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▣ BRIEF COMMUNICATION

# Molecular Identification of *Taenia hydatigena* from Sheep in Khartoum, Sudan

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**Abstract:** The cestode *Taenia hydatigena* uses canids, primarily dogs, as definitive hosts, while the metacestode larval stage cysticercus infects a range of intermediate hosts, including domestic animals such as goats, sheep, and pigs. Cysticercosis due to *T. hydatigena* has large veterinary and economic drawbacks. Like other taeniids, e.g., *Echinococcus*, intraspecific variation is found among the members of the genus Taenia. In Africa, few studies are available on the epidemiology and distribution of *T. hydatigena*, and even fewer studies are available on its genetic variation. In this study, we molecularly identified 11 cysticerci from sheep in Sudan and demonstrated the genetic variation based on the NADH dehydrogenase subunit 1 (*nad*1) and cytochrome c oxidase subunit 1 (*cox*1) mitochondrial genes. The isolates were correctly identified as *T. hydatigena* with more than 99% similarity to those in the GenBank database. Low diversity indices and insignificant neutrality indices were observed, with 3 and 2 haplotypes for the *nad*1 and *cox*1 genes, respectively. The results suggest the presence of unique *T. hydatigena* haplotypes in Sudan, as haplotypes with 100% similarity were not found in the GenBank database. With few available studies on the genetic variation of *T. hydatigena* in Africa, this report represents the first insights into the genetic variation of *T. hydatigena* in Sudan and constitutes useful data.

Key words: *Taenia hydatigena*, genetic variation, cysticercus, Khartoum

The cestode *Taenia hydatigena* uses canids, primarily dogs, as definitive hosts, while the metacestode larval stage infects a range of intermediate hosts, including goats, sheep, and pigs. Cysticercosis due to *T. hydatigena* is of veterinary and economic importance, especially in small ruminants, where it causes production loss through clinical disease or damage to infected organs [1-3].

In Africa, a few studies are available on the epidemiology and distribution of *T. hydatigena* [4]. Prevalence reports, where available, have demonstrated high infection rates in sheep and goats and low infection rates in pigs [4-8]. In Sudan, data on the prevalence of *T. hydatigena* in livestock are scarce. However, a study conducted by El Badawi et al. [9] reported a high prevalence of *T. hydatigena* in sheep (32.4%) and goats (29.0%) and emphasized its economic importance and implications for livestock production.

Genetic variation has been well studied in most cestodes species, e.g., *Echinococcus granulosus* [10,11], and correlates with parasite morphology, host infectivity, drug and vaccine development, and ultimately control. Similarly, mitochondrial genome studies of *Taenia* spp. have also confirmed the existence of genetic variation within species [13,14], and in some cases, such variation has been suggested to correlate with pathological differences in different hosts [15,16]. The molecular epidemiology and genetic variation of *T. hydatigena* in Africa are underinvestigated; however, a few studies in countries such as Nigeria, Egypt and Tanzania have demonstrated nucleotide variation between isolates from sheep, goats, and pigs [4,17,18]. Meanwhile, studies describing the genetic population structure of *T. hydatigena* in Sudan and how they differ from other geographical regions, as well as the epidemiological significance, are lacking. Therefore, the aim of this study was to molecularly identify *T. hydatigena* of Sudanese origin and to investigate possible genetic variation based on the NADH dehydrogenase subunit 1 (*nad*1) and cytochrome c oxidase subunit 1 (*cox*1) mitochondrial genes.

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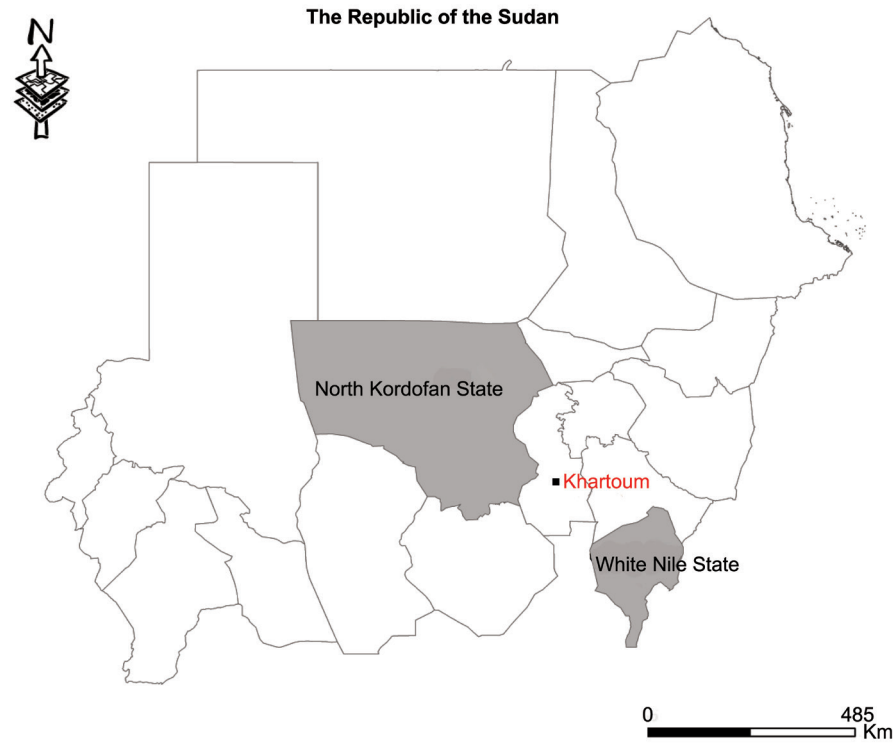


**Fig. 1.** Map showing study areas. Black dot, region where sheep were slaughtered. Grey region, sheep origin.

Thirty cyst samples (2-5 cm in size) from liver were harvested from slaughtered sheep in various homes in the Western Nile (Al-Hajyosif and Al Gouz)- Khartoum, Sudan, during a meat/slaughtering inspection for the Addha festival (September 2017). A total of 1,500 sheep were examined during the inspection. However, the slaughtered sheep were said to have originated from North Kordofan State and the west White Nile State of Sudan (Fig. 1). DNA was extracted from a portion of each cysticercus using the phenol-chloroform extraction method. Extracted genomic DNA samples were stored at −20℃ until use. PCR amplification of the mitochondrial *nad*1 and *cox*1 genes was performed using the following primer pairs specific for cestode parasites: forward 5′CARTTTCGTAAGGGBCCWAAWAAGGT and reverse 5′-CCAATTTCYTGAAGTTAACAGCATCA [19]; and a newly designed forward (5′-AGTCCTGATGCTTTTGGGTTCTATGGA-3′) and a previously reported reverse primer (5′-AAGCATGATGCAAAAGGCAAATAAACC-3′) [20], for the *nad*1 and *cox*1 genes, respectively, with expected fragment sizes of 871 and 939 bp, respectively. PCR was conducted in a 25 µl reaction mixture containing 12.5 µl Premix Ex Taq™ version 2.0 (Takara Bio, Japan), 10 pmol of each primer, 0.5 µl of genomic DNA extract (20-200 ng), and RNAse free water up to the final concentration of 25 µl. The reaction was performed under the following conditions: initial denaturation at 95℃ for 5 min, 35 cycles of denaturation at 95℃ for 30 sec, annealing at 55℃ for 30 sec and elongation at 72℃ for 60 sec, and a final extension at 72℃ for 10 min. Then, 5 µl of the PCR products were analyzed in a 1.5% (w/v) agarose gel stained with GelRed™ while the remainder was sequenced in an ABI3730Xl DNA Analyser (Beijing Tsingke Biotechnology Co., Beijing, PR China).

DNA sequences were viewed and manually corrected for any misread nucleotide and aligned using BioEdit software [21]. The identity of each isolate was confirmed in a BLAST search in the GenBank database using the NCBI BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The diversity (nucleotide and haplotype diversity) and neutrality (Fu's Fs and Tajima's D) indices were estimated in DnaSP v.6 [22]. Bayesian phylogeny was inferred based on the *nad*1 and *cox*1 dataset using MrBayes v.3.1.2. Markov chain Monte Carlo (MCMC) sampling was used to assess the posterior distribution of the parameters with a chain length of 2,000,000 states, and 10% was discarded as burn-in. Parameters were logged every 1,000 states. TreeView v.1.6.6. (http://taxonomy.zoology.

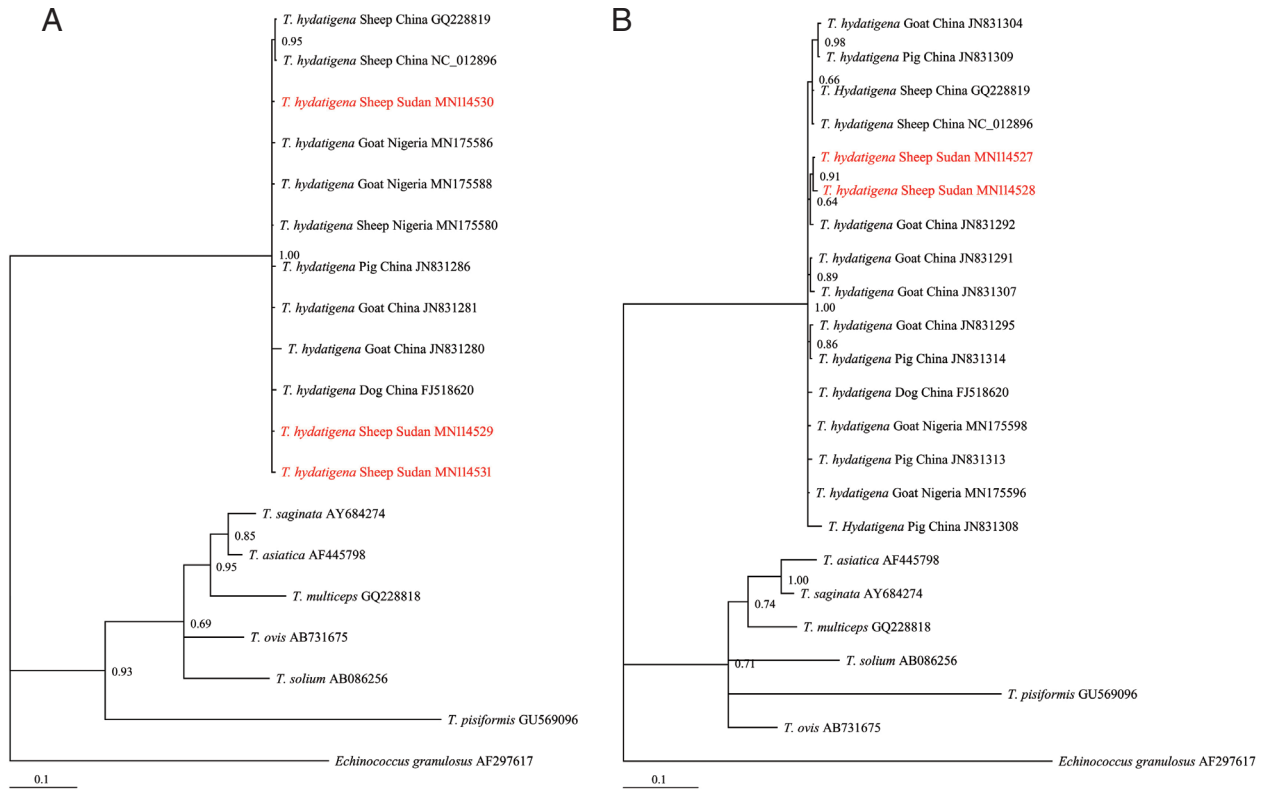


**Fig. 2.** Bayesian phylogenetic relationship of *T. hydatigena* isolates with other *Taenia* species. (A) *nad*1. (B) *cox*1. Red, Sudanese haplotype representing sheep isolates from this study.

gla.ac.uk/rod/treeview.html) was used to draw/display trees.

Of the 30 cyst samples, successful amplification and sequencing of the *nad*1 and *cox*1 genes identified 11 cysts as *T. hydatigena*. No amplification was observed for the other 19 cysts, even after the process was repeated. The useable nucleotide sequences after correction and editing were 764 and 735 bp for *nad*1 and *cox*1, respectively. These sequences were more than 99% similar to those in the GenBank repository. The Sudanese *T. hydatigena* isolates showed the highest similarity (99.73-99.87%) to the Chinese and Nigerian isolates according to the BLAST query. Due to availability of partial *nad*1 and *cox*1 genes from different geographical locations, phylogenetic analysis was conducted using 677 and 735 bp of the *nad*1 and *cox*1 genes, respectively. The Bayesian phylogeny further confirmed identity of the isolates, as they clustered with other reference *T. hydatigena* sequences from the GenBank database (Supplementary Table S1) and exhibited significant distances from other *Taenia* species (*T. saginata, T. ovis, T. pisiformis, T. solium, T. multiceps*, and *T. asiatica*) (Fig. 2A, B), indicating that all isolates were clearly *T. hydatigena*. This result was also confirmed by the posterior probability (PP) values between the isolates, although a few had PP values < 0.70. This low nodal support value between *T. hydatigena* species was also recently reported in Nigeria [18]. The nucleotide sequences of *cox*1 and *nad*1 genes representing *T. hydatigena* isolates from the present study were deposited in GenBank under the following accession numbers (*cox*1: MN114527 and MN114528; *nad*1: MN 114529-MN114531).

**Table 1.** Diversity and neutrality indices of a *Taenia hydatigena* population from Sudan based on partial *nad*1 (764 bp) and *cox*1 genes (735 bp)

| Index | *nad*1 | *cox*1 |
|---|---|---|
| No. of isolates | 11 | 9 |
| No. of mutations | 4 | 2 |
| Parsimony informative site | 1 | 0 |
| No. of haplotypes | 3 | 2 |
| Haplotype diversity (Hd) | 0.345 ± 0.172 | 0.222 ± 0.166 |
| Nucleotide diversity ($\pi$) | 0.00114 ± 0.00059 | 0.00060 ± 0.00045 |
| Tajima's D | -1.32167 | -1.3624 |
| Fu's Fs | 0.323 | 0.671 |

Analysis on the *nad*1 (764 bp) and *cox*1 (735 bp) mitochondrial gene sequences revealed 4 (1 parsimony informative) and 2 mutations (not parsimony informative), respectively. No deletions or insertions were observed. Low haplotype (Hd) and nucleotide (π) diversities were observed (Table 1). Regardless of small sample size, the observed *nad*1 Hd was comparable to *T. hydatigena* populations reported in dogs and goats, although with a lower π compared to the *T. hydatigena* populations from the same study [29]. A comparison of the *cox*1 diversity indices also revealed lower Hd and π than the populations from Italian, Iranian, and Palestinian sheep [29]. Meanwhile, comparable data from an African host on the diversity indices of *T. hydatigena* are lacking [4,17]. However, compared with the recent report from Nigeria [18], the Sudanese *T. hydatigena* isolates showed lower genetic variation.

Based on the *nad*1 gene, 3 haplotypes were observed, with the nSDN1 haplotype constituting 81.8% (9/11) of the population. All haplotypes were separated from each other by no more than a 3-point mutational difference. The number of haplotypes based on the *cox*1 gene was lower compared to the number of haplotypes (4) reported among a *T. hydatigena* population of Tanzanian sheep and goats [4] but similar to the number of haplotypes reported in Nigerian sheep [18]. The comparison of the nucleotide sequences from this study with the deposited sequences in the GenBank database yielded no haplotype with 100% identity, which could suggest the presence of unique haplotypes in Sudan and is consistent with previous reports of the existence of geographically distinct species of *T. hydatigena* [29]. Nonetheless, with limited sample size and the use of short mitochondrial DNA (mtDNA) fragments, further investigation would be necessary to understand and clearly describe the genetic differences and relationships between Sudanese *T. hydatigena* isolates and those from different geographical regions. Furthermore, we observed negative insignificant values of Tajima's *D* and insignificant positive Fu's Fs values (Table 1), indicating inconsistencies with population expansion or a non-significant deviation from neutrality. As expected, although the low sample size may not reflect the actual status of the parasite population in Sudan, it provides a clue to the genetic status of the *T. hydatigena* population in the country.

The genetic ecology of most parasites of medical and veterinary importance has been found to contribute to understanding the epidemiology and control of parasitic infections. In this study, the results suggest some degree of genetic variation among Sudanese *T. hydatigena* isolates based on partial *nad*1 and *cox*1 mitochondrial genes. While the isolates were highly similar to isolates from other locations, no 100% match was observed. Globally, there still is a dearth of information on the genetic variation of *T. hydatigena* species, and with few available studies from Africa, this report contributes significant preliminary data on the genetic variation of *T. hydatigena* species in Sudan. Finally, we recommend that molecular investigations that include large sample sizes and utilize complete mitochondrial gene markers in addition to the *nad*1 and *cox*1 genes be considered in the future to provide further insight regarding the genetic variation and population structure of *T. hydatigena* in Sudan.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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**Supplementary Table S1.** Characteristics of *Taenia* spp. isolates used in this study

| Origin | Specie | *nad*1 | | | | *cox*1 | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Parasite stage | Host | No. of isolates | Accession number | Parasite stage | Host | No. of isolates | Accession number | |
| Nigeria | *T. hydatigena* | Cysticercus | Goat | 2 | MN175586, MN175588 | Cysticercus | Goat | 2 | MN175598, MN175596 | [18] |
| | *T. hydatigena* | Cysticercus | Sheep | 1 | MN175580 | | | | | [18] |
| China | *T. hydatigena* | Cysticercus | Sheep | 1 | GQ228819 | Cysticercus | Sheep | 1 | GQ228819 | [19] |
| | *T. hydatigena* | Cysticercus | Sheep | 1 | NC_012896 | Cysticercus | Sheep | 1 | NC_012896 | [19] |
| | *T. hydatigena* | Cysticercus | Pig | 1 | JN831286 | Cysticercus | Pig | 4 | JN831308, JN831309, JN831313, JN831314 | [23] |
| | *T. hydatigena* | Cysticercus | Goat | 2 | JN831280, JN831281 | Cysticercus | Goat | 5 | JN831291, JN831292, JN831295, JN831304, JN831307, | [unpublished] |
| | *T. hydatigena* | Tapeworm | Dog | 1 | FJ518620 | Tapeworm | Dog | 1 | FJ518620 | [23] |
| N/A | *T. saginata* | Tapeworm | Human | 1 | AY684274 | Tapeworm | Human | 1 | AY684274 | [24] |
| Korea | *T. asiatica* | Tapeworm | Human | 1 | AF445798 | Tapeworm | Human | 1 | AF445798 | [25] |
| China | *T. multiceps* | Tapeworm | Dog | 1 | GQ228818 | Tapeworm | Dog | 1 | GQ228818 | [19] |
| New Zealand | *T. ovis* | N/A | N/A | 1 | AB731675 | N/A | N/A | 1 | AB731675 | [26] |
| China | *T. solium* | Cysticercus | Pig | 1 | AB086256 | Cysticercus | Pig | 1 | AB086256 | [27] |
| China | *T. pisiformis* | Tapeworm* | Dog | 1 | GU569096 | Tapeworm | Dog* | 1 | GU569096 | [19] |
| United Kingdom | *Echinococcus granulosus* | Protoscoleces | N/A | 1 | AF297617 | Protoscoleces | N/A | 1 | AF297617 | [28] |

*Dog fed cysticercus from rabbit.