Two cases of human thelaziasis as confirmed by mitochondrial *cox1* sequencing in China

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Two special cases of human thelaziasis were reported in China: an old farmer with heavy infection by 36 worms and a 7-year-old boy with infection by eight worms. *Thelazia callipaeda* was morphologically identified and confirmed by mitochondrial *cox1* gene sequencing.

Keywords: Human thelaziasis, *cox1* gene

Introduction

Thelaziasis is an insect-borne zoonosis. More than 30 species of *Thelazia* were identified in the world and only two species of them, *Thelazia callipaeda* (*T. callipaeda*, Railliet and Henry, 1910) and *Thelazia californiensis* (*T. californiensis*, Price, 1930), may cause human infection. Both differ in morphology and geographical distribution.¹ *T. callipaeda*, also called the oriental eyeworm, is common in Southeast Asia^{2–5} and Europe,^{6–8} and *T. californiensis* has been reported to infect human beings occasionally in the United States.⁹ Human thelaziasis may reach higher prevalence in poor socioeconomic settings. The incidence appears to be increasing, and more than 500 cases of human thelaziasis have been reported in China so far. The majority of Chinese cases were identified as *T. callipaeda* and only one was described as *T. californiensis*.¹⁰ All of the patients were diagnosed based on morphology of the worms or the clinical features. Here, we described two cases of human thelaziasis in China. *T. callipaeda* was determined by morphologic structure, and by mitochondrial *cox1* gene sequencing as well. Additionally, a video was presented to demonstrate worms crawling in the human conjunctival sac and producing larvae *in vitro*.

Cases Description

A 76-year-old man from rural area in Liaoning province (41°8' N, 123°4' E) in the northeast of

China visited his doctor at the First Hospital of China Medical University in August 2013, with the 10-day history of foreign-body sensation and itching, conjunctival hyperemia (HC), increased secretions, and blurred vision in eyes. The patient had removed four thread-like worms from his right eye with his finger a week ago. He did farming in the mountains with poor sanitary conditions, and surrounding him were raised two dogs and three cats. He was unaware of the eyes ever being attacked by any flies. Physical examination of the patient showed no abnormalities. Ophthalmologic examination revealed several thread-like worms moving in the outer canthus of the eyes. The vigorous movement of the worms resulted in conjunctival HC (Fig. 1, video (see supplementary material online for this article at www.maneyonline.com/doi/suppl/10.1179/2047773214Y.0000000153)). All of 32 worms, six in the left eye and 26 in the right eye, were removed with intraocular forceps under anesthesia with oxybuprocaine. The symptoms disappeared 2 days later after the patient was prescribed eyedrops of levofloxacin.

The second case was a 7-year-old boy who lived in rural Changzhi, Shanxi province (36° 5'N, 113°2'E) in the northwest of China. In August 2012, unaware of his eyes ever having been attacked by flies, the boy was admitted to Changzhi Hospital with the 14-day history of foreign-body sensation, itching, and increased secretions in his left eye. The boy's dog showed conjunctival redness and abundant secretions. Ophthalmologic examination displayed lacrimation and conjunctival exudates, and eight slender

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Figure 1 A group of *Thelazia callipaeda* parasitized the outer canthus of the patient's eye. The worms (W) parasitized the outer canthus of the eye of the patient with heavy *T. callipaeda* infection. The parasites' movements actively resulted in the conjunctival hyperemia (HC). P refers to pupil (image taken by the slit lamp microscope inspection, 75 $\times$).

worms on the conjunctival sac of the left eye were found. No additional abnormal physical examination was noted. Symptoms disappeared after the worm removal with forceps.

Identification of Parasites

The worms harvested from the patients were examined under a phase difference microscope. Thread-like, semi-transparent worms with distinctive scalariform buccal cavities (BCs) were seen (Fig. 2). Many round eggs and coiled embryonic larvae were visible within the utero of the worms. The worms were actively moving, and the male squirmed more quickly than the pregnant female (video (see supplementary material online for this article at www.maneyonline.com/doi/suppl/10.1179/2047773214Y.0000000153) and Fig. 3). Interestingly, a female worm was found to be producing the larvae individually while it was removed from the boy's eye (video (see supplementary material online for this article at www.maneyonline.com/doi/suppl/10.1179/2047773214Y.0000000153) and Fig. 3D). The vulva became more open like a rectangle during larval delivery. The worms were identified as *T. callipaeda* based on morphologic characters.¹¹ The species was verified with polymerase chain reaction (PCR) amplification. Total genomic DNA was extracted using sodium dodecyl-sulfate (SDS)/proteinase K digestion and mini-column purification (TIANamp Genomic DNA Kit, Tiangen Technology, Beijing, China). The specimen was then verified by PCR-based sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*, 689 bp) using primers NTF and NTR (GeneBank, No: AM042555).¹² The *cox1* from two nematodes isolated from each case was sequenced, followed by alignments using the Clustal X program.¹³

Figure 2 Light micrograph of the head end of *Thelazia callipaeda* showing the distinctive scalariform buccal cavity (BC) and the serrated cuticle with transverse striations (TS) were visible in the head end of the worm (400 $\times$).

Alignments were compared with a *cox1* sequence available for *Caenorhabditis elegans* (accession no. AY171197).¹⁴ The result showed 98.7–99.0% of homology with *T. callipaeda* haplotypes found in Europe and 99.0–99.3% of homology with the isolate in China and Korea.¹⁵ Among the six nucleotide positions in *cox1* (i.e. G/A at positions 89, 149, 206, 257, 539, and 608), worms from Europe and Asia were different from each other, but each group was internally consistent. These six positions may be considered as markers for the differentiation of European and Asian *T. callipaeda* from either human beings or animals.

Discussion and Conclusions

Human thelaziasis was first described in India in 1910, and reported in China in 1917, where an increasing number of cases were found since then. According to the cumulative reports before 2013, over 500 cases have been recorded,² nearly all of the nematodes found in human beings from China were identified as *T. callipaeda* except one that was described as *T. californiensis*.¹⁰ However, the previous case reports were primarily based on morphological or clinical features, and the diagnosis were undefined due to the maldevelopment or unisexual male of parasite, so their reliability is questionable. The mitochondrial cytochrome *c* oxidase I subunit gene (*cox1*) has been widely used as a genetic marker and DNA barcode for species identification because of its less variation in interspecies and intraspecies.^{12,15} Herewith, we described two special cases of human thelaziasis and identified *T. callipaeda* based on morphologic features, and mitochondrial *cox1* sequencing first in China. The results showed 1.0–1.3% of *cox1* haplotype polymorphisms compared with *T. callipaeda* found in Europe and 0.1–0.7% with

Figure 3 Light micrograph of the *Thelazia callipaeda* female worm. (A) The middle portion containing numerous coiled embryonic larvae, in multiple rows in the proximal area of the uterus; (B) The anterior portion containing numerous rounded first-stage larvae, in a single line in the distal area of the uterus, near the vulva; (C) The anterior portion shows that the vulva was located anterior to the esophagus-intestine junction, and it broadened notably during childbirth; (D) The anterior portion shows the female worm and newborn larvae with sheaths. Abbreviations: L: larvae; TS: transverse striations; V: vulva; E: esophagus; I: intestine; J: junction; M: female worm; S: sheaths. Magnification: A, B, and C: Aberration micrograph, 400 $\times$ and D: Phase contrast micrographs, 200 $\times$.

that reported in China and Korea. The worms obtained from Europe and Asia differ but each group was highly homologous.

Dogs and cats are important reservoir hosts and infective sources of human thelaziasis and fruit flies such as *Amiota okadae* or *Phortica variegata* (Drosophilidae) for *T. callipaeda*; *Musca* spp. or *Fannia* spp. for *T. californiensis* act as vectors for the transmission of the infectious larvae into the eyes of human beings and animals.^{16–19} However, Yang *et al.* described a case of human thelaziasis through a potentially contaminated towel,³ while Koyama *et al.* reported five cases of thelaziasis in Japan who had close contact with pets such as dogs, cats, and cows.⁴ It has been clarified that *T. callipaeda* is transmitted by the non-biting diptera in which the parasite goes through larval development stages. The third-stage larvae are released from the vector when the latter feeds on ocular secretions of human beings or animals,^{17–19} although a considerable number of patients (60%), particularly of children, were unaware of their eyes ever having contact with flies (data unpublished).

The active reproductive status of this nematode *in situ* indicates that human beings are competent hosts for this parasite.⁸ We recorded the female worm that is producing larvae and defined the ovoviparous²⁰ process of the nematode, presenting further evidence that human beings may act as definitive hosts or even transmission sources of *T. callipaeda*.

Thelaziosis is characterized by a range of sub-clinical to clinical signs. Here, a heavy infection of patients with 36 worms, resulting in conjunctivitis and other significant clinical manifestations is reported. This is the first report of abundant worms residing in the eyes of individual patient.

Disclaimer Statements

Contributors XL Wang and CL An designed the study, identified parasites, acquired, analyzed, and interpreted data, wrote and approved the manuscript; JY Guo and XL Wang acquired and interpreted the gene data, gene sequence; Y Wang and XL Ma acquired the clinical data and collected the parasites and took photos.

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Conflicts of interest There are no conflicts of interest in this research.

Ethics approval This research has been passed by The Hospital Ethics Committee of the 1st Hospital of China Medical University. The patient gave her informed consent for the publication of this article. No approbation from ethical committees was needed for this study.

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