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Taeniasis in Mongolia, 2002–2006

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Abstract. Survey on secondary data of taeniasis/cysticercosis was carried out in Mongolia in 2002–2006. A total of 118 taeniid proglottids, a diphyllobothriid segment, and 59 serum samples from 118 taeniasis cases were collected at National Center for Communicable Diseases, Ulaanbaatar, Mongolia. In 2006, 14 serum samples were collected from local people who had histories of epileptic seizures in Selenge Province where pig husbandry was the main business. The 118 proglottids were confirmed to be *Taenia saginata* by mitochondrial DNA analysis using *cytochrome c oxidase subunit 1* and *cytochrome b* genes. *T. saginata* taeniasis was widely distributed at least in 10 of 21 provinces. No variation in the nucleotide sequences of the two genes was observed among *T. saginata* isolates from Mongolia. There was no evidence of *Taenia solium* taeniasis/cysticercosis or *Taenia asiatica* taeniasis. A diphyllobothriid segment was confirmed to be *Diphyllobothrium latum* by mitochondrial DNA analysis.

INTRODUCTION

Mongolia is located in the northern part of Central Asia bordering with the Russian Federation and the People's Republic of China (Figure 1), and is mountainous country with an average altitude of 1,580 m above sea level. The geography of the country is characterized by great diversities. From north to south, it can be divided into four areas: mountain-forest steppe, mountain steppe, semi-desert, and desert.[1] Mongolia consists of 21 provinces, the population of Mongolia is ~2.8 million, and the average population density was 2.5 persons/km^2 in 2004. Ulaanbaatar, the capital of Mongolia, has a population of ~1 million. Nearly 40% of the rural population is nomadic or semi-nomadic herdsmen. Buddhist (Lamaist), Shamanist/Christian, Muslim, and other religions account for 50%, 6%, 4%, and 40%, respectively, in 2004.

The national pattern of diseases in Mongolia showed that cardiovascular diseases are the major causes of mortality (5,808 cases, 23.06 per 10,000 population), followed by neoplasm (3,062 cases, 12.16/100,000), injury, poisoning, and certain other consequences of external causes (2,603 cases, 10.34/100,000), diseases of the digestive system (1,213 cases, 4.82/100,000), diseases of the respiratory system (764 cases, 3.03/100,000), certain conditions originating in the prenatal period (472 cases, 1.87/100,000), certain infectious and parasitic diseases (378 cases, 1.50/100,000), and others (995 cases, 6.54/100,000).[2]

Although there has not yet been a national report on taeniasis/cysticercosis in Mongolia, Dovdon reported a 5.5% prevalence of *Taenia saginata* taeniasis (Dovdon Y, unpublished). A recent questionnaire survey on taeniasis in Mongolia reported that 13.4 ± 0.8% and 7.2 ± 0.5% of the Mongolian population were infected with *T. saginata* in the forest-steppe and steppe regions, respectively (Temuulen D, unpublished). A special issue by Cross[3] may be the only source for information on the general situation of parasitic diseases in Mongolia. At present, *Enterobius vermicularis* infection is the most common parasitic disease in Mongolia, followed by taeniasis (National Center for Communicable Diseases, Ulaanbaatar, unpublished data).

This paper reports a wide distribution of *T. saginata* taeniasis, one diphyllobothriasis case caused by *Diphyllobothrium latum*, and the present situation of *Taenia solium* cysticercosis in Mongolia during 2002–2006.

MATERIALS AND METHODS

Cestode and serum samples. Secondary data of helminthiases or helminthes confirmed at local health centers in each province are reported to the National Center for Communicable Diseases, Ulaanbaatar, Mongolia every year. A survey on secondary data of taeniasis/cysticercosis was carried out for this study in Mongolia during 2002–2006 when A. Ito visited Mongolia and launched a joint project on taeniasis/cysticercosis and echinococcosis in Mongolia with A. Davaajav in 2002. Samples were collected at the Laboratory of Parasitology, National Center for Communicable Diseases, Ulaanbaatar: 118 taeniid proglottids (19, 24, 23, 38, and 14 proglottids in 2002, 2003, 2004, 2005, and 2006, respectively), 59 serum samples from the 118 taeniasis cases, and a segment sample from a diphyllobothriasis patient who visited the laboratory in 2003 were examined. In 2006, an additional 11 and 3 serum samples were collected from local people who had histories of epileptic seizures at local health centers in Sukhbaatar and Darhan, Selenge Province, respectively, where pig husbandry is the main business. The taeniid proglottids and a diphyllobothriid segment were kept in 99.5% ethanol for mitochondrial (mt)DNA analysis after collection. Serum samples were kept at –30°C until use.

Serology for cysticercosis. For a total of 73 serum samples including 14 serum samples from people with histories of epileptic seizures, enzyme-linked immunosorbent assay (ELISA) and immunoblot were carried out using glycoproteins (GPs) purified by preparative isoelectric focusing from *T. solium* cyst fluids[4,5] and a recombinant chimeric *T. solium* antigen.[6] These assays were performed at Asahikawa Medical College, Japan, in 2004 and 2006.

Molecular identification of cestode samples by mtDNA analysis. mtDNA analysis was done at Asahikawa Medical College, Japan, in 2004 and 2006. mtDNA samples from tae-

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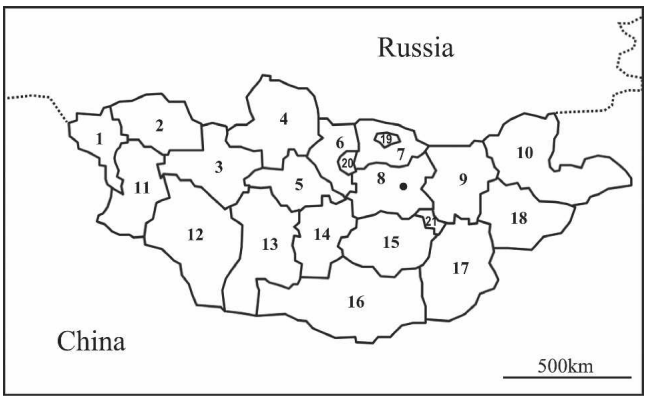

FIGURE 1. Taeniasis cases ($N = 118$) reported from 10 provinces of Mongolia during 2002–2006: 4, Khovsgol ($N = 1$); 17, Dornogobi ($N = 1$); 11, Khovd ($N = 2$); 15, Dundgobi ($N = 2$); 14, Suhbaatar ($N = 3$); 5, Arkhangay ($N = 5$); 9, Khentii ($N = 7$); 10, Dornod ($N = 8$); 7, Selenge ($N = 10$); 8, Tuv ($N = 79$). 1, Bayan-Ulgii; 2, Ubs; 3, Zavkhan; 6, Bulgan; 12, Gobi-Altai; 13, Bayarkhongor; 14, Ovorkhangai; 16, Omnogobi; 19, Darkhan-Uul; 20, Orkhon-Uul; 21, Gobi-Sumber. A closed circle in Tuv indicates Ulaanbaatar, the capital of Mongolia.

niid progrottids and a diphyllobothriid segment were prepared using a DNeasy tissue kit (Qiagen, Hilden, Germany). For molecular identification of the taeniid parasites, multiplex polymerase chain reaction (PCR) using cytochrome *c* oxidase subunit 1 gene (*cox1*) was performed according to method reported previously[7] except for the use of a forward primer (5′-TTATTTATTTACGTCAATCTTATTG-3′) for *Taenia asiatica* (T Wandra, unpublished data). For the complete nucleotide sequence analyses of *cox 1* and cytochrome *b* gene (*cob*) from the taeniid proglottids, both genes were amplified using two primer sets as described previously.[8] *Cox1* from the diphyllobothrid species was amplified by PCR using a forward primer (5′-CATAAGCGTATTGGTATGATTTA-3′, positions 43-65) and a reverse primer (GACATTGTAGTAAATACTTATTCA-3′, positions 1,240-1,217) based on the nucleotide sequence of *cox1* from *Diphyllobothrium nihonkaiense*.[9] *Ex Taq* DNA polymerase (hot start version; TaKaRa BIO, Kyoto, Japan) was used. The cycling conditions were 98°C for 30 seconds (activation of the enzyme), followed by 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 90 seconds, and a final extension at 72°C for 5 minutes.

The PCR products were directly sequenced using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (ABI, Applied Biosystems, Foster City, CA) for taeniid samples[7] and DYEnamic ET terminator (Amersham Biosciences, Buckinghamshire, UK) for diphyllobothriid cestode using Genetic Analyzer (ABI PRISM 310; Applied Biosystems, Foster City, CA).[10]

Statistic analysis. Statistic data analysis was performed by univariate analysis using EpiInfo version 6.

RESULTS

Molecular identification of Mongolian taeniid cestodes. A total of 118 proglottids from 10 different provinces were identified by multiplex PCR using *cox1*. Figure 2 shows the representative results of 20 of the 118 progrottids. The PCR

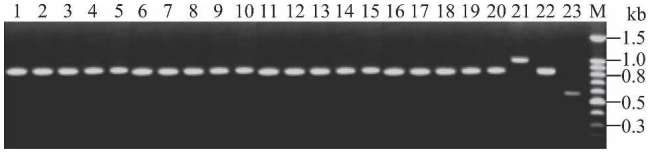

FIGURE 2. Molecular identification of taeniid proglottids expelled from 118 worm carriers by multiplex PCR using *cox1*. PCR products were run on a 1% agarose gel at 100 V and stained with ethidium bromide. Lanes 1–20, Mongolian taeniid proglottids; lanes 21–23, positive controls for *T. solium* Asian genotype (984 bp), *T. saginata* (827 bp), and *T. asiatica* (588 bp), respectively. M, 100-bp ladder DNA size markers (Promega).

products with molecular sizes of 827 bp were amplified from all samples, and the taeniid cestodes were identified to be *T. saginata*. DNA sequencing of *cox1* and *cob* also supported that the taeniid proglottids were *T. saginata.* In the case of *cox1* (3A), the nucleotide at position 723 was adenine conserved in *T. saginata.* The complete nucleotide sequences of *cox1* (1,620 bp) of 20 *T. saginata* isolates sequenced were identical (AB271695), but intraspecific variations (0.1~0.7%) were observed in 17 compared with those of *T. saginata* isolates from other Asian regions (Figure 3A). As in the case of *cob* (1,068 bp), *T. saginata* isolates from Mongolia had identical nucleotide sequences (AB271696), but slight differences (0.3~0.5%) were observed among *T. saginata* isolates from China and Cambodia (Figure 3B). In the deduced amino acid sequences of two target gene products, amino acid residues were substituted at 4 and 5 positions for (*cox1*) and (*cob*), respectively (Figure 4). Sequence divergences among the Asian *T. saginata* isolates were 0.2~0.6% and 0.6~1.1% for *cox1* and *cob*, respectively. Nucleotide sequence data reported in this study were deposited in DDBJ/EMBL/GenBank databases as accession numbers AB271695 and AB275143 for *cox1* gene and AB271696 and AB274525 for *cob* of *Taenia saginata* from Mongolia and Cambodia, respectively.

Distribution of *T. saginata* taeniasis patients in Mongolia. It has been confirmed that *T. saginata* taeniasis is distributed in at least 10 of 21 provinces in Mongolia by molecular analysis of proglottids (Figure 1): Khovsgol and Dornogobi (1 case each), Khovd and Dundgobi (2 cases each), Sukhbaatar (3 cases), Arkhangai (5 cases), Khentii (7 cases), Dornod (8 cases), Selenge (10 cases), and Tuv (79 cases). The highest proportion was in Tuv (66.9%, 79/118), where the capital of Mongolia is situated, whereas the lowest proportion was in Khovsgol and Dornogobi (0.8%, 1/118).

The prevalence of *T. saginata* taeniasis cases was not significantly different in men (47.5%, 56/118) and women (52.5%, 62/118; $P = 0.52$). The *T. saginata* carriers were abundantly found in the 15- to 29-year age group (35.6%, 42/118), followed by the 30- to 44-year group (33.0%, 39/118), the ⩾45-year group (22.0%, 26/118), the 5- to 14-year group (7.6%, 9/118), and the 1- to 4-year group (1.7%, 2/118; $P = 0.01$). The youngest *T. saginata* carriers were a 3-year-old boy and girl, and the oldest one was an 88-year-old woman.

The taeniasis patients occurred among people with various occupations. The prevalence rate of taeniasis was highest in herdsmen (12.7%, 15/118), followed by school girls and boys (junior high school; 11.8%, 14/118), drivers and persons including housewives, small children, and housemaids (8.5%, 10/118), cookers (7.6%, 9/118), pensioners (5.9%, 7/118),

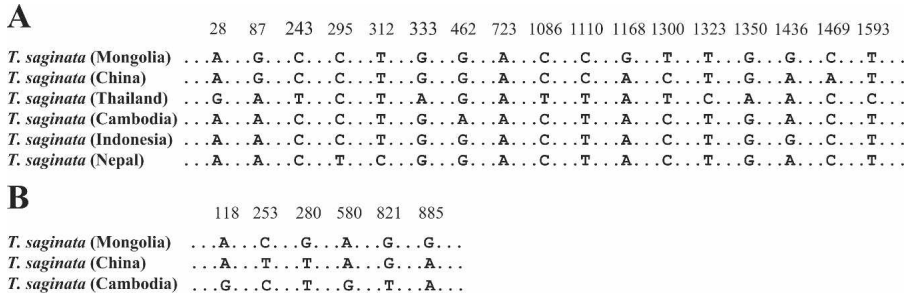

FIGURE 3. Multiple alignments of the partial nucleotide sequences of *cox1* and *cob* of *T. saginata* from Mongolia and other Asian countries. **A**, *cox1*. Adenine at position 723 is conserved only in *T. saginata*. Nucleotide sequence data of *cox1* used for comparison are from China (AB066495), Thailand (AB107245), Cambodia (AB275143), Indonesia (AB107240) and Nepal (AB107243). **B**, *cob*. Two nucleotide sequences from China (AB066581) and Cambodia (AB274525) are available. Dots indicate identical nucleotides. The numbers above nucleotides denote positions of each gene.

teachers (5.1%, 6/118), university students (4.2%, 5/118), accountants (3.4%, 4/118), repairmen, saleswomen, and policemen (2.5%, 3/118), engineers, correspondents, tailors, directors of private companies, mechanics, and health workers (1.7%, 2/118), and miners, translators, milkmaid, geologist, conductor, musician, builder, joiner, electrician, hairdresser, and soldier (0.8%, 1/118). School girls and boys infected with *T. saginata* were from Selenge (2/14), Dornod (2/14), Tub (1/14), Khentii (1/14), and Uraanbaatar (8/14).

***Taenia solium* cysticercosis.** A total of 73 serum samples including 14 serum samples from people with histories of epileptic seizures from Selenge were examined by both ELISA and immunoblots using native and recombinant *T. solium* antigens, but there were no positive cases (data not shown).

Diphyllobothriasis. A diphyllobothriid strobila expelled spontaneously from a Russian man was identified by molecular analysis. A 1,198-bp *cox1* fragment was amplified by PCR (data not shown), and the nucleotide sequence analysis revealed the diphyllobothriid cestode was *Diphyllobothrium latum* (AB269325).[10]

DISCUSSION

This study is the first report describing current situations of taeniasis/cysticercosis and diphyllobothriasis in Mongolia based on mtDNA analysis and/or serology. A total of 118 taeniid proglottids collected during 2002–2006 were confirmed to be *T. saginata* by molecular analysis, and it was shown that *T. saginata* taeniasis is widely distributed in Mongolia. There were no variations in the nucleotide sequences of both *cox1* and *cob* among 20 *T. saginata* isolates from Mongolia. However, considering the slight intraspecific variations of these genes (0.1~0.7% for *cox1* and 0.3~0.5% for *cob*) compared with five *T. saginata* isolates from other Asian regions, minor genetic divergences might have occurred in different regions of Asia. The difference of variant numbers in *cox1* and *cob* genes indicates that *cob* is more conservative than *cox1* in *T. saginata*.

Taenia saginata taeniasis has been found in a wide range of ages and in various occupations. In this study, two taeniasis cases of a 3-year-old boy and girl were found. The prevalence in herdsmen seemed to be higher than other occupations. Nomadic or semi-nomadic people (usually consisting of three to four families of each group) move four to six times a year from one natural pasture to another carrying domestic animals such as sheep (80–200), goats (20–40), horses (9–15), and cattle (8–28), except pigs, for each family. The family groups build simple latrines (non-septic tank) and use them during their staying periods. They keep their cattle in open common pastures, and periodically slaughter these domestic animals for their own food or distribution for the market. However, it is unclear why herdsmen are highly exposed to the infection of *T. saginata*. The reason why the prevalence rate of taeniasis in herdsmen is higher might be related to their lifestyle. Both men and women, especially in the age groups of 15–29 (36%) and 30–44 (33%), had the highest risk of becoming infected with *T. saginata* taeniasis. However, these data do not show any real epidemiologic information without population denominators. In Mongolia, the source of infection for *T. saginata* has not yet been specified, but it is probably beef and/or yak meat cooked/steamed using *horhog* (hot stones) during traditional ceremonies and/or roast beef (*shashlick*), which is one of the popular dishes sold in the small markets. The eating habits of Mongolian people may be closely related to infection with *T. saginata*. In this study, taeniasis was recorded based on the proglottids recognized and kept by the carrier themselves and brought to the National Center for Communicable Diseases directly or through local health centers. Based on the message from the National Center for Hygiene, Epidemiology, and Microbiology, Mongolia 1995, the infection rate of *T. saginata* in 1964 was 15.7%. By 1994,

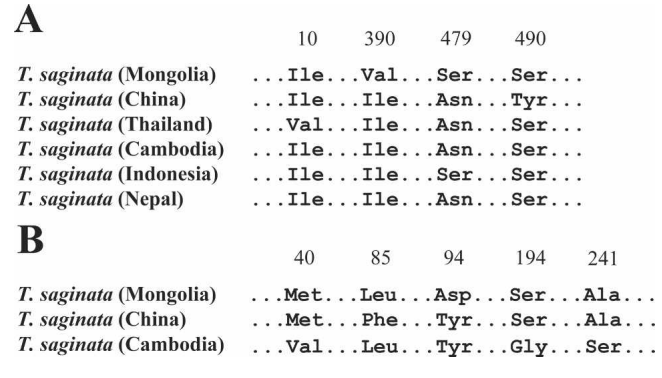

FIGURE 4. Deduced amino acid sequences of *cox1* and *cob* of *T. saginata* from Mongolia and Asian other countries. Amino acid residues are substituted at 4 and 5 positions for cox1 (539 amino acid residues, **A**) and cob (355 amino acid residues, **B**), respectively. Dots indicate identical amino acid residues. The numbers above amino acids denote positions of each protein.

the rate dropped to 0.04%. This decline was attributed to a 1974–1976 program of administering free medical treatment in both urban and rural areas.[3] However, taeniasis cases analyzed in this study are not rare but rather common, especially in and around Ulaanbaatar. Relatively high number of taeniasis from school girls and boys (8/14, 57.1%) was seen in the capital city of Ulaanbaatar. Therefore, taeniasis of *T. saginata*, the second helminthic disease in Mongolia, should have priority in treatment as in 1974–1976, not only for adults but also for school children, with more attention given to improvement of the quality of life.

The number of samples for serology to detect cysticercosis was too small, but all samples from local people with a history of epilepsy were from the Selenge province, along the north border to Russia, where pig husbandly is the main business. Pork sold in the local markets appeared to be free of cysticerci by the naked eye. There was no indication of *T. solium* cysticercosis by serology using both native *T. solium* glycoprotein antigens[4] and recombinant antigen.[6] In Mongolia, pigs are kept indoors, and pig owners (non-nomadic or semi-nomadic people = settlement) use simple latrine facilities with little free access to pigs. Nobody had a habit of consuming uncooked or undercooked pork. Pork is not the main cuisine in Mongolia. Therefore, those with histories of epilepsy but were sero-negative were considered to have idiopathic epilepsy.

There are crucial differences between Mongolia and the neighboring country, China, including Inner Mongolia. In China, the main cuisine is pork, and therefore, *T. solium* cysticercosis is a public health issue nationwide, except minorities in Muslim societies.[11,12] In Inner Mongolia, *Taenia solium* cysticercosis is a serious public health issue.[13] In recent work in the Sichuan province, three human *Taenia* species, *T. solium*, *T. asiatica,* and *T. saginata*, have been confirmed to be distributed sympathrically.[14] Therefore, the fact that taeniasis in Mongolia is exclusively *T. saginata* seems to be unique to Mongolia and caused by the lifestyle and eating customs that are different from China. Further comparative studies between Mongolia and the northern part of Inner Mongolia with Mongolian populations may be interesting.

Detection of *T. saginata* is much easier than that of *T. solium*, because singled proglottids of *T. saginata* are actively and spontaneously voided from the anus almost every day, and therefore, easily detected by patients themselves.[15] There is no evidence of *T. solium* cysticercosis in Mongolia as far as we know. Therefore, when we detect even a single cysticercosis case in Mongolia, we have to carry out a survey for detection of *T. solium* taeniasis by other methods including copro-ELISA[16] and copro-DNA tests[7] and for detection of cysticercosis again.

A diphyllobothriasis case was first diagnosed by morphology and confirmed to be *D. latum* by mtDNA analysis.[10,17] The patient was a 24-year-old Russian man. We speculate that the infection did not occur in Mongolia but Russia, perhaps around Lake Baikal, because *D. latum* is endemic in areas where people frequently consume raw trout in lakes and rivers. Such raw/uncooked fish are not served as dishes in Mongolia.

To estimate epidemiologic situation on taeniasis/cysticercosis, further field surveys in different provinces in Mongolia are needed. Active case detection, establishment of an effective treatment of the tapeworm carriers, and establishment of a system to inspect the quality of beef meat would be expected to have priority for control of *T. saginata* taeniasis.

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