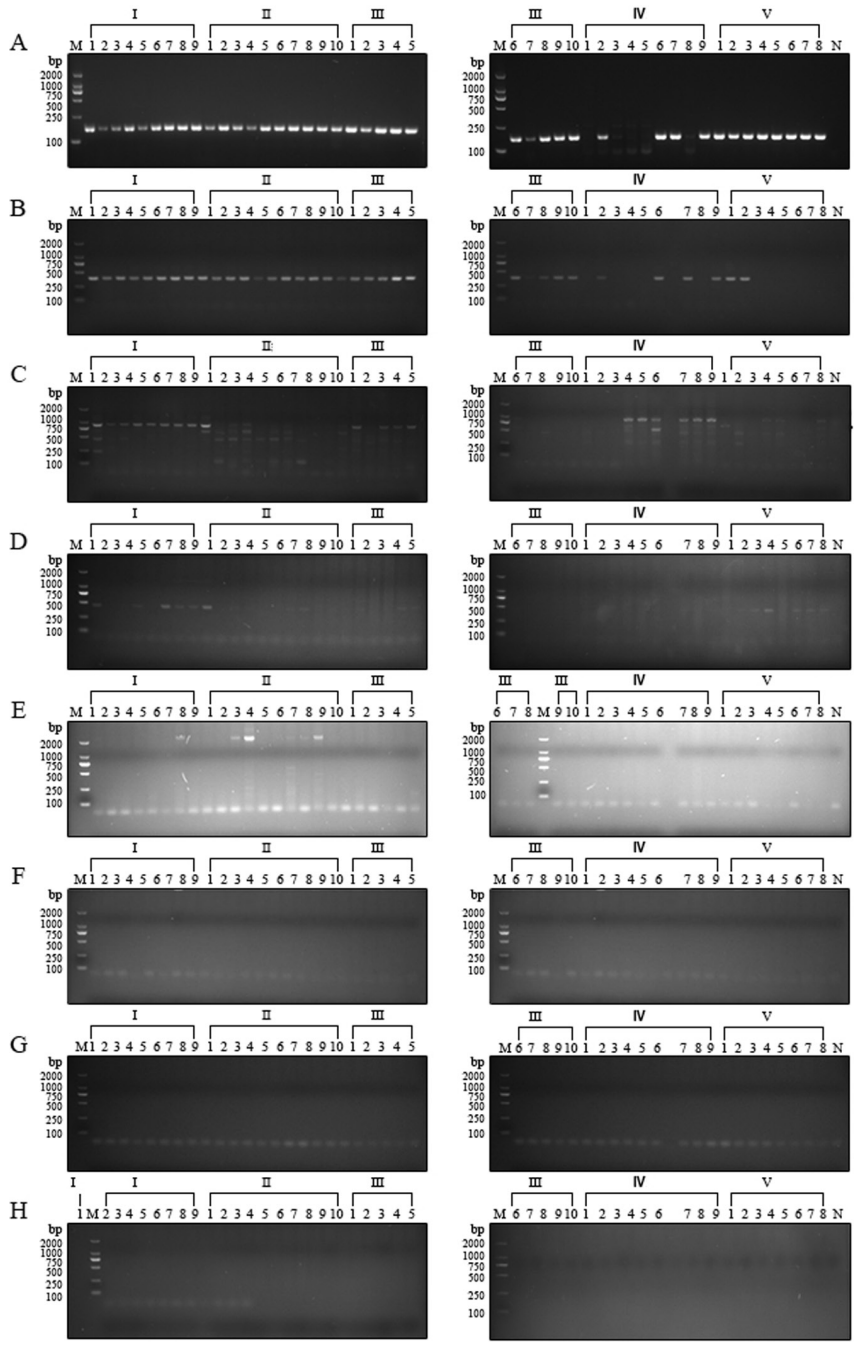

Fig 6. Electropherogram of PCR amplification results.
M: DNA Maker DL2000; A. *Trichostrongylus* spp. B. *Ostertagia ostertagi*. C. *Nematodirus* spp. D. *Chabertia* spp. E. *Haemonchus contortus*. F. *Trichuris ovis*. G. *Parabronema skrjabini* H. *Strongyloides* spp.; I, II, III, IV and V are subgroups; N: negative control.

were 10 350, 1 800, 7 850, 6 050 and 5 750, with average infection intensities of 1 810, 396, 1 409, 3 246 and 3 043, respectively.

Results of egg, larval hatching and adult worm dissection and identification

According to microscopic examination results, the most prevalent nematode species observed were from the Trichostrongylidae, mainly *Trichostrongylus* spp. (Figs 1,2,4) and *Ostertagia ostertagi* (Fig 3), followed by the *Nematodirus* spp (Figs 1,5).

The eggs of *Trichostrongylus* spp. measure 76-92×37-46 µm, with relatively well-filled contents. The eggs of *Nematodirus* spp. measured 165-175×76-86 µm and contained 6-8 embryonated cells (Fig 1).

The larvae have a relatively stout body, with a clearly visible excretory pore and 16 distinct triangular intestinal cells. The tail is thick and blunt, with either one or two segments or an indistinct rounded tail tip and a very short tail sheath. Measurements indicate a total length of 622-706 µm and a length from caudal end to tip of caudal sheath 31-39 µm.

The larvae were of moderate size, with two refractile bodies or a bright bar between the mouth and esophagus visible. The excretory pore was clearly visible and the tail was relatively thick with a short tail sheath.

Results of PCR Test

PCR testing was conducted on 46 faecal samples from Bactrian camels in Sonid, revealing 4 species of nematodes tested positive (Fig 6). *Trichostrongylus* spp. had the highest infection rate, followed by *Ostertagia ostertagi*. Among the 46 faecal samples from Sonid Bactrian camels, 41 were positive for *Trichostrongylus* spp., resulting in a total infection rate of 89.1% (41/46). The infection rates for Groups I, II, III, IV and V were 100%, 100%, 100%, 44.4% and 100%, respectively.

Ostertagia ostertagi. had a total infection rate of 76.1% (35/46), with infection rates in Groups I, II, III, IV and V being 100%, 100%, 100%, 44.4% and 25%, respectively.

Nematodirus spp. had a total infection rate of 54.3% (25/46), with infection rates in Groups I, II, III,

IV and V being 100%, 20%, 40%, 66.6% and 37.5%, respectively.

Chabertia spp. had a total infection rate of 13% (6/46), with infection rates in Groups I, II, III, IV and V being 44.4%, 10%, 0%, 0% and 10%, respectively.

Nematodes such as *Haemonchus contortus, Trichuris ovis, Parabronema* spp. and *Strongyloides* spp. all tested negative.

Discussion

Due to the rampant prevalence of gastrointestinal nematodes in Bactrian camels in the Inner Mongolia autonomous region of China in recent years, the productivity and product quality of Bactrian camels have declined, causing serious economic losses to the camel husbandry industry. This study conducted a preliminary investigation and analysis of the prevalence of gastrointestinal nematodes in Sonid Bactrian camels in Durbed Banner of Ulanqab City and Sonid West Banner, Sonid East Banner, Abaga Banner and West Uzumqin Banner of Xilin Gol city in Inner Mongolia. The results showed that the overall infection rate of gastrointestinal nematodes in 307 faecal samples from Sonid Bactrian camels was as high as 87.6% (269/307), indicating a severe situation of gastrointestinal nematodes in Sonid Bactrian camels in Inner Mongolia. The infection rates of Bactrian camels in the 5 investigated areas, namely Durbed Banner, Sonid West Banner, Sonid East Banner, Abaga Banner and West Uzumqin Banner, were 98.6% (71/72), 65.6% (67/102), 98.1% (101/103), 100% (15/15) and 100% (15/15), respectively. The highest EPG values were 10 350, 1 800, 7 850, 6 050 and 5 750, respectively. Morphological identification of eggs, infective larvae and adult nematodes revealed that the most commonly infected nematode was *Trichostrongylus* spp., followed by *Ostertagia ostertagi*. and *Nematodirus* spp.

In previous studies, the prevalence of gastrointestinal nematodiasis in Bactrian camels was highly common, with both high infection rates and intensity (Abubakr *et al,* 2000; Fraser and Craig, 1997; Abd El-Wahed, 2005). *Trichostrongylus* spp. was identified as the predominant species, which aligns closely with the findings of this study. For instance, El-Alfy *et al* (2019) using different molecular markers, identified infection rates of 26%, 65.2%, 60.8% and 95.6% for *Trichostrongylus axei, Trichostrongylus colubriformis, Cooperia oncophora* and *Haemonchus contortus,* respectively (El-Alfy *et al,* 2019) in faecal samples from 101 single-humped camels in Egypt. Similarly, researchers conducted PCR amplification and sequencing on faecal samples from 160 camels in central Iraq, revealing an infection rate of 18.13% (29/160) (Rasool *et al,* 2021). Research on gastrointestinal nematodes in Bactrian camels in China has primarily focused on the *Parabronema skrjabini* (Zhou, 2021; Li *et al,* 2020; Zhao *et al,* 2012). An epidemiological survey by Zheng (2015) reported a significant decrease in the prevalence of *Parabronema skrjabini* infection in Bactrian camels in Sonid, Xilin Gol League, from 4,696 worms per camel in 1988 to 16 worms per camel. In this study, *Parabronema skrjabini* was not detected in the genetic analysis of 46 faecal samples from Bactrian camels in Sonid West Banner, indicating some effectiveness in the prevention and control of *Parabronema skrjabini* disease in the Xilin Gol area over the past two decades.

In conclustion, the prevalence of gastrointestinal nematodes in Bactrian camels in Sonid, Inner Mongolia, is relatively common, especially with severe infections of *Trichostrongylus* spp., which significantly hinder the healthy development and economic benefits of camel husbandry in the region. The epidemiological findings of this study are of great significance for understanding the prevention and control of gastrointestinal nematodes in Sonid Bactrian camels, providing scientific evidence and technical support for the protection of Bactrian camel health and the promotion of sustainable development in animal husbandry.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgements

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