Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.149

RESEARCH ARTICLE

Molecular Investigation of *Bartonella melophagi* and the First Report of *Trypanosoma melophagium* in *Melophagus ovinus* from Southern Xinjiang, China

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ARTICLE HISTORY (23-554)

Received: December 25, 2023
Revised: March 14, 2024
Accepted: March 15, 2024
Published online: April 01, 2024

Key words:
Bartonella melophagi
Melophagus ovinus
One Health
PCR
Southern Xinjiang
Trypanosoma melophagium

ABSTRACT

Bartonella melophagi and *Trypanosoma melophagium* are intracellular bacteria and blood protozoan parasites, respectively, that can be transmitted by *Melophagus ovinus* and pose risks to human and animal health. Eight pupae and 214 *M. ovinus* were collected from three sampling sites in southern Xinjiang, China, between March 2019 and June 2023. The morphological characteristics and 18S rRNA sequences were used to identify *M. ovinus*. The *gltA* gene was used to detect *B. melophagi* and the 18S rRNA gene was used to detect *T. melophagium* in all samples. The DNA of *B. melophagi* was detected in 214 (100.00%, 214/214) samples and the DNA of *T. melophagium* was detected in 101 (47.20%, 101/214) samples using PCR and sequencing. Eight pupae produced two different outcomes: *B. melophagi* was positive and *T. melophagium* was negative. This study presents conclusive evidence for the presence of *B. melophagi* and *T. melophagium* in *M. ovinus*. To the best of our knowledge, this is the first report of *T. melophagium* in *M. ovinus* in China. These findings offer a significant reference for the advancement of the knowledge and prevention of *M. ovinus*, *B. melophagi*, and *T. melophagium* in China.

To Cite This Article: Huang K, Xiong N, Sun L, Zhang X, Zhao X, Zhou K and Wu J, 2024. Molecular investigation of *Bartonella melophagi* and the first report of *Trypanosoma melophagium* in *Melophagus ovinus* from southern Xinjiang, China. Pak Vet J, 44(2): 384-390. http://dx.doi.org/10.29261/pakvetj/2024.149

INTRODUCTION

Melophagus ovinus, also known as sheep ked, is a blood-sucking ectoparasite that primarily infests sheep. It is a wingless fly belonging to the family Hippoboscidae (Diptera, Hippoboscoidea) (Small, 2005). *M. ovinus*, which is 4–6 mm in size, has an adamantine exoskeleton, densely distributed bristles, three pairs of legs terminated with hook-like claws, and robust, sharp mouthparts (Li *et al.*, 2023b). Their life cycle comprises three definite stages: larva, pupa, and wingless adult. They usually parasitize the animal's head, neck, shoulder, abdomen, hind legs, perineal regions, and tail, and by direct contact, they can be transferred from one sheep to another (Zhang *et al.*, 2023). Although *M. ovinus* mainly parasitizes the body surface of sheep, it can also parasitize humans, domestic animals (goats, dogs, and rabbits), and wild animals (Tibetan antelope, red foxes and European bisons) (Werszko *et al.*, 2021).

M. ovinus blood-feeding behavior harms its host in two ways. On the one hand, the bite of *M. ovinus* cause itching, pain, and inflammation in the host. The host relieves the discomfort caused by gnawing, scratching, and rubbing; however, this process may lead to skin damage and wool shedding, creating conditions for secondary microbial infections and cutaneous myiasis. If *M. ovinus* parasitizes sheep, it will seriously damage their health and affect the economy of the livestock industry. (Duan *et al.*, 2017a). On the other hand, according to reports, *M. ovinus* acts as a vector for the spread of zoonotic pathogens such *Borrelia burgdorferi*, *Theileria* spp, *Bartonella* spp., *Trypanosoma* spp., *Rickettsia* spp., *Anaplasma* spp., Border disease virus, Bluetongue virus, *et al.* (Li *et al.*, 2023b). The majority of sheep-rearing locations have been reported to have *M. ovinus* present in Asia, North America, Europe, Africa, and Oceania; it has been reported in Tibet, Gansu, Xinjiang, Qinghai, and Liaoning, China, and has been found in sheep and their products that are imported to China's quarantine ports (Liu *et al.*, 2018; Werszko *et al.*, 2021).

Belonging to the family Bartonellaceae, *Bartonella* spp. are arthropod-borne intracellular Alphaproteobacteria that require haemotropic gram-negative bacteria. It infects the endotheliocytes and erythrocytes in mammalian hosts.

They are aerobic bacilli that are slow-growing, fastidious, and highly adapted to vertebrate hosts (Kumsa *et al.*, 2014). Ever since *Bartonella* was reclassified in 1993. Within the family Bartonellaceae, there is only one genus, *Bartonella* (Brenner *et al.*, 1993). At present, the genus comprises 39 species and three subspecies that hold an effective taxonomic position, with at least 20 of them being linked to human ailments (Gonçalves-Oliveira *et al.*, 2023). They have been associated with endocarditis and/or persistent bacteremia, retinitis, peliosis, hepatitis, myocarditis, uveitis, and bacillary angiomatosis. However, *Bartonella* spp. normally cause continuous asymptomatic bacteremia (Kumsa *et al.*, 2014). *Bartonella* spp. use humans, cats, dogs, mice, cows, sheep, horses, and various wild animals as hosts worldwide. It can be transmitted via insect vectors such as ticks, *M. ovinus*, human body lice, sand flies, and cat fleas. Currently, five types of *Bartonella* are linked to ruminants: *B. melophagi*, *B. bovis*, *B. capreoli*, *B. schoenbuchensis*, and *B. chomelii* (Ni *et al.*, 2021). Among these, Chinese scholars detected *B. melophagi* in *M. ovinus* from Gansu, Xinjiang, and Tibet (Duan *et al.*, 2017a; Liu *et al.*, 2018; Liu *et al.*, 2022). Maggi *et al.* detected and cultured *B. melophagi* in the blood of two female patients with dry cough, fatigue, muscle pain, muscle weakness, shivering, and pericarditis. Both patients had been in contact with, or even scratched and bitten by, domestic or wild animals and had a history of being bitten by mosquitoes, flies, fleas, or ticks (Maggi *et al.*, 2009). Although a causal relationship between the development of clinical symptoms and *B. melophagi* in these two female patients has not been established, it highlights the potential harm caused by *B. melophagi* as a zoonotic pathogen.

Among the most significant and widespread parasites worldwide, trypanosomes (Euglenozoa, Kinetoplastea, and Trypanosomatida) are responsible for several serious diseases in humans and animals. These digenetic blood parasites are mostly spread by insects that feed on the blood. In the *T. theileri* group, *T. melophagium* is species-specific to sheep and is only transmitted by *M. ovinus* (Brotankova *et al.*, 2022). Infection in *T. theileri* group is usually implicit and nonpathogenic but has been detected in ruminants with fever, anorexia, malnutrition and anemia (Sood *et al.*, 2011; Hajihassani *et al.*, 2020; Bittner *et al.*, 2021). Thus, the nonpathogenic *T. theileri* group may still cause minor damage to the host, especially in highly prevalent host populations that are easily overlooked (Oldrieve *et al.*, 2022). Although *T. melophagium* may cause serious infections in humans and animals, it is harmless to *M. ovinus* and does not cause insect-to-insect transmission. Sheep become infected when they consume *M. ovinus* (Martinkovic *et al.*, 2012). A sheep experimentally infected with *T. melophagium* had a maximum infection duration of three months, and there was no permanent immunity because the sheep could contract *T. melophagium* again after being isolated for several months (Gibson *et al.*, 2010). *T. melophagium* uncertainty is a threat to human and animal public health.

With a total area of approximately 1,664,900 km^2, the Xinjiang Uyghur Autonomous Region is the largest autonomous region in China, comprising approximately one-sixth of the total area of mainland China. The length of the land border exceeds 5,700 km. It is a vital export channel for worldwide trade and adjoins many nations, including Pakistan, Mongolia, Russia, Kazakhstan, and India. Southern Xinjiang occupies an area of approximately 1,020,000 km^2 and is surrounded by various landforms, including the Gobi Desert, flatlands, grasslands, mountains, and valleys (Li *et al.*, 2023c). The main economic industries in Southern Xinjiang are agriculture and livestock production. The main livestock species include cattle (e.g., Kazakh and Mongolian) and sheep (e.g., Hotan and Duolang). Therefore, insect vectors and insect-borne diseases have a significant impact on the livestock economy. At the same time, zoonotic diseases transmitted by *M. ovinus* must be considered.

The number of reports on *M. ovinus* and the different pathogens it carries has gradually increased in recent years, indicating that it poses a risk to human and animal health. (Hao *et al.*, 2020; Li *et al.*, 2023a; Liu *et al.*, 2022; Zhang *et al.*, 2021). In the current study, to better understand the genetic heterogeneity of *B. melophagi* and *T. melophagium* in southern Xinjiang, China, we conducted comprehensive molecular detection and analysis of *B. melophagi* and *T. melophagium* in *M. ovinus* samples obtained from this area and determined the prevalence and geographical distribution of *M. ovinus* in the region.

MATERIALS AND METHODS

Study area and sample collection: The selection of sites for the collection of *M. ovinus* were based on the presence of sheep ked, good flock health, lax implementation of deworming measures, and obtained consent from the head of the sheep farm. From March 2019 to June 2023, 214 *M. ovinus* and eight pupae were collected from 146 sheep captured at three sheep farms in three counties in southern Xinjiang: CeLe (CL), WuShi (WSW), and WenSu (WSE) (Table 1 and Fig. 1). The research locations were all situated in southern Xinjiang's border regions, and they served as central points for animal husbandry in these areas. The head, neck, shoulder, abdomen, hind legs, perineal regions, and tail were carefully examined by separating the wool in the direction opposite to the fleece rest. All pupae and adult *M. ovinus* samples were collected using fine-tipped forceps, preserved in 75% alcohol and kept at 4°C. Finally, the samples were sent to the Tarim Animal Disease Diagnosis and Control Engineering Laboratory (Alar, China). Information on the samples, such as the number of samples, hosts and locations, was recorded.

Morphological identification and total DNA extraction of *M. ovinus*: Using a Leica stereomicroscope M165 C (Solms, Germany), the morphological features of *M. ovinus*, including its basic capituli, ventral surface, dorsal surface, legs, tarsi, anal groove, and scutum, were identified. The main points of identification were as follows: the compound eyes were small and degenerated; the wings were completely degenerated; the head and thorax were densely covered with bristles; and there were no spots or stripes (Duan *et al.*, 2017b). All *M. ovinus* were washed in a thermostatic culture shaker with 90%, 70%, 50%, 30%, and 10% ethanol at 37°C, 180 rpm,

Table 1: Molecular detection of *B. melophagi* and *T. melophagium* in *M. ovinus*.

Cities	Year	Number of hosts	Number of *M.ovinus*	pupa	*B. melophagi* Positive Number(%)	*T. melophagium* Positive Number(%)
CeLe (CL)	2023	27/68	143	4	143 (100%)	73 (51.05%)
WuShi (WSW)	2021	9/31	28	0	28 (100%)	15 (53.57%)
WenSu (WSE)	2019	16/47	43	4	43 (100%)	13 (30.23%)
Total		52/146	214	8	214 (100%)	101 (47.20%)

Note: Number of hosts: *M. ovinus* number of infected sheep/ total number of sheep; eight pupae produced two different outcomes: *B. melophagi* was positive and *T. melophagium* was negative.

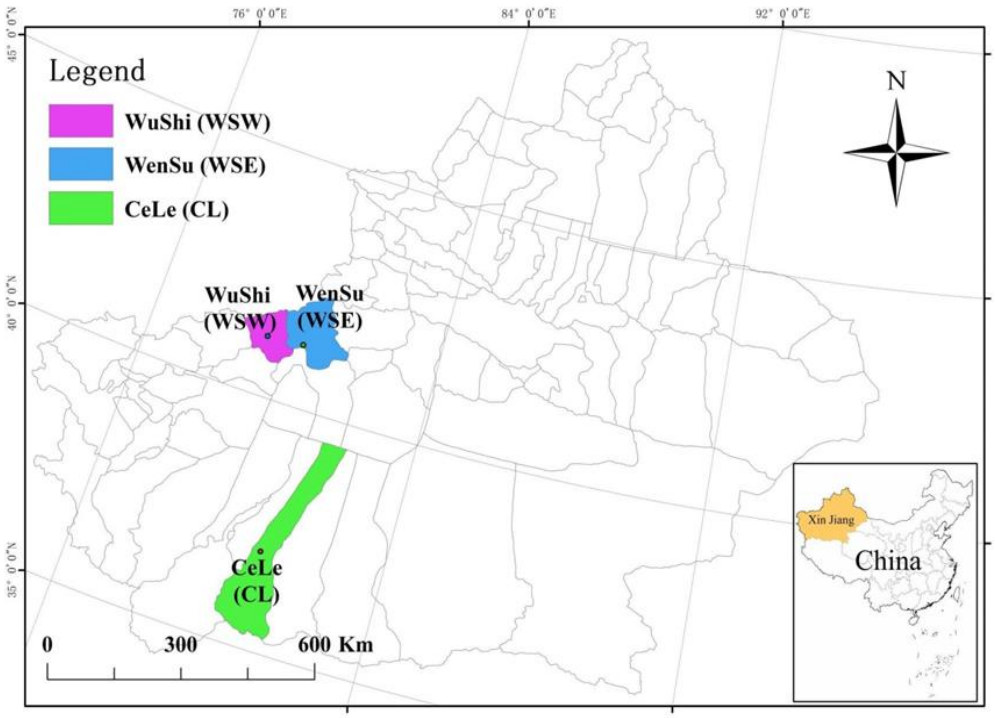

Fig. 1: Distribution map of sampling districts of three locations in southern Xinjiang, China.

in each of which *M. ovinus* was washed for 30 minutes respectively. They were then cleaned three times in sterile distilled water to remove any remaining debris and finally dried on filter paper. They were then placed in a 1.5 mL sterile centrifuge tube and cut into the smallest pieces using sterilized surgical scissors. Following the provided instructions, we extracted DNA from the processed *M. ovinus* samples utilizing the TIANamp Genomic DNA Kit (manufactured by TIANGEN Corporation, Beijing, China). Before PCR was carried out, the extracted DNA was kept at -20°C.

Molecular identification of *M. ovinus*, *B. melophagi* and *T. melophagium*: To identify *M. ovinus*, polymerase chain reaction (PCR) targeting the Hippoboscidae 18S rRNA gene was performed. The detection of *B. melophagi* was performed using the *gltA* gene and *T. melophagium* using the 18S rRNA gene. Table 2-1 shows the primer sequences used for amplification of 520bp, 379bp and 650bp fragments. The PCR mixture, which had a total volume of 25 µL, contained 9 µL nuclease-free deionized water, 13 µL of 2× *Taq* PCR Master Mix (Tiangen), 1 µL of the relevant primers (10 µM final concentration) and 1 µL template DNA, and was performed under the following amplification conditions in Table 2-2.

To monitor for contamination, nuclease-free sterile distilled water was used as a negative control, and the DNA of *B. melophagi* and *T. melophagium* stored in our laboratory was used as a positive control. For each experiment, 5 µL of the PCR product was applied to a 1.5% agarose gel that contained GelStain (Beijing TransGen Biotech Co., Ltd., Beijing, China) for gel electrophoresis. Experimental data were evaluated using a gel documentation system (FluorChem, ProteinSimple, CA, USA). The PCR products were sent to GENEWIZ Inc. (Suzhou, China) for sequencing. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare and analyze the genomic sequences. Finally, the genotypes of *B. melophagi* and *T. melophagium* based on *gltA* and 18S rRNA sequences were determined using DNAStar and MegAlign software (DNASTAR, Inc., USA). The positivity rate was calculated using the following equation:

$$\text{Positivity rate (\%)} = \frac{\text{number of positive pools}}{\text{number of examined pools}} \times 100$$

The sequences of the *B. melophagi* and *T. melophagium* nucleotides were inferred from their respective genomic sequences using DNAStar and MEGA 7.0 software. Using maximum likelihood algorithms and 1000 bootstrap repetitions, a phylogenetic tree was constructed using the MEGA software (version 7.0) (Kumar *et al.*, 2018; Saitou and Nei, 1987). Representative nucleotide sequences from this study were submitted to the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/) (18S rRNA of *M. ovinus*: OR921412 and OR921413; *gltA* of *B. melophagi*: OR936726, OR936727, OR936728, OR936729, OR936730, and OR936731; 18S rRNA of *T. melophagium*: OR921417, OR921418, OR921419, OR921420, and OR921421).

Table 2-1: Details of primers, target gene, nucleotide sequence and product size of PCR assays.

Species	Target gene	Primer	Nucleotide sequences（5'-3'）	Product size (bp)	Reference
Melophagus ovinus	18S rRNA	45F	AAC TTG TGC TTC ATA CGG G	520	(Duan *et al*., 2017b)
		564R	GCG ACT GAG AGA GCC ATA A		
Bartonella	*gltA*	BhCS.781p	GGG GAC CAG CTC ATG GTG G	379	(Li *et al*., 2015)
		BhCS.1137n	AAT GCA AAA AGA ACA GTA AAC A		
Trypanosomes	18S rRNA	TrypF 150	GAA ACA CGG GAG CGG TTC CTT	650	(Werszko *et al*., 2020)
		TrypR 800	ACC TCA AAG CTT TCG CGT GAA G		

Table 2-2: Amplification conditions (temperature and time) corresponded to each set of primers.

Primer	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Final extension
45F 564R	95°C/5 min	95°C/30 s	55°C/30 s	72°C/30 s	38	72°C/8 min
BhCS.781p BhCS.1137n	94°C/2 min	94°C/30 s	53°C/30 s	72°C/1 min	30	72°C/5 min
TrypF 150 TrypR 800	94°C/3 min	94°C/30 s	62°C/30 s	72°C/1 min	35	72°C/4 min

RESULTS

Identification of *M. ovinus*: In the present study, 52 (35.6%) sheep were infested with *M. ovinus* between March 2019 and June 2023 at three sampling points in southern Xinjiang. A total of 214 *M. ovinus* and eight pupae were collected from the infested sheep (Table 1 and Fig. 2). Each sheep was infected with M. ovinus at an average of 1.5 *M. ovinus*. Through the identification of their morphological features and molecular biological studies using 18S rRNA, all these ectoparasites were verified to be *M. ovinus*. The 18S rRNA sequences found in *M. ovinus* were identical according to molecular analysis. BLAST analysis of the 18S rRNA gene sequence of *M. ovinus* showed 100% nucleotide identity with GenBank reference sequences (*M. ovinus*: ON211915 and FN666411).

Prevalence of *B. melophagi*: Overall positivity rates for *B. melophagi* in *M. ovinus* and pupae were 100% (214/214) and 100% (8/8), respectively (Table 1). In this study, the *gltA* gene sequence of *B. melophagi* was amplified from the total DNA extracted from *M. ovinus*. After BLAST comparison, these sequences showed 98.60%~100.00% similarity to the *gltA* gene sequences of *B. melophagi* reported elsewhere (e.g., MT154631, OQ924085, AY724768, OQ924089, and OQ924091). Based on the *gltA* of the characteristic *Bartonella* spp., a phylogenetic tree revealed that all sequences acquired for our examination belonged to the same clade, encompassing strain K-2C (*B. melophagi*: AY724768) previously discovered in the USA (Fig. 3).

Prevalence of *T. melophagium*: The prevalence of *T. melophagium* was 51.05% (73/143), 53.57% (15/28) and 30.23% (13/43) in *M. ovinus* at the CeLe, WuShi and WenSu, respectively (Table 1). The total positivity rate was 47.20%, and the positive results did not include pupae. The PCR products were subjected to Sanger sequencing, and BLAST analysis of the 18S rRNA gene sequence revealed the presence of 101 *T. melophagium* isolates (47.20%, 101/214). Through phylogenetic analysis of the *Trypanosomes* spp., it was discovered that our sequences exhibited 99.11~100.00% similarity to the accession numbers of *T. melophagium* ON637625, HQ664912, OM256700, and FN666409 (Fig. 4).

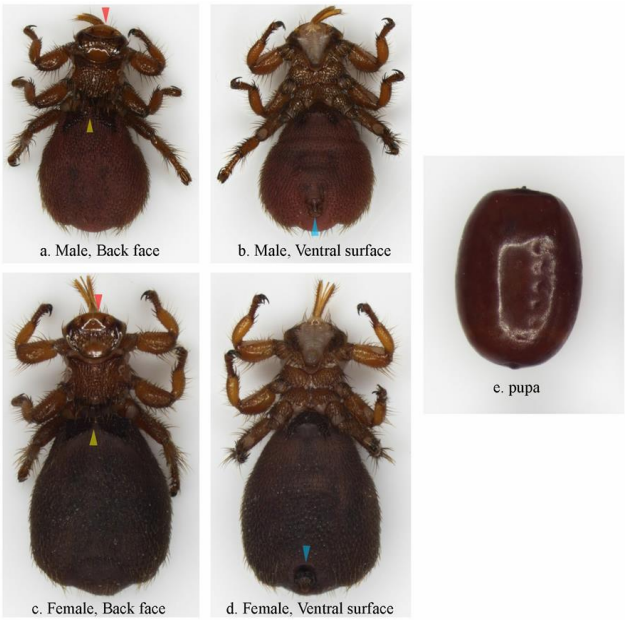

Fig. 2: The *M. ovinus* and pupae collected from sheep, in southern Xinjiang, China. Features of identification: The compound eyes on the head are small and degenerated (red arrows); Wings are fully degraded (yellow arrows); Males have convex ends and elongated genitalia, females have concave ends (blue arrows)

DISCUSSION

The presence of *B. melophagi* and *T. melophagium* in our samples was verified by molecular identification analysis of *Bartonella spp.* and *Trypanosoma spp.* in *M. ovinus*, utilizing *gltA* and 18S rRNA genes. As China's largest province, Xinjiang is home to most of the country's arid regions, and *M. ovinus* has been reported multiple times (Liu *et al.*, 2019; Zhao *et al.*, 2020; Li *et al.*, 2023b). Studies have indicated that *M. ovinus* is present in Xizang, Gansu, the Eastern Tibetan Plateau, and other regions of China (Duan *et al.*, 2020; Zhang *et al.*, 2021; Liu *et al.*, 2022). Approximately 75% of emerging infectious diseases are zoonotic and 28% are vector-borne (Regier *et al.*, 2016). Therefore, a one-health strategy is required to reduce the risk of zoonotic illnesses in both humans and animals.

Based on the results of standard PCR, sequencing, and sequence analyses conducted at three sampling locations in southern Xinjiang, China, we determined that 214 *M. ovinus* and eight pupae contained *B. melophagi*. The prevalence of *B. melophagi* DNA in *M. ovinus*

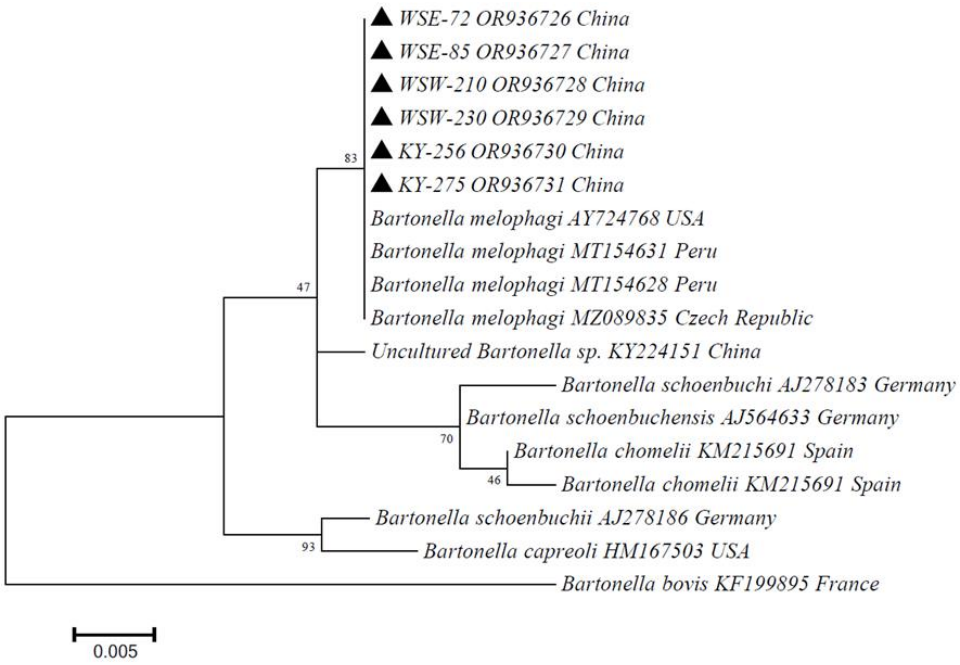

Fig. 3: Phylogenetic analysis of *B. melophagi* was conducted using concatenated fragments of partially sequenced *gltA* genes found in *M. ovinus* from southern Xinjiang, China. The maximum likelihood phylogenetic tree with 1000 bootstrap replicates was created using MEGA 7.0 software. The identified *B. melophagi* sequences are represented by a triangle (▲) in the constructed tree.

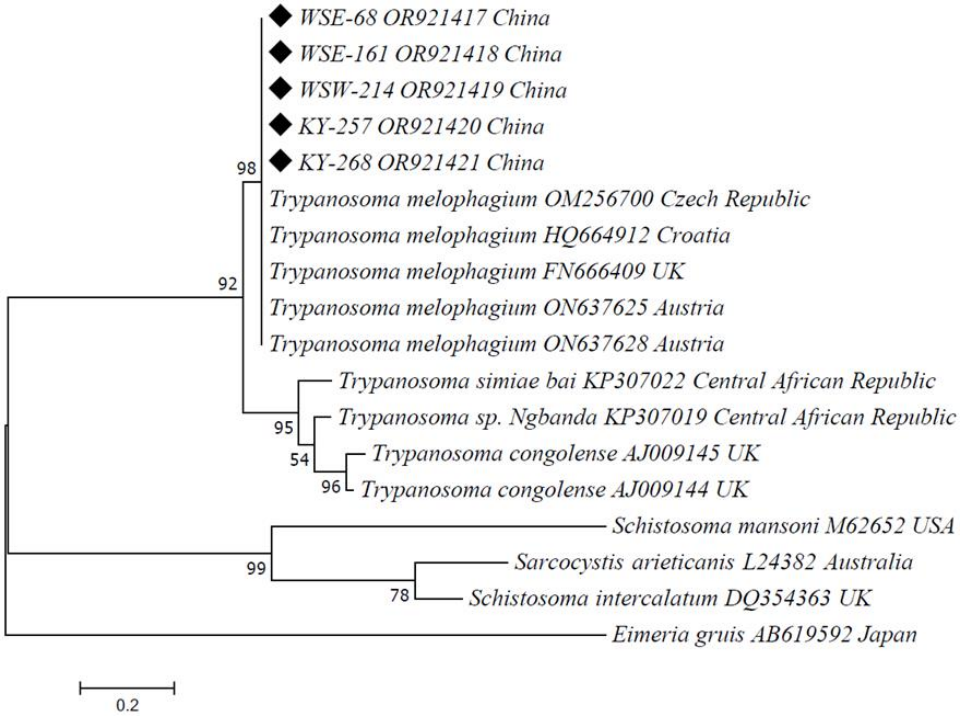

Fig. 4: Phylogenetic analysis of *T. melophagium* was conducted using concatenated fragments of 18S rRNA partial gene sequences. These sequences were detected in *M. ovinus* from southern Xinjiang, China. Maximum likelihood phylogenetic analysis with 1000 bootstrap replicates was employed to construct the tree, using MEGA 7.0 software. The identified *T. melophagium* sequences are represented by a diamond (◆) in the constructed tree.

obtained from sheep in this study was 100.0%, which is similar to the prevalence of *B. melophagi* in *M. ovinus* reported previously in Ethiopia (Kumsa *et al.*, 2014), the Czech Republic (Rudolf *et al.*, 2016), the USA (Kosoy *et al.*, 2016), Poland (Werszko *et al.*, 2021) and China (Liu *et al.*, 2018). However, their prevalence in Algeria is low (Boucheikhchoukh *et al.*, 2019). This difference in prevalence can be attributed to differences in animal hosts, living conditions, vectors, and geographical and environmental factors. The identification of *B. melophagi* in non-feeding pupae of *M. ovinus* suggests the potential vertical transmission of *B. melophagi* in *M. ovinus*. In the USA, two female patients with pericarditis and skin lesions with a history of exposure to wild or domesticated animals were found to be infected with *B. melophagi* in 2009 (Maggi *et al.*, 2009). Kosoy *et al.* (2016) detected not only the DNA of *B. melophagi* in sheep blood in the USA but also demonstrated that *M. ovinus* served as the primary vector, and its natural hosts were sheep, which was also shown to be zoonotic but requires further investigation.

A total of 214 *M. ovinus* and eight pupae were examined by PCR, and the average positivity rate was 47.2%. All the pupae tested negative. To date, there are no reports of *T. melophagium* being transported by *M. ovinus* in Asia. To our knowledge, this is the first study to report the presence of *T. melophagium* in *M. ovinus* and to provide molecular evidence that *M. ovinus* carried *T. melophagium*. This is the first report of *T. melophagium* being found in *M. ovinus* around the Taklimakan Desert. But its positivity rate was lower than 67.0% at Hořice of the Czech Republic, 86.0% in the Republic of Croatia and

82.4% in Scotland (Brotankova *et al.*, 2022; Gibson *et al.*, 2010; Martinkovic *et al.*, 2012). *T. melophagium* positivity rate in our study was lower than that reported previously. This may be related to the characteristics of *T. melophagium*, sheep feed management, and disease prevention. *T. melophagium* was not detected in the non-feeding pupae of *M. ovinus*, suggesting that vertical transmission of *T. melophagium* in *M. ovinus* may not occur. This is also the first report.

Experimental infections with *M. ovinus* isolates were unsuccessful, in agreement with *M. ovinus* as a specific vector for *T. melophagium* (Brotankova *et al.*, 2022). In an investigation of organic sheep farms, *T. melophagium* was present in 86% of *M. ovinus* but was not detected in sheep blood smears (Martinkovic *et al.*, 2012). Another investigation using blood cultures revealed that 7.8% of sheep had *T. melophagium* infections (Nalbantoglu and Karear, 2008). These sporadic reports are associated with difficulties in the detection of *T. melophagium* in sheep blood samples, possibly because of low transitory parasitemia (Magri *et al*., 2021).

M. ovinus spends its entire life on sheep wool and skin. An overwhelming majority of male and female *M. ovinus* feed on sheep blood. This makes it easy for *M. ovinus T. melophagium* to spread in sheep blood. In contrast, sheep can also be infected by eating *M. ovinus* that carries *T. melophagium*, such as by eating dropped *M. ovinus* or *M. ovinus* that are easily exposed after shearing. Most herders in southern Xinjiang perform one or two sheaths of sheep in April, July, or September. A medicinal bath was used to remove ectoparasites. The differences in prevalence among these studies can be attributed to factors such as livestock transport, field grazing, varying climatic conditions, seasonal changes, feeding patterns, and the number of samples analyzed. All of these factors can influence the transmission of pathogens between hosts and vectors. As previously noted, *T. melophagium* has been identified in the blood of symptomatic ruminants, suggesting a potential risk to both livestock production and public health.

M. ovinus is known to have a direct impact on human health, animal health and economic industries. Therefore, the control of *M. ovinus* populations is a key measure. Sheep herds with *M. ovinus* can be exterminated using effective insecticides for whole-body spraying, soaking in medicinal baths, and other measures after spring shearing. Pyrethroid insecticides and avermectin have been found to have a relatively positive effect on killing *M. ovinus*. *B. melophagi* is an intracellular bacterium and *T. melophagium* is a blood protozoan parasite. However, there is a lack of knowledge and molecular biology data regarding these species in China. They may be zoonotic pathogens; however, their pathogenic mechanisms are unclear. Regular molecular biology testing is valuable for monitoring the prevalence of relevant pathogens in herds in various regions, as these pathogens have the potential to harm both human health and the economy.

Finally, a limitation of this study is that we did not collect blood from the sheep to analyze *B. melophagi* and *T. melophagium* in *M. ovinus*. First, we could not determine the prevalence of these two pathogens in sheep flocks. However, we could not determine whether *T. melophagium* was derived from sheep blood or whether *T. melophagium* had already established a symbiotic relationship with *M. ovinus*. For exact species differentiation of these parasites, amplification and sequence analysis of other genes have been suggested, such as the ITS and SSU rRNA genes for *Trypanosoma*; *FtsZ* gene, *ribC* gene and *rpoB* genes for *Bartonella*; and the 12S and 16S rDNA genes for *Melophagus*. Therefore, the role of *M. ovinus* in the transmission of *B. melophagi* and *T. melophagium* needs further study, which is our next research direction.

Conclusions: To our knowledge, this is the first report of *T. melophagium* infection in China. Additionally, it has been proven that *B. melophagi* and *T. melophagium* can coexist in *M. ovinus* located in southern Xinjiang, China. These diseases have not been previously reported in this region, indicating potential negligence among local medical professionals and public health workers. To maintain healthy and sustainable growth of the sheep farming business and reduce threats to human health, our findings are crucial for Xinjiang herders in managing insect-borne diseases.

Funding statement: This study was supported by grants awarded by the National Natural Science Foundation of China (Granted No. 32160841 and 31960705), Key Laboratory of Tarim Animal Husbandry Science and Technology, Xinjiang Production & Construction Group (HS201802), and Tarim University Graduate Student Research Innovation Project (TDGRI202240).

Author contributions: WJ provided the research idea. ZXQ and ZX performed the collection and assembly of data. ZK and SL performed the experiments. HK and XN performed the data analysis and interpretation. HK wrote the manuscript. WJ handled the critical revision of the article. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Bittner L, Krämer K, Wöckel A, *et al*., 2021. Malnutrition as the cause of recumbency in suckler cows associated with *Trypanosoma theileri* infection. Acta Vet Scand 63(1): 2.

Boucheikhchoukh M, Mechouk N, Benakhla A, *et al*., 2019. Molecular evidence of bacteria in *Melophagus ovinus* sheep keds and Hippobosca equina forest flies collected from sheep and horses in northeastern Algeria. Comp Immunol Microb 65: 103-109.

Brenner D, Oconnor S, Winkler H, *et al*., 1993. Proposals to unify the genera *Bartonella* and *Rochalimaea*, with descriptions of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov., and to remove the family Bartonellaceae from the order *Rickettsiales*. Int J Syst Bacteriol 43(4): 777-786.

Brotankova A, Fialova M, Cepicka I, *et al*., 2022. Trypanosomes of the *Trypanosoma theileri* Group: Phylogeny and New Potential Vectors. Microorganisms 10(2): 294.

Duan D, Liu G, Cheng T, *et al*., 2017a. Microbial population analysis of the midgut of *Melophagus ovinus* via high-throughput sequencing. Parasite Vector 10(1): 382.

Duan D, Wang Y and Cheng T, 2017b. Morphological and Molecular Identification of *Melophagus ovinus*. Acta Veterinaria et Zootechnica Sinica 48(11): 2181-2188. (in Chinese)

Duan D, Zhou H and Cheng T, 2020. Comparative analysis of microbial community in the whole body and midgut from fully engorged and unfed female adult *Melophagus ovinus*. Med Vet Entomol 34(2): 215-224.

Gibson W, Pilkington J and Pemberton J, 2010. *Trypanosoma melophagium* from the sheep ked *Melophagus ovinus* on the island of St Kilda. Parasitology 137(12): 1799-1804.

Gonçalves-Oliveira J, Gutierrez R, Schlesener C, *et al*., 2023. Genomic Characterization of Three Novel *Bartonella* Strains in a Rodent and Two Bat Species from Mexico. Microorganisms 11(2): 340.

Hajihassani A, Maroufi S, Esmaeilnejad B, *et al*., 2020. Hemolytic anemia associated with *Trypanosoma theileri* in a cow from Kurdistan province, West of Iran. Vet Res Forum 11(2): 191-193.

Hao L, Yuan D, Li S, *et al*., 2020. Detection of *Theileria* spp. in ticks, sheep keds (*Melophagus ovinus*), and livestock in the eastern Tibetan Plateau, China. Parasitol Res 119(8): 2641-2648.

Kosoy M, Bai Y, Enscore R, *et al*., 2016. *Bartonella melophagi* in blood of domestic sheep (*Ovis aries*) and sheep keds (*Melophagus ovinus*) from the southwestern US: Cultures, genetic characterization, and ecological connections. Vet Microbiol 190: 43-49.

Kumsa B, Parola P, Raoult D, *et al*., 2014. *Bartonella melophagi* in *Melophagus ovinus* (sheep ked) collected from sheep in northern Oromia, Ethiopia. Comp Immunol Microb 37(1): 69-76.

Kumar S, Stecher G, Li M, *et al*., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35(6):1547-1549.

Li D, Hou Y, Song X, *et al*., 2015. High prevalence and genetic heterogeneity of rodent-borne *Bartonella* species on Heixiazi Island, China. Appl Environ Microb 81(23): 7981-7992.

Li S, Zhang L, Li Z, *et al*., 2023a. Distribution and Molecular Epidemiology of *Anaplasma ovis* in *Melophagus ovinus* and Small Ruminants in Border Regions of South Xinjiang, China. Vector-Borne Zoonot 23(1): 1-8.

Li S, Zhang L, Li Z, *et al*., 2023b. Detection of *Rickettsia* spp. and *Anaplasma ovis* in *Melophagus ovinus* from southern Xinjiang, China. Med Vet Entomol.

Li S, Zhang L, Zhou P, *et al*., 2023c. Detection of *Babesia* Spp., *Theileria* Spp., and *Anaplasma Ovis* in *Ornithodoros Lahorensis* from Southern Xinjiang, China. J Vet Res 67(1): 79-85.

Liu Y, He B, Li F, *et al*., 2018. Molecular Identification of *Bartonella melophagi* and *Wolbachia* Supergroup F from Sheep Keds in Xinjiang, China. Korean J Parasitol 56(4): 365-370.

Liu Y, He B, Li K, *et al*., 2019. First report of border disease virus in *Melophagus ovinus* (sheep ked) collected in Xinjiang, China. Plos One 14(8): e0221435.

Liu Y, Ma Y, Tian H, *et al*., 2022. First determination of DNA virus and some additional bacteria from *Melophagus ovinus* (sheep ked) in Tibet, China. Front Microbiol 13: 988136.

Maggi R, Kosoy M, Mintzer M, *et al*., 2009. Isolation of Candidatus *Bartonella melophagi* from human blood. Emerg Infect Dis 15: 66-68.

Magri A, Galuppi R and Fioravanti M, 2021. Autochthonous *Trypanosoma* spp. in European Mammals: A Brief Journey amongst the Neglected Trypanosomes. Pathogens 10(3): 334.

Martinkovic F, Matanovic K, Rodrigues A, *et al*., 2012. *Trypanosoma (Megatrypanum) melophagium* in the sheep ked *Melophagus ovinus* from organic farms in Croatia: phylogenetic inferences support restriction to sheep and sheep keds and close relationship with trypanosomes from other ruminant species. J Eukaryot Microbiol 59(2): 134-144.

Nalbantoglu S and Karear Z, 2008. *Trypanosoma melophagium* in blood cell culture. Ankara Univ Vet Fak 55: 173-176.

Ni J, Ren Q, Lin H, *et al*., 2021. Molecular Evidence of *Bartonella melophagi* in Ticks in Border Areas of Xinjiang, China. Front Vet Sci 8: 675457.

Oldrieve G, Malacart B, López-Vidal J, *et al*., 2022. The genomic basis of host and vector specificity in non-pathogenic trypanosomatids. Biol Open 11(4).

Regier Y, O'Rourke F and Kempf V, 2016. *Bartonella* spp. - a chance to establish One Health concepts in veterinary and human medicine. Parasite Vector 9(1): 261.

Rudolf I, Betásová L, Bischof V, *et al*., 2016. Molecular survey of arthropod-borne pathogens in sheep keds (*Melophagus ovinus*), Central Europe. Parasitol Res 115(10): 3679-3682.

Saitou N and Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406-25.

Small R, 2005. A review of *Melophagus ovinus* (L.), the sheep ked. Vet Parasitol 130(1-2): 141-155.

Sood N, Singla L, Singh R, *et al*., 2011. Association of *Trypanosoma theileri* with peritonitis in a pregnant cross-bred cow: a case report. Vet Med-Czech 56(2): 82-84.

Werszko J, Asman M, Witecka J, *et al*., 2021. The role of sheep ked (*Melophagus ovinus*) as potential vector of protozoa and bacterial pathogens. Sci Rep 11(1): 15468.

Werszko J, Szewczyk T, Steiner-Bogdaszewska Z, *et al*., 2020. Molecular detection of *Megatrypanum* trypanosomes in tabanid flies. Med Vet Entomol 34(1): 69-73.

Zhang Q, Wang Y, Li Y, *et al*., 2021. Vector-Borne Pathogens with Veterinary and Public Health Significance in *Melophagus ovinus* (Sheep Ked) from the Qinghai-Tibet Plateau. Pathogens 10(2): 249.

Zhang Q, Zhou Q, Han S, *et al*., 2023. The genome of sheep ked (*Melophagus ovinus*) reveals potential mechanisms underlying reproduction and narrower ecological niches. Bmc Genomics 24(1): 54.

Zhao L, Wang J, Ding Y, *et al*., 2020. *Theileria ovis* (Piroplasmida: Theileriidae) Detected in *Melophagus ovinus* (Diptera: Hippoboscoidea) and *Ornithodoros lahorensis* (Ixodida: Argasidae) Removed From Sheep in Xinjiang, China. J Med Entomol 57(2): 631-635.