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Molecular Identification of *Taenia hydatigena* from Goats in Khishig-Undur, Mongolia

(IDENTIIKASI SECARA MOLEKULER TAENIA HYDATIGENA DARI KAMBING ASAL KHISHIG-UNDUR, MONGOLIA)

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ABSTRACT

Cysticercosis caused by the larval form of the *Taenia hydatigena* tapeworm poses a global challenge to the livestock industry. A total of 56 goats slaughtered in Khishig-Undur, Mongolia were evaluated for the presence of cystic lesions. Collected cysts were assessed using the mitochondrial 12S rRNA gene. In total, 46.4% (26/56) of evaluated goats were positive for *T. hydatigena* infection, with most cysts attached to the omentum, mesentery, liver, or spleen. Partial 12S rRNA gene sequences were obtained from all evaluated cysts and aligned with known sequences for *T. hydatigena*. Infection prevalence was higher in goats three years of age and older (50.0%; 17/34) compared to goats less than three years of age (40.0%; 9/22) (p=0.035). Infection with *T. hydatigena* appears to be highly prevalent in goats in Khishig-Undur. Additional studies are needed to evaluate local parasite transmission dynamics and the impact of this parasite on local livestock production.

Keywords: *Taenia hydatigena;* goat; 12S rRNA gene; Mongolia

ABSTRAK

Cysticercosis disebabkan oleh bentuk larva cacing pita *Taenia hydatigena* yang kini menjadi tantangan sedunia dalam industry peternakan. Sebanyak 56 ekor kambing yang disembelih di Khishig-Undur, Mongolia dievaluasi terhadap keberadaan lesi berbentuk kista. Kista-kista yang dikoleksi dinilai menggunakan gen mitochondria 12S rRNA. Secara ksdluruhan, 46,4% (26/56) kambing-kambing yang dievalusi ternyata positif terinfeksi *T. hydatigena*, dan sebagian besar kista menempel pada omentum, mesenterika, hati atau limpa. Sekuens gen secara parsial terhadap 12S rRNA diperoleh dari seluruh kista yang dievaluasi dan dijajarkan/*aligned* dengan sekuens yang telah diketahui untuk *T. hydatigena*. Prevalensi infeksi ditemukan lebih tinggi pada kambing umur tiga tahun dan di atas tiga tahun, yakni 50,%; atau 17/34 dibandingkan dengan kambing di bawah tiga tahun, dengan prevalensi 40.0% atau 9/22, (p=0.035). Infeksi oleh *T. hydatigena* ternyata sangat prevalent pada kambing di Khishig-Undur. Studi lanjutan perlu dilakukan untuk mengevaluasi dinamika transmisi parasit lokal dan dampak parasit-parasit tersebut terhadap produksi peternakan di daerah tersebut.

Kata-kata kunci: *Taenia hydatigena;* goat; gen 12S rRNA; Mongolia

INTRODUCTION

Animal husbandry is the backbone of Mongolia's economy, accounting for more than 20% of its gross domestic product (Temuujin *et al.* 2021). Mongolia has over 70 million livestock, including 32.3 million sheep, 29.3 million goats, 4.7 million cattle, 4.2 million horses, and 0.5 million camels. The country is also the world's largest cashmere producer, accounting for half of the global production. As a result, the population of cashmere goats in Mongolia is increasing yearly (Wei and Zhen, 2020; FAO, 2021).

Taenia hydatigena is a common tapeworm found in domestic and wild animals worldwide and is considered a major obstacle to livestock production in lower-middle-income countries (Rostami *et al.*, 2015) including Mongolia (Ulziijargal *et al.*, 2020; Temuujin *et al.* 2021). Carnivores such as foxes, wolves, snow leopards, corsac foxes, and dogs act as definitive hosts of *T. hydatigena* (Ulziijargal *et al.*, 2020). This species is also commonly found in goats from Iran (Oryan *et al.,* 2012), China (Xia *et al*., 2014), Ethiopia (Admasu *et al*., 2019), Bangladesh (Islam, 1995), and Brazil (Morais *et al.,* 2017) whose intermediate hosts are wild and domestic hooved mammals. Domestic livestock, including sheep, goats, cattle, and wild hoofstock, including argali, ibex, and roe deer, serve as intermediate hosts for *T. hydatigena* in Mongolia (Tinnin *et al.*, 2011). This cestode has medical, veterinary and economic importance due to its hepatic migration, especially in intermediate hosts (Nourani *et al*., 2010). The intermediate host becomes infected by ingesting eggs contained in canid feces that contaminate pastures or feeding areas (Miran *et al*., 2017). The larvae then penetrate the intestinal wall and migrate to the liver. The larvae can remain in the liver or migrate to the surface of the liver, mesentery, or omentum, where they attach and develop into fluid-filled vesicular cysts called cysticerci. *Taenia hydatigena* infection can result in economic losses due to reduced weight gain and other production losses (Debas and Ibrahim, 2013). This species is also capable of infecting humans and causing lethal coenurosis (Deplazes *et al.*, 2019).

Taenia. hydatigena has recently been found in Mongolian sheep dogs (16.2%) (Temuujin *et al.,* 2021), as well as wildlife, including gray wolves (19.9%), red foxes (13.8%), corsac foxes (4.8%), and snow leopards (7.5%) (Ulziijargal *et al.*, 2020). The number of parasitic disease cases in humans and domestic animals has increased annually in Mongolia (Myadagsuren *et al.,* 2007; McFadden *et al*., 2016); however, the diversity, distribution, and prevalence of cestode infections in livestock and companion animals have not been sufficiently investigated (Ulziijargal *et al.,* 2020). To date, the diversity, distribution, and prevalence of cestode infections in livestock and companion animals have not been sufficiently investigated in Mongolia and little is

known about the molecular characteristics of *T. hydatigena* infecting livestock, especially goats (Ulziijargal *et al.*, 2020). Therefore, this study was designed to molecularly characterize *T. hydatigena* cysts obtained from goats using the mitochondrial 12S rRNA (12S ribosomal RNA) gene and describe the characteristics of infected goats.

RESEARCH METHODS

Study Area

This study was conducted in the Mongolian administrative district (soum) of Khishig-Undur, which is located in the province of Bulgan and surrounded by three mountain ranges: the Darkhan-Khaan, the Burenkhaan, and the Khishig-Undur (Fig. 1). The region has an average altitude of 1300 m above sea level and an approximate area of 245,500 hectares. Water from local rivers makes it an ideal location for livestock rearing. At the last count, Khishig-Undur had 173,272 heads of livestock, including 15,611 horses, 12,052 cattle, 85,427 sheep, and 60,182 goats (FAO, 2021). At the time of this study, Khishig-Undur was home to 601 herder households and 417 livestock-owning households, with a total population of 3,171. Herders tend to live in more remote areas, whereas non-herder livestock owners tend to live near the center of the soum and often hold full-time non-livestock-rearing-related positions, such as teachers and government employees.

Sample Collection

Herders often come together to group slaughter their animals after receiving government approval to sell their meat in the capital. During a single day in October 2013, 56 goats were examined for the presence of cystic lesions during routine government-approved slaughter by a group of herders in Khishig-Undur. The animals were selected in a purposive manner from a total of 85 goats slaughtered that day. Information collected for each enrolled slaughtered animal included the goat's age (<3 years of age versus >3 years of age), sex, and cyst location(s). For all collected cysts, cyst fluid was drained and cysticerci were stored in 70% ethanol at −20 °C until processing. This study was approved by the Medical Ethical Committee of the Mongolian National University of Medical Sciences (METc No. 11-02/2A).

DNA Extraction and PCR

Genomic DNA was extracted from an excised portion of each cysticercus using the DNeasy Blood and Tissue Kit (Qiagen®, Hilden, Germany) according to the manufacturer's instructions. The mitochondrial 12S ribosomal RNA gene (12S rRNA) (von Nickisch-Rosenegk *et al.,* 1999) was amplified using primers as described previously. The PCR (30 µL) was carried out using 10× PCR buffer (Takara®, Shuzo Co., Tokyo, Japan) (3 µL), 2.5 mM deoxynucleoside triphosphate mixture (2.4 µL), 20 µM forward primer (0.38 µL), reverse primer (0.38 µL), EX Taq polymerase (Hot start version, Takara, Japan) (0.15 µL), template DNA (3 µL), and double-distilled H_2O (20.7 µL). The following cycling conditions were used: 98 °C for 30 s, followed by 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 35 s for 30 cycles. A Biometra T-Gradient thermocycler (T Gradient®; Biometra, Göettingen, Germany) was used for amplification. Genomic DNA from *Echinococcus granulosus* was used as a positive control. Template DNA samples for DNA sequencing were prepared using the DyeTerminator Cycle Sequencing kit (Applied Biosystems®, Foster City, CA, USA), and all amplicons were sequenced in both directions using a 3100-Advant Genetic Analyzer (ABI PRISM, Applied Biosystems).

Phylogenetic Analyses

Sequencing data were assembled and edited using ClustalW implemented in DNAStar software (DNAStar®, Madison, WI, USA). The distance matrix of nucleotide divergence was calculated according to the Kimura's two-parameter model provided by MEGA 11 (http://www.megasoftware.net/). Evolutionary distances were computed using the maximum composite parsimony method (Tamura *et al.,* 2021).

Statistical Analyses

Data were entered into a Microsoft Excel spreadsheet, with statistical analyses performed using SPSS® (version 22) (IBM®, Armonk, NY, USA). Prevalence was defined as the number of animals with confirmed *T. hydatigena* cysts/ total number of sampled animals × 100. The chi-square test was used to detect differences in infection by age and sex. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Twenty-six of the 56 goats examined (46.4%) had cysts visible on routine slaughter-based inspection. All evaluated cysts were confirmed to be *T. hydatigena* via PCR. Table 1 shows infection prevalence by age and sex. Infection prevalence was higher in goats aged >3 years (50.0%; 17/34) than in goats aged <3 years (40.0%; 9/22) (p=0.035). Females had a higher prevalence (47.4%; 18/35) than males (38.1%; 8/21), but there was no significant difference between the two groups (p=0.225) (Table 1). Cysts were found in the omentum (n=9), mesentery (n=6), liver (n=4), spleen (n=4), lungs (n=2), tongue (n=1), and diaphragm (n=1). A 414 base pair PCR product of the 12S rRNA gene was successfully obtained for all 26 samples. Acquired sequences were unique but aligned with known sequences for *T. hydatigena* (Table 2, Fig. 2).

Goats are common production animals in Mongolia. However, parasite infections in Mongolian goats have yet to be extensively investigated. This study found that *T. hydatigena* infection in goats was prevalent in Khishig-Undur, Mongolia, with an overall prevalence of 46.4%, which was low compared to 72.3% in goats from Dessi, Ethiopia (Gessese *et al*., 2015) 66.5% in dogs from Dagestan, Russia (Moskvina *et al*., 2016), 46.94% in goats, 62.77% in sheep from Tibet, China (Xia *et al.,* 2014). This may be due to high levels of environmental pollution (Abbas *et al*., 2021). The lowest recorded *T. hydatigena* infection rates in goats ranged from 1.6% in Andhra Pradesh, India (Rao *et al* ., 2003), to 2.2% in Aswan, Egypt (Elshahawy *et al.,* 2014), 2.4% in Queensland, Australia (Jenkins *et al*., 2018), 2.6% in Kurdistan, Iraq (Ahmed Hama *et al.,* 2018), 4.9% in Tabriz Iran (Mirzaei *et al*., 2015). Differences in prevalence between countries and regions are likely a result of differences in grazing and deworming (or lack of deworming) practices. The propensity for livestock owners to feed raw offal to shepherd dogs in the study region likely contributes to local transmission (Temuujin *et al.*, 2021). In addition, the privatization of livestock owners in the early 1990s (versus government ownership) has resulted in increased livestock numbers in Mongolia. As herders and other private livestock owners are now also responsible for the veterinary care of their animals, vaccination and deworming coverage has likely decreased.

Goats older than three years of age had a higher infection prevalence than those younger than three years of age, which is consistent with the findings of previous studies (Singh *et al*., 2015; Omar *et al.*, 2016). This may be because older goats are more likely to come into contact with parasite eggs in grazing areas, whereas younger goats are often confined in enclosures. No difference in infection prevalence by sex was found, which is also consistent with the findings of other studies (Mirzaei *et al.*, 2015). Several studies have reported a significantly higher prevalence in females (Khanjari *et al.,* 2015; Omar *et al.*, 2016), which may be due to females being kept in the herd longer than males. The liver had the highest infection rate among the organs in this study, which is consistent with the findings of Mekuria *et al.* (2013) whereas the omentum was the preferred site in several previous studies (Braae *et al*., 2015; Dyab *et al.,* 2017).

In previous studies, *T. hydatigena* was identified using morphological and serological methods in various Mongolian intermediate hosts, including sheep, horses, camels, cows, goats, red deer, wild sheep, and mountain goats (Tinnin *et al.*, 2011). However, to date, mitochondrial DNA analysis has only been used to identify *T. hydatigena* from definitive hosts in Mongolia (Ulziijargal *et al.*, 2020; Temuujin *et al.*, 2021). A single isolate of *T. hydatigena* was identified from goats by evaluating the 12S rRNA gene. The sequence data from the *T. hydatigena* isolate from Mongolian goats were unique but aligned with reference sequences in GenBank, including sequences from goats and sheep from China, Egypt, Iran, and Iraq (Table 2, Fig 2). Additional studies are needed to further evaluate differences between circulating isolates.

Study limitations include the small sample size and non-random sampling method. Logistical constraints make large-scale studies challenging in the study area. However, additional studies covering a larger geographic region are needed to determine any molecular, genetic, and spatial differences in *T. hydatigena* infection, as well as determine risks for infection in other domestic and wild herbivores. Since *T. hydatigena* is also considered zoonotic, a more holistic One Health approach should be taken for future research and control initiatives, with collaboration between the agricultural, public health, and environmental/wildlife sectors.

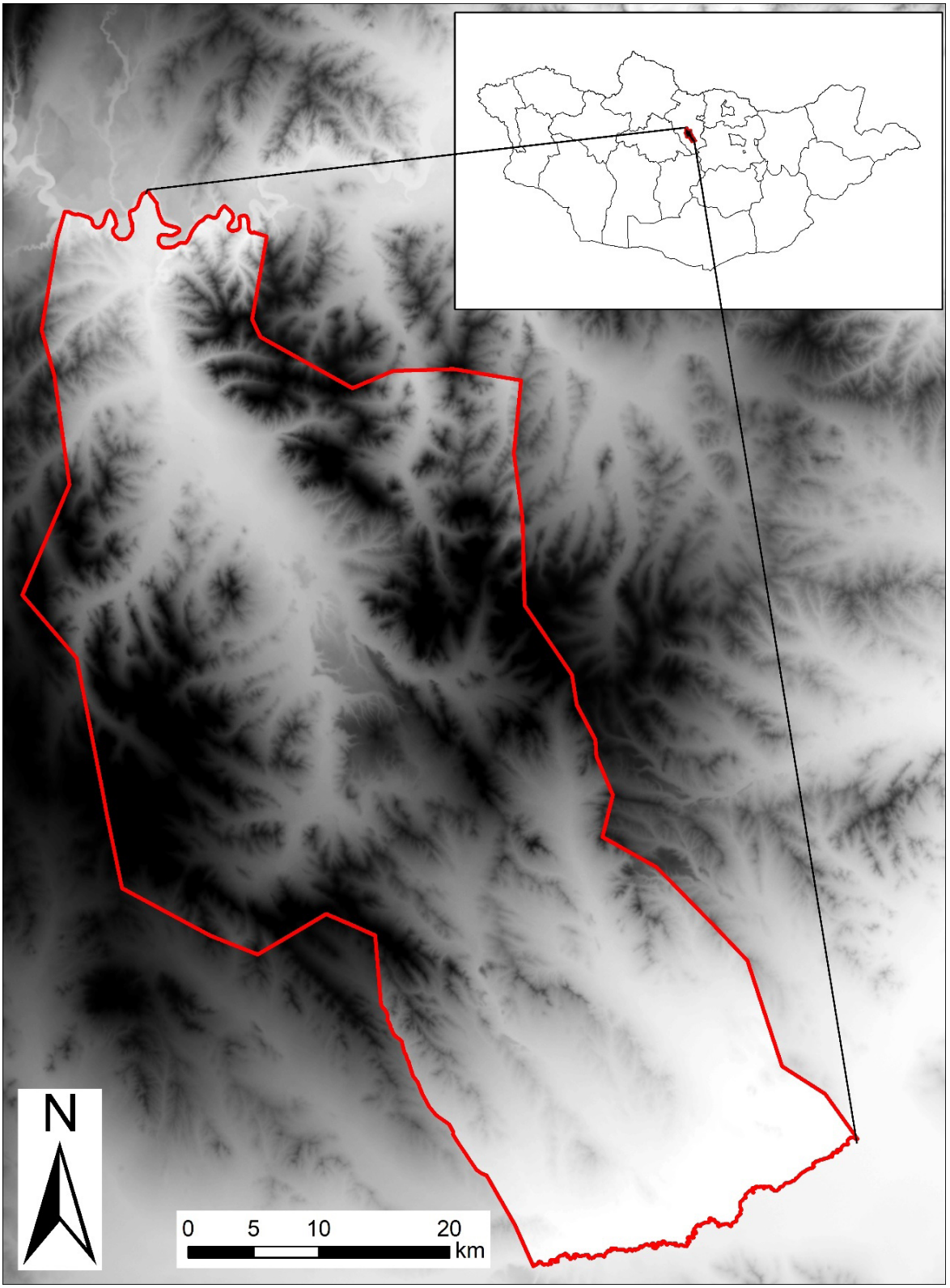

Figure 1. Mongolian map depicting the study area in the Khishih-Undur soum.

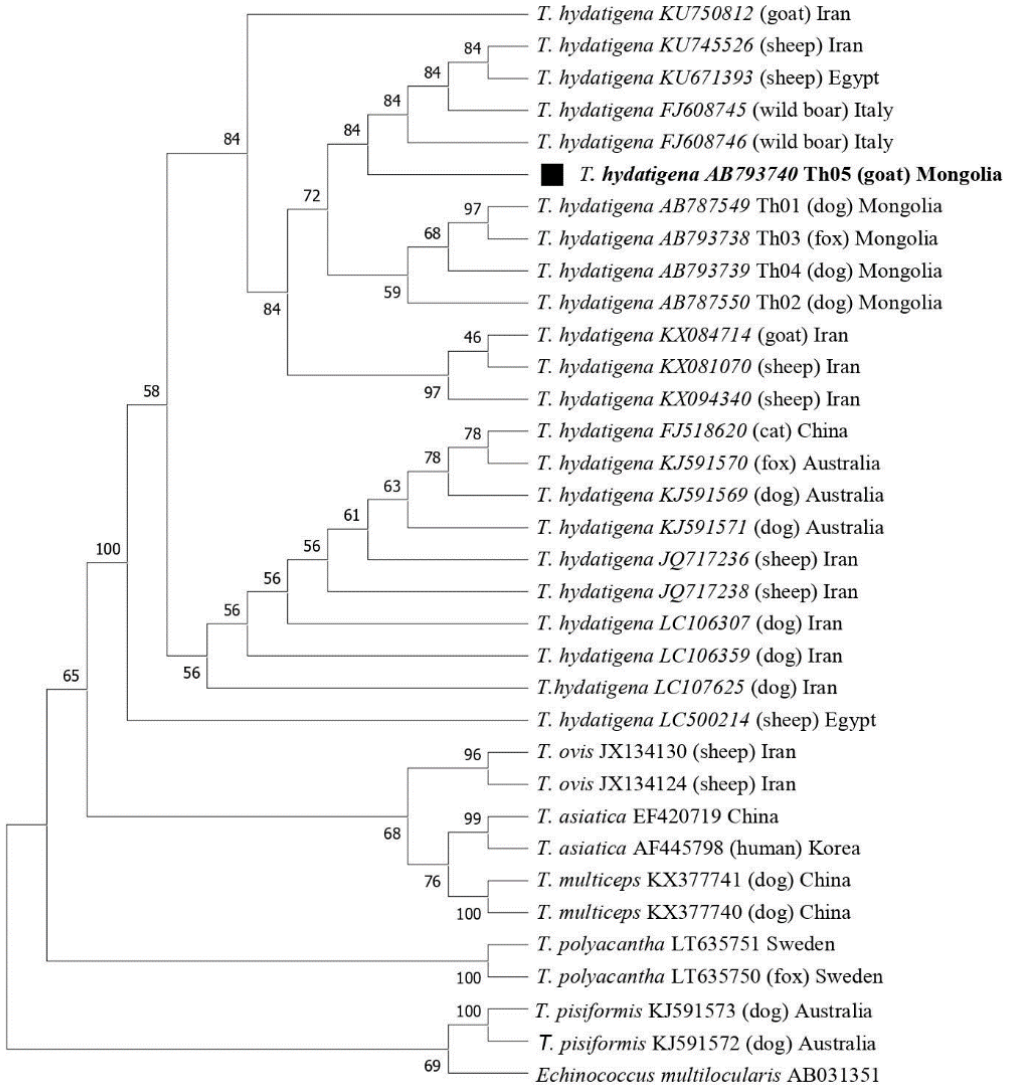

Figure 2. Phylogenetic tree constructed using the maximum likelihood method based on partial 12S rRNA sequences of *Taenia hydatigena.* Sequences reported in the present study are shown in bold. HKY + G was used as the best substitution model. Numbers at the end of branches are percent bootstrap values based on 1000 replicates. An *Echinococcus multilocularis* sequence was used as the outgroup.

Table 1. *Taenia hydatigena* infection prevalence in goats by age and sex in Khishig-Undur soum, Bulgan Province, Mongolia (n=56).

Age and sex	No. of samples examined	No. of positive samples	%	p-*value**
Age (year)				
<3	22	9	40,9	0.035
≥3	34	17	50.0	
Sex				
Female	35	18	51.4	0.225
Male	21	8	38.1	

*Using the chi-square test

Table 2. Pairwise genetic distance in the 12S rRNA gene among *Taenia hydatigena* isolates from Mongolia and related taxa.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*T. hydatigena* KU750812 (goat) Iran																							
2	*T. hydatigena* KU745526 (sheep) Iran	0.000																						
3	*T. hydatigena* FJ518620 (cat) China	0.013	0.013																					
4	*T. hydatigena* KX084714 (goat) Iran	0.000	0.000	0.013																				
5	*T. hydatigena* LC107625 (dog) Iran	0.000	0.000	0.013	0.000																			
6	*T. hydatigena* LC500214 (sheep) Egypt	0.000	0.000	0.013	0.000	0.000																		
7	*T. hydatigena* AB787549 (dog) Mongolia	0.033	0.033	0.047	0.033	0.033	0.033																	
8	*T. hydatigena* AB787550 (dog) Mongolia	0.000	0.000	0.013	0.000	0.000	0.000	0.033																
9	*T. hydatigena* AB793738 (fox) Mongolia	0.027	0.027	0.040	0.027	0.027	0.027	0.006	0.027															
10	*T. hydatigena* AB793739 (dog) Mongolia	0.006	0.006	0.020	0.006	0.006	0.006	0.027	0.006	0.020														
11	***T. hydatigena* AB793740 (goat) Mongolia**	**0.000**	**0.000**	**0.013**	**0.000**	**0.000**	**0.000**	**0.033**	**0.000**	**0.027**	**0.006**													
12	*T. hydatigena* FJ608746 (wild boar) Italy	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000												
13	*T. hydatigena* FJ608745 (wild boar) Italy	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000	0.000											
14	*T. hydatigena* LC106359 (dog) Iran	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000	0.000	0.000										
15	*T. hydatigena* LC106307 (dog) Iran	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000	0.000	0.000	0.000									
16	*T. hydatigena* KX094340 (sheep) Iran	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000	0.000	0.000	0.000	0.000								
17	*T. hydatigena* KX081070 (sheep) Iran	0.006	0.006	0.020	0.006	0.006	0.006	0.040	0.006	0.033	0.013	0.006	0.006	0.006	0.006	0.006	0.006							
18	*T. hydatigena* KU671393 (sheep) Egypt	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.006						
19	*T. hydatigena* JQ717238 (sheep) Iran	0.006	0.006	0.020	0.006	0.006	0.006	0.040	0.006	0.033	0.013	0.006	0.006	0.006	0.006	0.006	0.006	0.013	0.006					
20	*T. hydatigena* JQ717236 (sheep) Iran	0.006	0.006	0.020	0.006	0.006	0.006	0.040	0.006	0.033	0.013	0.006	0.006	0.006	0.006	0.006	0.006	0.013	0.006	0.013				
21	*T. hydatigena* KJ591571 (dog) Australia	0.006	0.006	0.020	0.006	0.006	0.006	0.040	0.006	0.033	0.013	0.006	0.006	0.006	0.006	0.006	0.006	0.013	0.006	0.013	0.013			
22	*T. hydatigena* KJ591569 (dog) Australia	0.000	0.000	0.013	0.000	0.000	0.000	0.033	0.000	0.027	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.006	0.006	0.006		
23	*T. hydatigena* KJ591570 (fox) Australia	0.006	0.006	0.020	0.006	0.006	0.006	0.040	0.006	0.033	0.013	0.006	0.006	0.006	0.006	0.006	0.006	0.013	0.006	0.013	0.013	0.013	0.00	

CONCLUSION

Taenia hydatigena appears to be a common parasitic infection in goats raised in Khishig-Undur soum of Mongolia. Additional studies are needed to evaluate the socioeconomic impact of this parasite on local livestock production, as well as better understand regional domestic and wildlife hosts and any molecular differences in circulating parasites.

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