HUMAN THELAZIASIS FROM ZUNYI, CHINA: A CASE REPORT

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Abstract. Human thelaziasis has not been previously reported from Zunyi, Guizhou Province, China. We report here the case of thelaziasis from Zunyi, China. A 72-year-old male, who did farming in the mountains with poor sanitary conditions, and owned one raised dog, reported to the doctor with complaints of foreign body sensation, and occasionally some black object moving in front of the left eye. He had removed two thread-like worms from the left eye with his finger the week before coming to the hospital. Ophthalmologic examination revealed several thread-like worms moving in the outer canthus of his left eye. A total of 10 worms were found and retrieved. The worms were morphologically identified as *Thelazia callipaeda* (*T. californiensis*) and confirmed by mitochondrial *cox1* gene sequencing. Gene sequencing revealed to the worms have a novel haplotype not previously described. Thelaziasis due to *T. callipaeda* can infect humans in Zunyi, China. Physicians should be alerted to include thelaziosis as the possible cause of ocular infections in differential diagnosis.

Keywords: Human thelaziasis, *cox*1 gene, human case, Zunyi

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INTRODUCTION

Thelazia nematodes (*Spirurida*, *Thelazioidea*), phylogenetically closely related to *Spirocerca lupi* (Iorio *et al*, 2009), due to its distribution in East Asia, has been called the oriental eye worm (Handique *et al*, 2014). More than 30 species of *Thelazia* have been identified world-wide but only *Thelazia callipaeda* (*T. callipaeda*) and *Thelazia californiensis*

(*T. californiensis*), can cause human infection (Viriyavejakul *et al*, 2012). *T. callipaeda* is a parasitic nematode usually causing *thelaziasis* in humans, dogs, cats and several species of wild carnivores (Handique *et al*, 2014).

Telaziasis is an ocular arthropod-borne disease of the eye infesting the conjunctival sac and lacrimal duct (Sah *et al*, 2018). Telaziasis is capable of causing conjunctivitis, keratitis, corneal opacities and corneal ulcers (Sah *et al*, 2018). In China, *T. callipaeda* is transmitted by *Phortica okadai* (Drosophilidae, Steganinae) drosophilids, which deposit infective third-stage larvae while feeding on ocular secretions of the hosts (Wang *et al*, 2002). The incidence appears to be increasing, and more than 500 cases of human thelaziosis have been reported in China so far (Wang *et al*, 2014). These cases are usually located in central China near the Yellow River and the Yangtze River systems, however, in recent years, the number of cases of *T. callipaeda* infection has been continuously increasing in Zunyi, where the first case reported in 2010, as of 2016, more than 30 cases had been treated (Zheng *et al*, 2017).

The worms in the previously reported cases were mainly identified based on their morphology or by the clinical features (Wang *et al*, 2014). However, these worms may have been misidentified due to an abnormal worm appearance caused by worm developmental abnormalities or worm differences by sex (Wang *et al*, 2014). The polymerase chain reaction (PCR) and Western blot testing has the ability to identify the worm (Mirahmadi *et al*, 2017). Genetic markers have several advantages such as good stability, great variability and may be free from many environmental influences (Otranto *et al*, 2005b), over morphological identification.

We describe here the case of human *thelaziasis* in a patient from Zunyi, China. The worm was identified as being *T. callipaeda* based on the morphology of the worm and genetic identification using the *cox*1 gene.

CASES REPORT

A 72-year-old man from rural area (Zheng'an) in Guizhou province (28°51 N, 107°41 E) in the southwestern of China visited his doctor at the First Hospital of Zunyi Medical University in June 2018, 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 two thread-like worms from his left eye with his finger a week ago. He did farming in the mountains with poor sanitary conditions, and one raised dog. 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 left eye resulted in conjunctival HC (Fig 1). Altogether 10 worms, six in the left eye and 4 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.

Morphological identification of the worms

The removed worms were immediately stored at -80°C until examined morphologically. They were placed in saline and examined directly under microscope (Olympus BX43, Japan). Microscopy revealed thread-like, semi-transparent worms with distinctive scalariform buccal cavities (BCs) (Figs 2 and 3). The vulvae of the adult female

Fig 1-The left eye of the subject with *Thelazia callipaeda* adult worms.

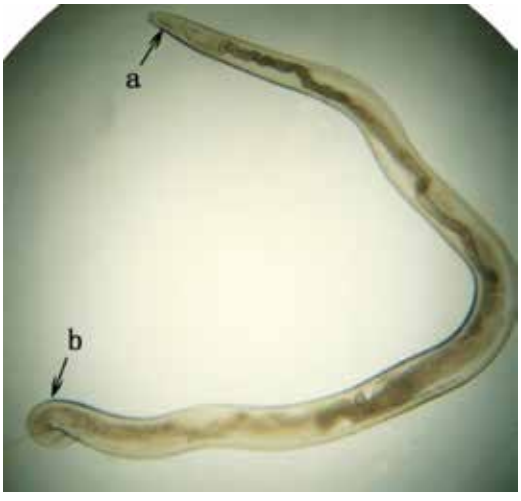

Fig 2-Light micrograph of *Thelazia callipaeda*
a: head.; b: tail.

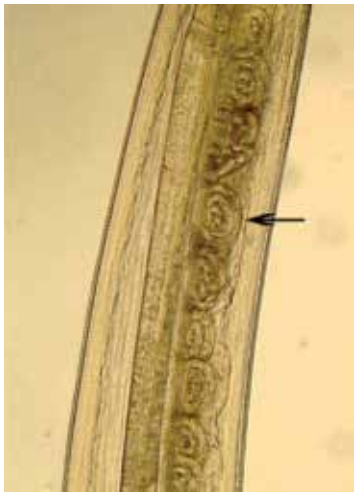

Fig 3-Light micrograph of the *Thelazia callipaeda* female worm.
The arrow points to numerous coiled embryonic larvae, in multiple rows in the proximal area of the uterus. (x40).

worms were open as in preparation for larval delivery. The worms were identified as *T. callipaeda* based on the above morphological characteristics (Otranto *et al*, 2003).

Genetic identification of the worms

The DNA of a whole female worm was extracted using a Genomic DNA Kit (Qiagen, Germany) following the manufacturer's protocol. The *cox*1 gene was amplified by PCR using specific primers (F: 5′AGATGGCGTTTCCTCGTCT 3′, R: 5′ GCAAAGAACCAATACCCACAG 3′) designed by partial sequences of the

*cox*1 gene (Gene Bank accession number AB538283). Polymerase chain reaction (PCR) was performed in 25 μL using the 2x Trans PCR SuperMix kit (TransGen, Beijing, PR China) at 0.5mM for each primer. The PCR amplicon was 202 bp. PCR conditions consisted of initial denaturation at 95°C for 2 minutes, following 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The PCR amplicon was sequenced using the Sanger method with a ABI 3730xl DNA Analyzer (Sangon Biotech, Shanghai, PR China). The DNA sequence obtained (202 bp) was aligned with the ten cox1 sequence (Table 1) and one outgroup species (*Gnathostoma spinigerum*, accession no. AB551552) was downloaded from Gene Bank. Sequence variation was calculated using BlastN from NCBI (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and genetic distance analysis was computed using MEGA 7.0 according to the Kimura 2-Parameter (K2P) model (Tamura *et al*, 2007). The result showed a lower (1.0%) sequence variation and smaller genetic distance on average (1.4%) for the cox1 than the *T. callipaeda* reported from Europe (Otranto *et al*, 2005b) and a higher sequence variation (3-4%) and larger genetic distance on average (2.9%) than that reported from Asia (Otranto *et al*, 2005b). This partial *cox*1 sequence (202 bp) suggests a novel haplotype.

DISCUSSION

T. callipaeda is an uncommon ocular parasite (Sah *et al*, 2018). Human thelaziasis may have a higher prevalence in rural communities with poor living conditions and a lower socioeconomic level. It may be more common in the elderly especially those who live in close contact with animals (Krishnachary *et al*, 2014). The widespread distribution of *T. callipaeda* is related to their widely distributed intermediate and final hosts (Otranto *et al*, 2009). Cats and dogs are

Table 1
Comparison of *Thelazia callipaeda* genetic sequences found in our subject with those reported in the Gene Bank.

Geographical origin	Host origin	Accession number	Sequence variation	Genetic distance
China (Anhui, Hefei)	Homo sapiens	MF795663	96%	0.043
China (Hubei, Wuhan)	*Homo sapiens*	MF795680	97%	0.032
China (Henan, Zhengzhou)	*Homo sapiens*	MF795693	97%	0.032
China (Liaoning, Dandong)	*Homo sapiens*	MF795670	97%	0.038
China (Shaanxi, Tongchuan)	Homo sapiens	MF795675	97%	0.032
Italy	*Canis familiaris*;	AM042549	99%	0.009
Japan (Tokyo)	*Canis lupus*	AB852543	97%	0.013
Korea (Kou Kuk)	*Canis familiaris*	AM042556	96%	0.016
Portugal	*Oryctolagus cuniculus*	KX033489	99%	0.016
Romania	*Canis familiaris*	KP087796	99%	0.016

important reservoir hosts for and infective sources of human thelaziasis (Zhang *et al*, 2019). Travel of reservoir hosts and illegal trade in pets has been reported to transmit the parasite from endemic to nonendemic areas (Graham-Brown *et al*, 2017).

T. callipaeda is transmitted by non-biting diptera, such as *Phorticaokadai* or *Phortica variegate,* in which the parasite goes through larval developmental stages (Otranto *et al*, 2013; Otranto *et al*, 2005a; Otranto *et al*, 2006). The third-stage larvae are released from the vector when the latter feeds on ocular secretions of human beings or animals (Otranto *et al*, 2005a; Otranto *et al*, 2006). Introduction of *T. callipaeda* from abroad by the vector seems less likely than from home, because fruit flies are not able to travel long distances (Ruytoor *et al*, 2010). Yang *et al*, (2006) described a case of human thelaziasis acquired through a contaminated towel.

The *cox*1 has been widely used as a genetic marker for species identification due to less variation interspecies and intraspecies (Otranto *et al*, 2005b; Liu *et al*, 2013; Zhang *et al*, 2018). Our case reported here shows *T. callipaeda* can cause thelaziasis in southwestern China. Our results suggest a novel haplotype.

This case suggests the possible need to educate the population in this area on how to reduce the risk for contracting this disease. Further incidence studies are needed to determine if such an education program is warranted in this area

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study

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