Journal of Helminthology

www.cambridge.org/jhl

First report of molecular confirmation and phylogenetic analysis of ocular seteriasis in buffalo in India using 12S rRNA

S. Anandu[1], S. N. Chaithra[2], K. M. Manjusha[2], V. K. Tiwari[1], A. K. Tewari[1], G. N. Tanuj[3], S. Samanta[1] and M. Sankar[1]

[1]Division of Parasitology, Indian Veterinary Research Institute, Uttar Pradesh, India; [2]Division of Surgery, Indian Veterinary Research Institute, Uttar Pradesh, India and [3]Division of Animal Biotechnology, Indian Veterinary Research Institute, Uttar Pradesh, India

Short Communication

Cite this article: Anandu S, Chaithra SN, Manjusha KM, Tiwari VK, Tewari AK, Tanuj GN, Samanta S and Sankar M (2023). First report of molecular confirmation and phylogenetic analysis of ocular seteriasis in buffalo in India using 12S rRNA. *Journal of Helminthology*, **97**, e70, 1–4
https://doi.org/10.1017/S0022149X23000512

Received: 26 June 2023
Revised: 05 August 2023
Accepted: 08 August 2023

Key words:
Setaria digitata; buffalo; ophthalmology; India; molecular diagnosis; 12S rRNA

Corresponding author:
M. Sankar;
Email: drsankarm@gmail.com

Abstract

An adult Indian buffalo (*Bubalus bubalis*) presented with corneal opacity, irritation, and excessive lacrimation from the left eye in the Referral Veterinary Polyclinic-Teaching Veterinary Clinical Complex (RVC-TVCC), Indian Veterinary Research Institute, Izatnagar. Clinical examination revealed a whitish thread-like worm in the left eye's anterior chamber. The worm was surgically removed from the eye with supportive nerve blocks. Light microscopy was used for parasite morphological identification, which provided insight into the worm as female *Setaria* sp. Genomic DNA was isolated, and polymerase chain reaction amplification of 12S rRNA was conducted for molecular confirmation of the parasite. The amplicon was sequenced and analysed by bioinformatics software. Sequence data showed an amplicon size of 243 bp. Phylogenetic analysis with reference data from the NCBI Genbank database revealed the worm was *S. digitata*, with a similarity of 99.17%. The common predilection site of *S. digitata* is in the peritoneal cavity of natural hosts like cattle and buffalo and is mostly non-pathogenic. The aberrant migration of the parasite larva to the brain and eye commonly occurs in goats, sheep, and horses, causing clinical conditions like cerebrospinal nematodiasis (lumbar paralysis) and ocular setariasis, respectively. Nevertheless, until now, there have been no reports of ocular setariasis in buffalo. This report is the first unusual occurrence of ocular setariasis in buffalo and its molecular confirmation and phylogenetic analysis using 12S rRNA.

Introduction

Setaria digitata belongs to the superfamily Filarioidea, the family Setariidae, and the genus *Setaria*. The life cycle involves ungulates as definitive hosts and mosquitoes as vectors. The first stage larvae are known as microfilariae, often sheathed and released into the bloodstream by the adult female worm. While taking a blood meal, mosquitoes take up the infective microfilariae, which are transmitted to the next natural host while feeding. The adult worm resides in the peritoneal cavity of cattle and buffalo and is often non-pathogenic, although in some cases, it causes mild peritonitis (Solusby 1982). The aberrant migration of third stage (L_3) larvae occurs in hosts like goats, sheep, and horses (Soulsby 1982; Mohan *et al.* 2009). Ophthalmic migration usually occurs in horses, and cerebrospinal migration occurs in horses, sheep, and goats, causing enzootic cerebrospinal nematodiasis/lumbar paralysis (Soulsby 1982; Maharana *et al.* 2020). The heterotrophic migration and swirling movement of the worm in the anterior chamber of the eye of horses, cattle, and goats can cause pathogenic changes to the cornea leading to corneal opacity and blindness (Mohan *et al.* 2009; Gautam *et al.* 2018). Also, there are reports of erratic migration of larvae of *Setaria* sp. to the urinary bladder, oviduct, liver, and heart (Yoshikawa *et al.* 1976; Fujita *et al.* 1985; Sundar *et al.* 2005). There are 43 species of *Setaria* reported worldwide, so accurate identification of the parasite with simple morphological features is insufficient (Wijesundera 2001). Therefore, molecular techniques, sequence data, and phylogenetic analysis will help in the confirmation of the parasite at the species level and also help in understanding the evolutionary pattern, relationship of species, and parasite biology (Senanayake *et al.* 2020). Tamilmahan *et al.* (2013) reported an incidence of 57.02% of equine ocular setariasis between 2002–2011. Here, we report the first case of ocular setariasis in a buffalo. Further, the parasite was confirmed as *S. digitata* by amplifying the 12S rRNA region, sequencing, and its phylogenetic analysis.

Materials and methods

The clinical case was presented at the referral veterinary polyclinic, Indian Veterinary Research Institute (IVRI). The parasite sample was analysed at the division of parasitology, IVRI, Izatnagar, Uttar Pradesh, India.

Case report

An adult Indian buffalo (*Bubalus bubalis*) presented with a history of restlessness, excessive lacrimation, and leukomatous cornea. On gross clinical examination, corneal opacity, blepharospasm, and discharge from the eye were observed (Figure 1a). A thin, milky white, thread-like, cylindrical, swirling worm was observed in the anterior chamber of the left eye. The blood sample was collected for wet film examination, peripheral blood smear, and complete blood count (CBC). No microfilariae could be detected in both wet film/blood smear, and CBC was also normal. Surgical intervention was preferred over medication. Proper restraining and irrigation of the eye with normal saline was done, and then auriculopalpebral and Peterson nerve block was given (Rafee 2016). A 5 mm incision was made at the 6 o'clock position at the limbus of the eye, and the worm was removed with the help of ocular forceps (Figure 1b). The surgical site was left to heal by administration of post-surgical antibiotic and anti-inflammatory drops.

Morphological identification

For morphological identification, the retrieved worm was washed thrice with phosphate-buffered saline (PBS), and both the anterior and posterior ends were observed under the light microscope. The identification was made based on criteria by Rhee *et al.* (1994).

Molecular Identification

Genomic DNA was isolated for molecular analysis using the phenol-chloroform-isoamyl alcohol method (Sambrook & Russel 2006). The concentration of DNA was measured using a nano-spectrometer (Qiagen, Hilden, Germany) and subsequently stored at -20°C for further molecular work. Polymerase chain reaction (PCR) was performed with the extracted genomic DNA using the primers: SDF: 5' -AGT CCT CCC TTG TTG CTG GT-3' and SDR: 5'-GGG TGG TTT GTA CCC CTC CG-3' (Peng *et al.* 2019). The amplification was done in a 50 uL reaction volume by adding 25 uL of 2X PCR master mix (Sapphire Amp Fast PCR Master Mix, TaKaRa, Shiga, Japan), 10 pmol each of forward primer and reverse primer, and 40 ng of template. The reaction conditions were: 94°C for 4 min, followed by 39 cycles of 94°C for 80 sec, 46°C for 80 sec, 72°C for 60 sec, and 72°C for 10 min (Yu *et al.* 2021). The amplicons were gel purified and custom sequenced. The sequences were analysed with published sequences available in the NCBI data bank. The ClustalW program (https://www.genome.jp/tools-bin/clustalw) was used to obtain multiple sequence alignment, and then phylogenetic analysis was done using MEGA11 software (MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al.* 2021)). A Tamura Nei substitution model was used to construct the distance matrix, and the phylogenetic tree was constructed using the maximum likelihood approach with a 500-bootstrap value (Yang 2007).

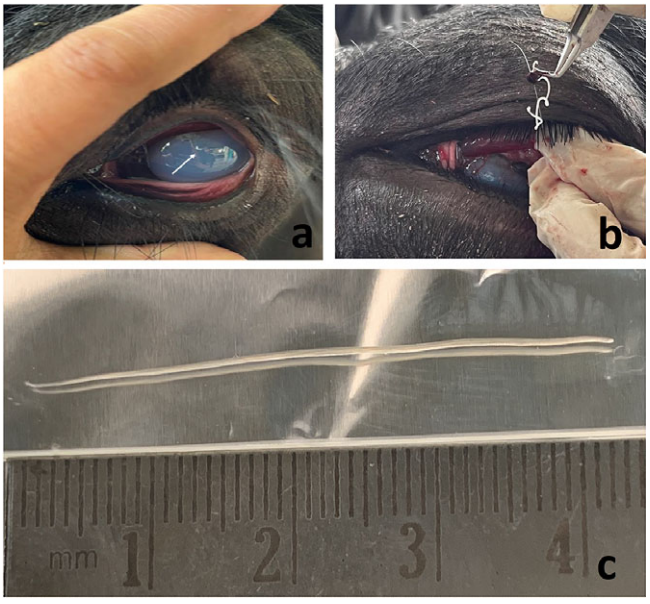

Figure 1. (a) Whitish, thread-like worm (arrow) present in the anterior chamber of left eye with leukomatous cornea; (b) Retrieval of worm with ocular forceps; (c) Gross morphology of worm retrieved measuring 43 mm long.

Results and discussion

Parasite

In gross examination: a 43 mm long, both-ends tapered, thread-like, milky white worm was observed (Figure 1c). The anterior end is round with two projections in the peri buccal area (Figure 2a), and the posterior end with a spherical bulb and lateral appendage is characteristic of female *Seteria digitata* (Figure 2b).

Seteria genus includes *S.digitata, S.labitopapilosa, S. equina*, and *S.cervi,* which are similar in morphology. Therefore, PCR was used to further confirm species by amplifying the 12S rRNA gene. After gel electrophoresis, the amplicons were purified and sequenced. The sequence result showed a 243 bp band, which confirmed the worm isolated was *S. digitata* by Basic Local Alignment Search Tool (BLAST) analysis and comparison with other sequences from the National Center for Biotechnology Information (NCBI).

After BLAST analysis, the query sequence showed more than 99% similarity to the *S. digitata* mitochondrion complete genome (KY284626.1) and *S. digitata* Thailand isolates (OP895162.1), which confirmed the sequence was of *S. digitata.* Phylogenetic analysis showed a major clade formed by the genus *Setaria* and another by the genus *Brugia* + *Wuchereria* + *Onchocerca*, and an outgroup was formed by *Thelazia gulosa* (Figure 3).

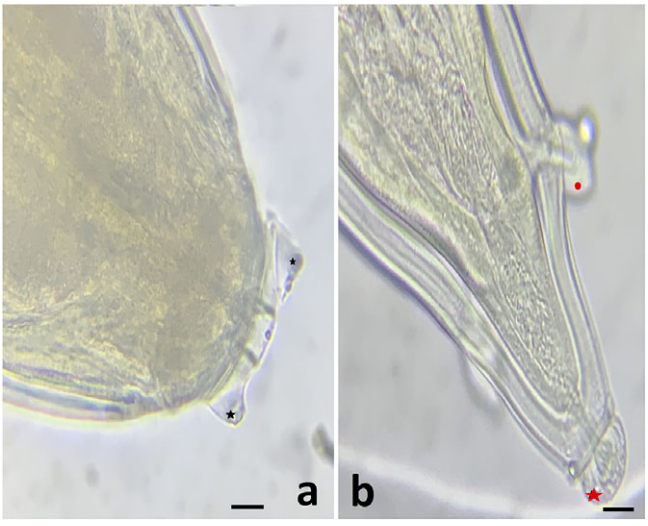

Figure 2. (a) Anterior end showing the presence of two tuberences (black star; dorsal projection and ventral projection); (b) Posterior end showing spherical bulb (red star) and lateral appendages (red round). Scale bar = 100 μm.

https://doi.org/10.1017/S0022149X23000512 Published online by Cambridge University Press

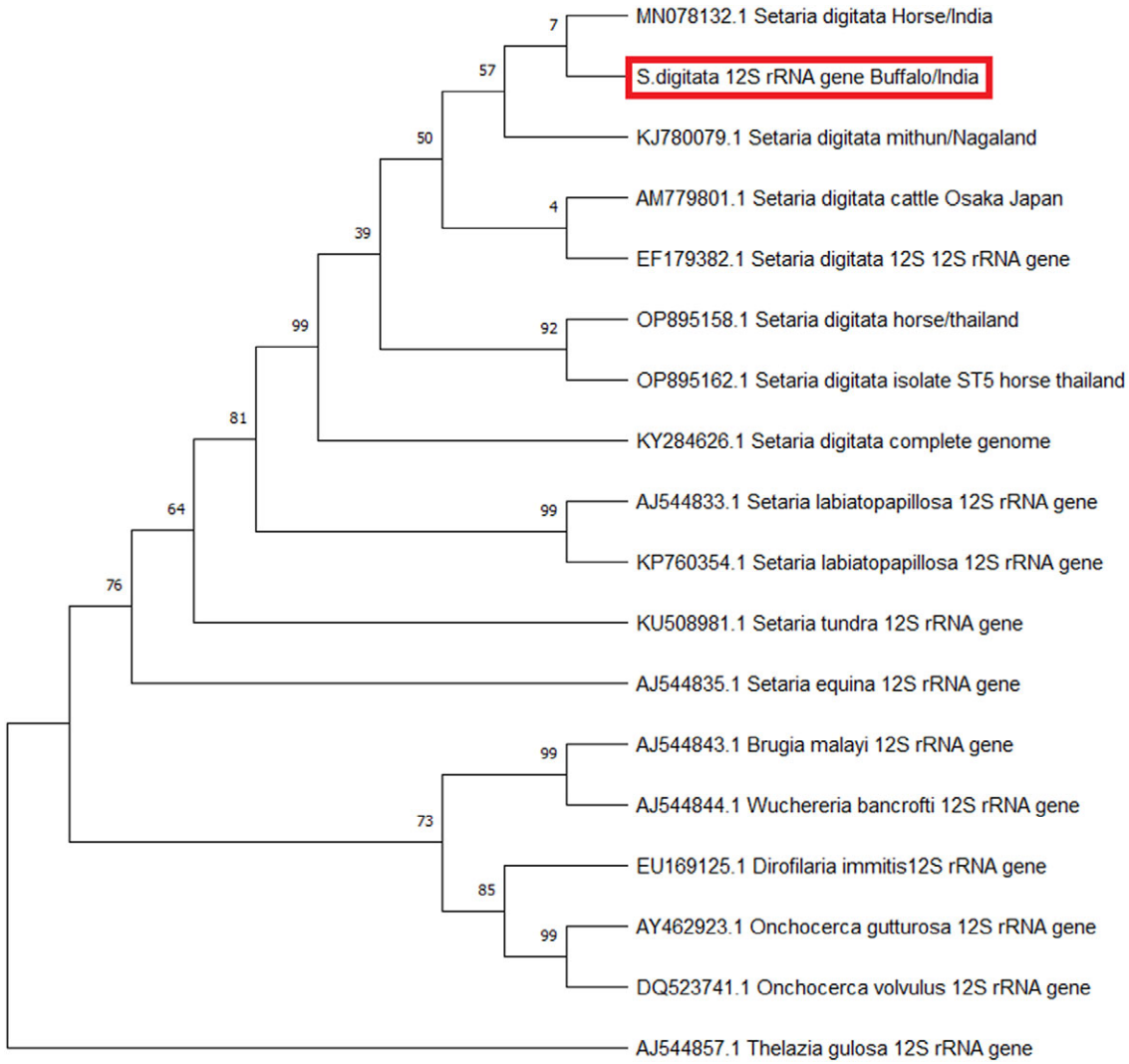

Figure 3. Phylogenetic tree constructed in MEGA 11 software with related *Setaria* species and *Thalezia gulosa* as an outgroup, using maximum likelihood method after Tamura Nei substitution model and 500 bootstrap values. The generated sequence is represented in the red rectangle and other species' accession numbers are also mentioned.

Occular setariasis caused by *S. digitata* mainly causes its pathogenic effect in equines. However, there are reports of ocular setariasis in goats and cattle (Mohan *et al.* 2009; Gautam *et al.* 2018; Shin *et al.* 2002) but not in buffalo. Sundar & D'Souza (2015) reported that the length of an adult male and female worm can reach up to 82 mm and 156 mm, respectively, and the length of fourth-stage larvae can be 22–23 mm (Tung *et al.* 2003). Here the retrieved worm was 45 mm long, suggesting it was an immature worm. The microscopic examination of the worm revealed ventral and dorsal protuberances at the anterior end and a smooth knob at the posterior end, suggestive of a female *S. digitata* worm. A surgical procedure was chosen over medication because of the lower risk and faster corneal opacity improvement. The presence of a dead worm in the eye can cause immunological reactions leading to further complications like degeneration of structures in the eye, enhancing intraocular pressure and eyeball rupture (Peng *et al.* 2019). Ocular setariasis is caused mainly by *S. digitata*, but morphological characters cannot differentiate *S. digitata* from its congeners (Jayasinghee *et al.* 2003). Thus, for molecular confirmation, the 12S rRNA gene is targeted, which is an evolutionary marker (Yatawara *et al.* 2007; Junsiri *et al.* 2023). Earlier, the sequence data analysis of the *S. digitata* showed 243 bp (Peng *et al.* 2019; Yu *et al.* 2021). Some potential mosquito vectors for the transmission of filarial nematodes are *Anopheles, Mansonia, Culex, Armigeres, Aedes*, and *Ochlerotatus. Anopheles* sp. and *Armigeres* sp. are mainly responsible for transmitting *Setaria* sp. (Siriyasatien *et al.* 2023). The area where this case was reported has a prevalence of 63 species of mosquitoes, which include 15 species of *Anopheles* and *Aedes*, 24 species of *Culex*, 3 species of *Mansonia*, and 2 species each of *Verrallina*, *Mimomyia*, and *Ochlerotatus* (Kanojia & Geevarghese 2005). In Asia, the occurrence of *S. digitata* is high, and there are many reports of aberrant ophthalmic migration in equines (Shin *et al.* 2017). However, this migration pattern is rare in buffalo; this the first report of an unusual occurrence of ocular setariasis in buffalo, which might be due to the high prevalence of vectors for *S. digitata* in the summer season. The accidental case may have occurred because of the vector's high prevalence in the summer (Tung *et al.* 2004).

Acknowledgements. The authors express their sincere gratitude to the Head of Division, Division of Parasitology, for providing research facilities.

Financial support. None.

Competing interest. None.

https://doi.org/10.1017/S0022149X23000512 Published online by Cambridge University Press

Ethical standard. All the authors here by declare that they have followed the ethical standard for this experiment.

References

Fujita J, Imai S, Ishii T, Nunoya T, Takahashi K, Tomita T, Oikawa R (1985). Heterotopic parasitism of Setaria digitata (Linstow, 1906) in the heart of a cattle. *Nihon Juigaku Zasshi* Dec;**47**(**6**), 999–1002. https://doi.org/10.1292/jvms1939.47.999.

Gautam D, Verma NK, Shah MA, Raghuvanshi PDS, Nehra A, Kumar N (2018). Successful management of the rare ocular setariasis in a goat. *Ruminant Science* **7**, **1**, 161–162.

Jayasinghe DR, Wijesundera WSS (2003). Differentiation of *Setaria digitata* and *Setaria labiatopapillosa* using molecular markers. *The Veterinary Journal* **165**, **2**, 136–142. https://doi.org/10.1016/s1090-0233(02)00157-0

Junsiri W, Kamkong P, Chinkangsadarn T, Ouisuwan S, Taweethavonsawat P (2023). Molecular identification and genetic diversity of equine ocular setariasis in Thailand based on the COI, 12S rDNA, and ITS1 regions. *Infection, Genetics and Evolution* **110**, 105425. https://doi.org/10.1016/j.meegid.2023.105425

Kanojia PC, Geevarghese G (2005). New mosquito records of an area known for Japanese encephalitis hyperendemicity, Gorakhpur District, Uttar Pradesh, India. *Journal of the American Mosquito Control Association* **21**, **1**, 1–4. https://doi.org/10.2987/8756-971X(2005)21[1:NMROAA]2.0.CO;2

Maharana BR, Potliya S, Ganguly A, Bisla RS, Mishra C, Ganguly I (2020). First report of the isolation and phylogenetic characterization of equine *Setaria digitata* from India based on mitochondrial COI, 12S rDNA, and nuclear ITS2 sequence data. *Parasitology Research* **119**, **2**, 473–481. https://doi.org/10.1007/s00436-019-06587-1

Mohan K, Ananda KJ, Shridhar NB, Puttalakshmamma GC, Placid E (2009). Corneal opacity due to *Setaria digitata* in a Jersey cross-bred cow and its surgical management. *Veterinary World* **2**, **2**, 69–70.

Peng TL, Armiladiana MM, Ruhil HH, Maizan M, Choong SS (2019). First report of equine *Setaria digitata* (von Linstow 1906) infestation in Malaysia. *Veterinary Parasitology: Regional Studies and Reports* **17**, 100310. https://doi.org/10.1016/j.vprsr.2019.100310

Prakash A, Jambulingam P, Rajavel A, Bhattacharyya D, Mahanta J, Mohapatra P (2014). Faunal richness and the checklist of Indian mosquitoes (Diptera: Culicidae). *Check List*, **10**, **6**, 1342–1358. https://doi.org/10.15560/10.6.1342

Rafee MA, Amarpal A (2016). Equine ocular setariasis and its management. *Journal of Experimental Biology and Agricultural Sciences* **4**, Spl-4-EHIDZ. https://doi.org/10.18006/2016.4(Spl-4-EHIDZ).S139.S143

Rhee JK, Choi EY, Park BK, Jang BG (1994). Application of scanning electron microscopy in assessing the prevalence of some *Setaria* species in Korean cattle. *The Korean Journal of Parasitology* **32**, **1**, 1–6. https://doi.org/10.3347/kjp.1994.32.1.1

Sambrook J, Russell DW (2006). *Purification of nucleic acids by extraction with phenol: chloroform. Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Protocols. https://doi.org/10.1101/pdb.prot4455

Senanayake KS, Söderberg J, Põlajev A, Malmberg M, Karunanayake EH, Tennekoon KH, Niazi A (2020). The genome of *Setaria digitata*: a cattle nematode closely related to human filarial parasites. *Genome Biology and Evolution* **12**, **2**, 3971–3976. https://doi.org/10.1093/gbe/evaa017

Shin J, Ahn KS, Suh GH, Kim HJ, Jeong HS, Kim BS, Shin SS (2017). First blindness cases of horses infected with *Setaria digitata* (Nematoda: Filarioidea) in the Republic of Korea. *The Korean Journal of Parasitology* **55**, **6**, 667–671. https://doi.org/10.3347/kjp.2017.55.6.667

Shin SS, Cho KO, Wee SH (2002). Ocular infection of cattle with *Setaria digitata*. *Journal of Veterinary Medical Science* **64**, **1**, 7–10. https://doi.org/10.1292/jvms.64.7

Siriyasatien P, Intayot P, Sawaswong V, Preativatanyou K, Wacharapluesadee S, Boonserm R, Sor-Suwan S, Ayuyoe P, Cantos-Barreda A, Phumee A (2023). Description of potential vectors of zoonotic filarial nematodes, *Brugia pahangi, Setaria digitata,* and *Setaria labiatopapillosa* in Thai mosquitoes. *Heliyon*, **9**, **2**, e13255. https://doi.org/10.1016/j.heliyon.2023.e13255

Soulsby EJL (1982) *Helminths, Arthropods and Protozoa of Domesticated Animals*. London: Baillière Tindall, 316–319.

Sundar SB, D'souza PE, Jagannath MS (2005). Prevalence of setariosis in cattle and buffaloes in Karnataka. *Journal of Parasitic Diseases* **29**, 147–149.

Sundar STB, D'Souza PE (2015). Morphological characterization of *Setaria* worms collected from cattle. *Journal of Parasitic Diseases* **39**, **3**, 572–576. https://doi.org/10.1007/s12639-013-0399-x

Tamilmahan P, Zama MMS, Pathak R, Muneeswaran NS, Karthik K (2013). A retrospective study of ocular occurrence in domestic animals: 799 cases. *Veterinary World* **6**, **5**, 274–276. https://doi.org/10.5455/vetworld.2013.274-276

Tamura K, Stecher G, Kumar S (2021). MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* **38**, 3022–3027.

Tung KC, Cheng FP, Lai CH, Wang KS, Wang JS, Lee WM (2004). Demonstration of vector competence of *Culex quinquefasciatus* (Diptera: Culicidae) for *Setaria digitata*. *Veterinary Parasitology* **123**, **3–4**, 279–284. https://doi.org/10.1016/j.vetpar.2004.07.001

Tung KC, Lai CH, Ooi HK, Yang CH, Wang JS (2003). Cerebrospinal setariosis with *Setaria marshalli* and *Setaria digitata* infection in cattle. *Journal of Veterinary Medical Science* **65**, **9**, 977–983. https://doi.org/10.1292/jvms.65.977

Wijesundera, WSS (2001). *The Encyclopedia of Arthropod-transmitted Infections*. Wellington: CABI Publishing.

Yang Z (2007). PAML 4: phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution* **24**, **8**, 1586–1591. https://doi.org/10.1093/molbev/msm088

Yatawara L, Wickramasinghe S, Nagataki M, Rajapakse RP, Agatsuma T (2007). Molecular characterization and phylogenetic analysis of *Setaria digitata* of Sri Lanka based on CO1 and 12S rDNA genes. *Veterinary Parasitology* **148**, **2**, 161–165. https://doi.org/10.1016/j.vetpar.2007.06.005

Yoshikawa T, Oyamada T, Yoshikawa M (1976). Eosinophilic granulomas caused by adult setarial worms in the bovine urinary bladder. *Nihon juigaku zasshi. The Japanese journal of veterinary science* **38**, **2**, 105–116. https://doi.org/10.1292/jvms1939.38.105

Yu F, Liu B, Chen S, Yi Z, Liu X, Zhu Y, Li J (2021). First molecular confirmation of equine ocular *Setaria digitata* in China. *Veterinary Sciences* **8**, **4**, 55. https://doi.org/10.3390/vetsci8040055

https://doi.org/10.1017/S0022149X23000512 Published online by Cambridge University Press