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Case Report

First report of equine *Setaria digitata* (von Linstow 1906) infestation in Malaysia

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ABSTRACT

The occurrence of *Setaria digitata* in a horse is reported for the first time in Malaysia. An 8-year-old Thoroughbred cross mare was referred to the University Veterinary Clinic with the primary complaint of corneal opacity and excessive eye discharge. After initial treatment with Terramycin eye ointment, corneal opacity cleared partially to reveal a moving thread-like cylindrical worm in the anterior chamber of the eye. The parasite was successfully removed surgically, and examination under the light microscope revealed that the isolated worm (length = 45 mm) was a 5th stage larva of *S. digitata* based on morphological criteria. Confirmation of the species of the worm was through molecular methods. The 12S rRNA gene was PCR-amplified, and the purified amplicon was directly sequenced. Phylogenetic analyses revealed that the isolated roundworm showed 100% sequence similarity with that of *S. digitata* in NCBI GenBank database (Accession no.: KY284626.1). This report is the first confirmed case of equine ocular setariasis by *S. digitata* in Malaysia. The current study provides evidence that *S. digitata* is an etiological agent of ocular infection and its presence in Malaysia.

1. Introduction

Ocular parasitism is a common vision impairing ophthalmic condition in equine resulting mainly from an ectopic infestation of *Setaria digitata, S. equina, S. marshalli* and *Thelazia lacrymalis* (Parrah et al., 2004; Sellon and Long, 2013). According to a retrospective study in India, there were 138 cases (57.0%) diagnosed as ocular setariasis among the 242 horses with ocular disorders examined (Tamilmahan et al., 2013). And the most commonly reported species in Asia is *S. digitata*, a parasite of the cattle as its permissive host and other hoofed animals.

Setaria (Nematoda: Filarioidea) is a genus of roundworm transmitted by biting insects, such as mosquitoes, and is commonly found free within the peritoneal cavity of ungulates (Al-Azawi et al., 2012; Soulsby, 1982). Larvae of *Setaria* frequently migrate to other organs and causes pathological lesions. Several reports have shown that this filarid can invade the eye of cattle and horses to cause blindness (Tamilmahan et al., 2013; Shin et al., 2002; Jemelka, 1976). As horses in this region are raised mainly for recreation and sports activities, impairment on visual ability can be detrimental to the animal and rider, also led to huge economic loses to the owners.

Filariasis is common in Malaysia and some mosquito species from the *Aedes*, *Anopheles*, *Culex* and *Mansonia* genus which act as vectors are easily found here (Kwa, 2008; Noordin, 2007). Equine setariasis has been reported in Malaysia (Noorashimah et al., 2015). However, confirmation of the species of *Setaria* has not been made, and the purpose of this communication is to report the first known case of setariasis by *S. digitata* in Malaysia.

2. Materials and methods

2.1. Case report

An 8-year-old Thoroughbred cross mare was referred to the University Veterinary Clinic with the primary complaint of corneal opacity and excessive eye discharge in the right eye. Upon examination, the horse showed signs of ocular pain indicated by blepharospasm, squinting and photophobia. Moderate conjunctivitis was also observed. However, further ophthalmic examination could not be conducted as the entire corneal surface was cloudy and opaque (Fig. 1). Initial treatment with Terramycin eye ointment resulted in partial corneal opacity clearing after two days and revealed the presence of intraocular eye worm in the anterior chamber of the right eye. The parasite had a white, thread-like appearance and was moving in a swirling motion in

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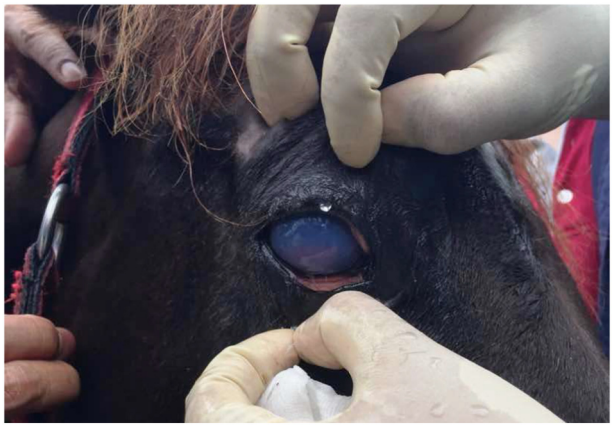

Fig. 1. Complete corneal opacity of the right eye during the first visit.

the aqueous humour. Nevertheless, examination of the blood films did not reveal the presence of microfilaria.

Surgical removal was opted as treatment for this case. The horse was sedated using Detomidine hydrochloride (0.01–0.02 mg/kg IV), and lidocaine 2% is administered locally to block the auriculopalpebral nerve and supraorbital nerve. The eye was prepared by flushing with sterile normal saline and 1% iodine solution. Proparacaine hydrochloride ophthalmic solution (Alcaine®, Alcon Inc. Texas, USA), a sterile aqueous ophthalmic topical anaesthetic solution was drop onto the cornea to desensitise the cornea and sclera. An 18-gauge needle was inserted into the anterior chamber of the eye at 7 o'clock position of the cornea, and 1 mm away from the limbus. The worm appeared at the puncture site due to aqueous humour pressure and can be easily removed.

This procedure caused only minimal aqueous leakage and the puncture site was left to heal without suturing. Terramycin eye ointment as prophylaxis was given four times daily for two weeks, and a single administration of non-steroidal anti-inflammatory drug (flunixin meglumine, 1.1 mg/kg) for reduction of intraocular inflammation and pain management.

2.2. Worm identification

The extracted worm was firstly examined under the light microscope, specifically at the anterior and posterior regions for the important morphological features according to the identification keys provided by Rhee et al. (1994) to identify the species.

This was followed by definitive confirmation through PCR identification. Genomic DNA was extracted from the nematode using Genomic DNA Mini Kit (Geneaid, USA) following the manufacturer's instruction. The 12S gene PCR assay used in this study was designed in-house using 12S rRNA gene. Primers sequence used in this study are SDF: 5′-AGT CCT CCC TTG TTG CTG GT-3′ and SDR: 5′-GGG TGG TTT GTA CCC CTC CG-3′. Polymerase Chain Reaction (PCR) amplification was performed using a Mastercycler Gradient (Biorad, USA). A final PCR volume of 25 µL contained 12.5 µL Go Taq® Green Master Mix (Promega, USA), one µL of each 20 pmol forward and reverse primer and five µL of DNA template. The PCR was performed under the condition of initial denaturation at 94 °C for 3 min for 40 cycles, denaturation at 94 °C for 45 s, annealing at 59.5 °C for 45 s, extension at 72 °C for 90 s followed by final 5 min extension at 72 °C. Amplified products were electrophoresed on 1.5% agarose gels. The gel was visualized and captured by using GelDoc (Biorad, USA). Then, the PCR products were purified from agarose gels using the GenepHlow TM Gel/PCR Kit (Geneaid, USA).

Nucleotide sequencing was conducted by Apical Scientific Sdn. Bhd. The sequence information of the nematode was compared with the sequences available in the GenBank of National Centre for Biotechnological Information (NCBI - www.ncbi.nim.nih.gov) using the Basic Local Alignment Search Tool (BLAST) program. Sequence similarity searching of the sample was also performed using the NCBI BLAST program. Analyses of multiple sequence alignments were done using the programs ClustalX (NCBI) and phylogenetic analysis was generated using Neighbour–joining method (NJ) (Saitou and Nei, 1987). The obtained 12SrDNA sequences were aligned with the available sequences of filarial worms in GenBank. For distance analyses, the Kimura 2-parameter model was used to construct the distance matrix. Bootstrap resampling (100 pseudoreplicates) was done and a bootstrap consensus tree was produced.

Fig. 2. *Setaria* worm extracted from the eye and preserved in tube.

3. Results and discussion

Ocular setariasis has been reported worldwide with few cases caused by *S. equina* in both animal and human (Nabie et al., 2017), while the others mainly by *S. digitata* (Jemelka, 1976; Yadav et al., 2006; Jaiswal et al., 2006; Marzok and Desouky, 2009; Rafee and Amarpal, 2016; Shin et al., 2017). The general appearance of the worm isolated in this case was thread like, milky-white, long with tapering ends (Fig. 2). Microscopic examination showed that the dorsal and ventral projection on the peribuccal crown was clearly visible from the side view, and the distance between the dorsal and ventral projection of this worm was relatively wide compare to other *Setaria* species; and the lateral lips were round (Fig. 3). A pair of prominent lateral appendages was found at the upper terminal end of the worm. Furthermore, the tapering tail was terminated with a smooth knob (Fig. 4). All these features indicated that the nematode species is *S. digitata*. On the other hand, the length of the isolated worm is around 45 mm, which suggested that it is a 5th stage larva. As according to the morphometric reported for adult *S. digitata*, male worm can grow up to the length of 82.0 ± 0.5 mm and 156.0 ± 0.7 mm for female worm (Sundar and D'Souza, 2015). Besides, it was also differentiated from 4th stage larva

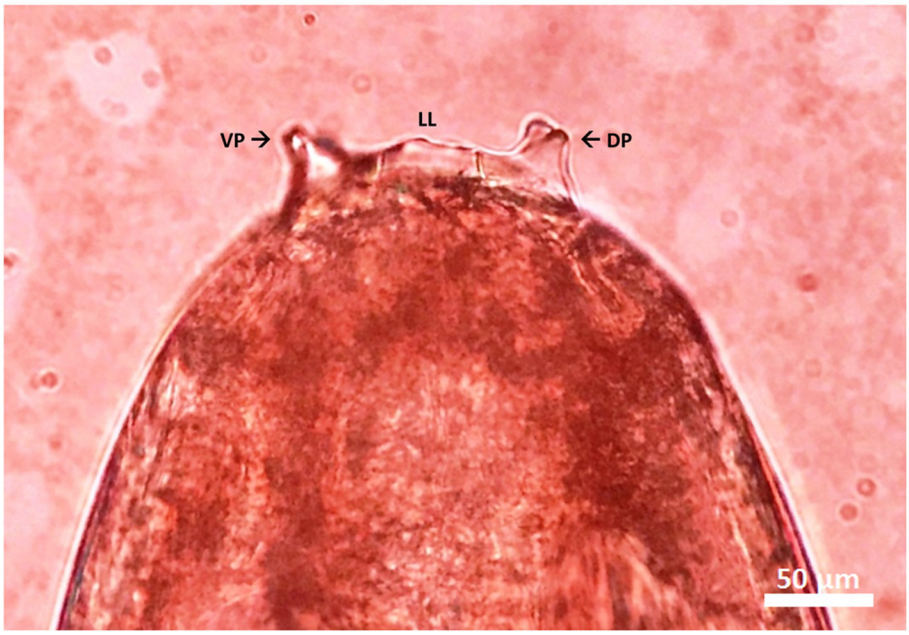

Fig. 3. Anterior region of *Setaria* shows the dorsal projection (DP); ventral projections (VP) and round lateral lips (LL) on the peribuccal crown.

base on its distinct spinous teeth at the apex of the anterior and morphometric in which the length for 4th stage larva is around 22–23 mm in length (Tung et al., 2003).

Surgical removal is the treatment of choice, compared to anthelmintic treatment, as this provide fast resolution of the infestation and avoid severe intraocular immunological tissue response to the dead eye worm, which may lead to degeneration and necrosis of structures within the globe, increased intraocular pressure, even rupture of the eyeball. The use of diethylcarbamazine (20 mg/kg IM) was shown to resolve microfilaremia. However, multiple treatments were required, and the drug is ineffective against adult parasites in the eye (Razig, 1989). Successful treatment of microfilariasis caused by *S. digitata* with a single subcutaneous dose of ivermectin (300 µg/kg) was reported. Yet, microfilaremia was only resolved 7 days post treatment, whereas the adult eyeworm was only killed after 15 days post treatment, and all ocular sings resolved after 90 days post treatment (Muhamad & Saqib, 2007). Additionally, the surgical procedure carries minimal risk and generally would not cause severe deviation in vision due to the small stab incision on the cornea. In this case, corneal opacity has cleared up post operatively, except at the point of incision, a common complication reported by other authors (Gopinathan et al., 2013; Patil et al., 2012). Furthermore, anti-inflammatory therapy and topical eye ointment are advocated to facilitate resolution of the clinical signs.

In view of the detrimental effects on animals and its economic impact for the owners, it is important to prevent seteriasis in horses. One of the suggested preventive measures is the use of ivermectin against circulating microfilaria, which was reported being effective in reindeer. On the other hand, controlling the vector population and preventing vector access to the horse may be an alternative to anthelmintic administration. As in other mosquito-borne diseases, such as dengue and

Fig. 4. Posterior region of *Setaria* with a pair of prominent lateral appendages (LA) and with a smooth knob at the posterior end (PE).

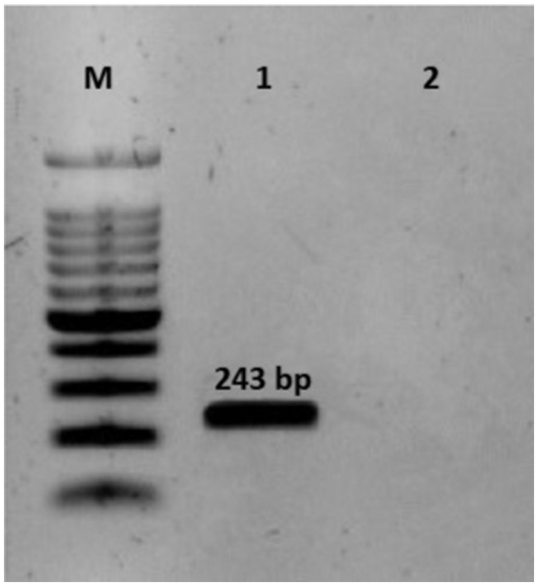

Fig. 5. Gel electrophoresis of *Setaria digitata*. (M: 100 bp ladder; 1: PCR product of *Setaria digitata*; 2: Negative control.)

malaria, destruction of the breeding grounds has proven to prevent the spread of these diseases. Covering the stables with fine wire mesh to prevent entry of mosquitoes would prevent horses from being bitten during the active hours of these vectors, thus preventing the spread of setariasis. The use of insecticide and pour-on mosquito repellent, deltamethrin, was found to be ineffective possibly due to its short lifespan, particularly during rainy seasons (Laaksonen et al., 2008). However, regular application may increase the effectiveness of deltamethrin.

The primer used in this study was designed specifically for the detection of *S. digitata* and it successfully produce a 243 bp band on gel electrophoresis (Fig. 5). This result was sequenced and confirmed as *S. digitata* when compared to database in NCBI GenBank using BLAST. The occurrence of *S. digitata* is concentrated in Asia and has been widely studied to the molecular level, in which the whole genome sequencing is available for identification and comparison purposes (Yatawara et al., 2010; Liu et al., 2017). The worm extracted from this case showed 100% similarity with the ones found in Sri Lanka and China (Yatawara et al., 2007; Liu et al., 2017). Phylogenetic analysis further support this finding in which the sequence of *S. digitata* isolated in this study was grouped together in the same clade as *S. digitata* from China and Sri Lanka (Fig. 6). However, it is in the different clade from *S. digitata* found in India, Japan and Thailand, where *S. digitata* in Thailand contain the same protein profiles as adult *S. digitata* in Japan through SDS-PAGE (Subhachalat et al., 1999). This may suggest that *S. digitata* in several Asian countries are of the same origin.

Confirmed setariasis due to *S. digitata* either through morphological identification or molecular confirmation has not been reported in Malaysia to date. Thus the endemic status and epidemiology of *S. digitata* have not been made available. Additionally, the currently available information could not determine the extent of the presence of this disease in Malaysia in the past, nor the appearance of the disease was due to a recent introduction through importation of animals. Thus, the present of this strain in this country which is closely related to *S. digitata* from China and Sri Lanka need to be further studied.

Kelantan being chiefly an agrarian state in Malaysia with abundant of small-holder ruminant (Ansari and Buchoo, 2005) and equine farms; the animals are usually kept in multi-species setup. Therefore, it is foreseeable that reports of setariasis will be increased as transmission of this parasite through mosquitoes can be achieved easily with the presence of various mosquito species in this country to serve as competent vectors (Tung et al., 2004). Inevitably, once the parasite is introduced into an area that is conducive to the propagation of vectors with the presence of suitable hosts, its transmission is virtually insured (Pereiraa et al., 2013). Difficulty in microfilariae detection and insignificant clinical manifestations of this disease is believed to be the reasons of low report rate in this country (Singh et al., 2013; Sabageh et al., 2014). Nevertheless, with the increasing clinical manifestations, such as the invasion of other vital organs and lumbar paralysis (Tung et al., 2003), the prevalence of *S. digitata* and its economic impact on the livestock and equine industry should no longer be neglected.

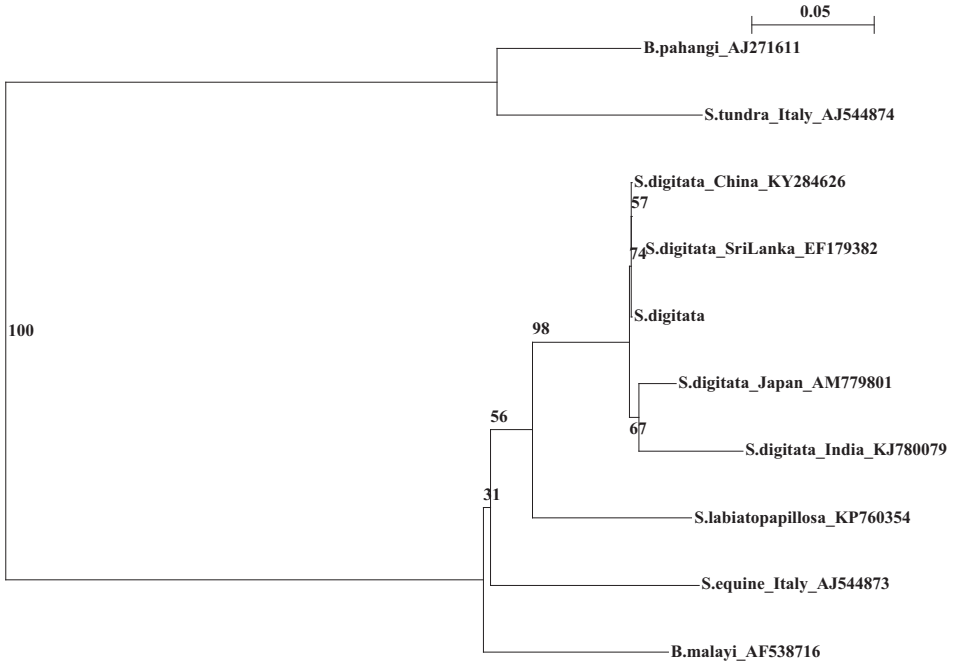

Fig. 6. A phylogenetic tree, inferred from 12SrDNA nucleotide sequences using the Neighbour Joining method after Kimura correction. Scale bar indicates the proportion of sites changing along each branch. Accession numbers of all sequences used for tree analyses are given.

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Conflict of interest

The authors declare no conflicts of interest.

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