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HELMINTHOLOGY - ORIGINAL PAPER

Setaria labiatopapillosa (Filarioidea, Nematoda) in Moroccan cattle: atypical localization and morphological characterization of females and microfilariae by light and scanning electron microscopy

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Abstract

Filarioid nematodes are parasites of the tissues and tissue spaces of all vertebrates except fish. Females produce microfilariae that enter the host's blood circulation or skin and may cause ocular and neurological pathology, leading to important implications in veterinary and public health. The present work is the first investigation on *Setaria labiatopapillosa* conducted in Morocco to characterize the morphological features of both adult and microfilaria forms. Two adult female nematodes were found free in the thoracic cavity of a slaughtered 3.5-year-old (6 teeth) Moroccan enhanced cross-breed bull which was born and raised in Morocco. The worms were identified as *S. labiatopapillosa* by light microscope (LM) and scanning electron microscopy (SEM) on the basis of their characteristic features of the anterior and posterior parts of the worms. The two *S. labiatopapillosa* worms measured 90 mm and 105 mm in length and 0.55 and 0.64 mm in width, respectively. Microfilariae were detected in the fully developed eggs contained in the uterus of both nematodes. A detailed morphology of both the adult females and larvae of *S. labiatopapillosa* is described using LM and SEM. Although the origin of *S. labiatopapillosa* analyzed in the present study is unknown and there is currently no evidence that *Setaria* spp. have invaded Morocco, further surveillance is warranted to determine the incidence of setariasis, identify its vectors, and take appropriate measures to protect the livestock and cattle industry of the country.

Keywords Blood · Cattle · Filaria · Microfilariae · Microscopy · Morocco

Introduction

Setaria spp. (Filarioidea, Nematoda) are vector-borne filarial nematodes transmitted through the bites of mosquitoes (*Aedes*, *Anopheles*, *Culex*, and *Mansonia*) and flies (*Haematobia stimulans*) (Azari-Hamidian et al. 2019). The mature adult roundworms measure between 5 and 10 cm and dwell primarily in the abdominal cavity of domesticated ungulates, such as cattle, camels, horses, goats, and sheep, as well as in wild mammals (Singh et al. 2014; Singla et al. 2014). Adult females produce larval infective forms, called microfilariae, that enter the blood stream of the infected host animal (i.e., microfilariosis). Many species of *Setaria* do not show any microfilarial periodicity, and only a few microfilariae are present in the blood. The microfilariae are ingested by insect vectors during blood meal, and the development of infective larvae (L3) occurs in the thoracic muscles of the vector (Shoho and Nair 1960). The infective stage microfilariae are transmitted through insect bites to other host animals and reach sexual maturity within 8–10 months, completing the life cycle (Nelson 1962).

Among *Setaria* worms, *S. labiatopapillosa*, *S. digitata*, *S. cervi*, *S. marshalli*, *S. equina*, and *S. tundra* have been reported worldwide. Several *Setaria* species infect cattle and buffaloes, including *S. labiatopapillosa*, *S. marshalli*, *S. cervi*, *S. nelsoni*, *S. leichungwingi*, *S. equina*, *S. tundra*, and

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S. digitata, the latter being the most common (Shoho 1958, 1976a; Becklund and Walker 1969; Kaur et al. 2015; Sundar and D'Souza 2015; Alborzi et al. 2020). *Setaria digitata*, *S. marshalli*, and *S. labiatopapillosa* are common nematodes found in cattle in Asia (Rhee et al. 1994; Tung et al. 2003; Nakano et al. 2007; Bazargani et al. 2008; Khedri et al. 2014). *Setaria labiatopapillosa* is a heteroxenous parasite of cattle with several species of mosquitoes belonging to the genus *Culex* and *Anopheles* acting as intermediate hosts. Adult worms in the peritoneal cavity are generally considered to be non-pathogenic in their natural host although they may cause a mild, clinically insignificant fibrinous peritonitis. The presence of microfilariae (L1) in the blood stream of bovine natural host leads to microfilariosis with little clinical consequence (Sigraskar et al. 1999). However, microfilariae may migrate into the eyes or to the central nervous system in unusual hosts, such as sheep, goats, horses, and humans, where ocular pathology, neurological disease, and even death may ensue (Rhee et al. 1994; Shin et al. 2002; Shin et al. 2017). Cerebrospinal nematodiasis may occur in epizootic proportions, leading to death in horses, sheep, and goats (Bazargani et al. 2008). The ubiquity and economic losses associated with *Setaria* spp. have rendered them among the most important parasites in unusual animal hosts. Rare occurrences of zoonotic transmission of *S. equina* (from horses and donkeys), *S. labiatopapillosa*, and *S. digitata* have also been reported, causing subconjunctival eye infection (Panaitescu et al. 1999; Ţălu et al. 2012; Nabie et al. 2017).

Setaria adult worms and their microfilariae occupy an important place in parasitological research as they are extensively used in diagnostic assays and are laboratory models used in drug studies of human filariasis due to their morphological, histological, and antigenic similarities (Murugananthan et al. 2010; Perumal et al. 2016). Investigations on *S. labiatopapillosa* have not been conducted in Morocco. The present study was carried out with the aim to identify and describe the morphological features of this nematode revealed by light microscopy (LM) and scanning electron microscopy (SEM).

Materials and methods

Female *Setaria* worms were found and collected from the thoracic cavity of a 3.5-year-old (6 teeth) cross-bred bull slaughtered at Sidi Yahia El Gharb slaughterhouse in May 2011 in Morocco and transported to the laboratory in 10% formalin. The bull was not imported. It was born and raised locally in a small town called Kceibya, located about 7 km from the city of Sidi Yahya El Gharb, northern Morocco. The worms were washed several times in physiological saline solution to remove host material and blood contamination. The anterior and posterior ends of the worms were cut and suspended in lactophenol solution for identification of the worm under light microscope. The dimensions of the worms, microfilariae, and eggs were measured using a Nikon Alphaphot YS microscope (Tokyo, Japan) equipped with an eyepiece micrometer, and the images were photographed using an Olympus μ700 digital camera (Olympus Corporation, Tokyo, Japan).

Unlike in the work of Ghahvei et al. (2020), which involved the preparation of the nematodes fixed with glutaraldehyde and osmium tetroxide, the adult worms were simply suspended in lactophenol solution before observation. The eggs and microfilariae were expelled spontaneously from the vulva of the female adult worms. They were suspended in lactophenol solution for further observation. Different parts of the nematodes and microfilariae were analyzed using a scanning electron microscope (SEM; model Quanta field emission gun [FEG] 200; ThermoFisher Scientific, Illkirch-Graffenstaden, France), at the National Center for Technical and Scientific Research (CNRST), Rabat, Morocco.

Results

The milky white, threadlike nematodes with rounded anterior end and coiled posterior end collected alive from the thoracic cavity of a slaughtered bull were tentatively identified as *S. labiatopapillosa* female worms. The worms were 90 mm and 105 mm long and 0.55 mm and 0.64 mm wide, respectively (Fig. 1A). Light microscopy revealed clearly visible dorsal and ventral projections on the peribuccal crown from the lateral view. The distance between the dorsal and ventral projection was narrow (Fig. 1B). Symmetrical, posterior papillae were observed to be located approximately at the beginning of the glandular esophagus (Fig. 1C). The shape of the vagina was distinctive with a simple, straight vaginal canal. The ovejector comprised of a large, pyriform sphincter within which the genital lumen turns (Fig. 1D). The uteri of both worms were filled with numerous fully developed eggs and eggs in different developmental stages (Fig. 1E). The posterior end was characterized by a roughly furcated terminus (Fig. 1F).

The eggs had an ovorectangular shape and contained embryonic mass (Fig. 2A). The embryonic mass slowly developed into a coiled larval form, i.e., microfilaria. Microfilaria inside the egg extended with round anterior and pointed posterior ends where the egg shell remained on the larvae forming a sheath around it (Fig. 2B–E). Microfilariae (220–260 μm × 5–6 μm) were detected in fully developed eggs and were observed under light microscope and SEM. The mean size of the eggs ($n = 40$) was 40 μm × 27 μm and ranged from 38 to 44 μm × 21 to 31 μm.

The SEM confirmed the characteristic feature of the peribuccal crown at the long, oval-shaped mouth

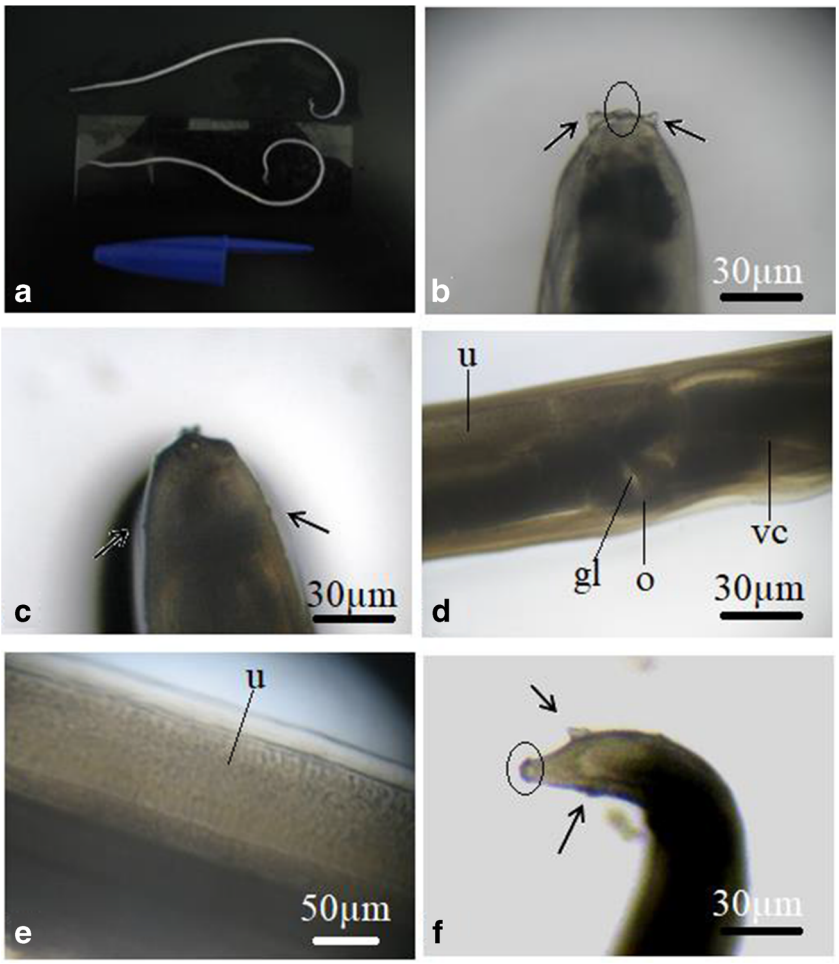

Fig. 1 Gross and light microscopy findings of adult female *S. labiatopapillosa*. (A) Two female adult worms of *S. labiatopapillosa* found in the thoracic cavity of a bull. (B) Light microscopy (LM) findings of the female; lateral view of the head region; lateral lips (circle); dorsal and ventral projections (arrow). (C) Ventral view; symmetrical papillae (arrow). (D) Vagina vera, lateral view; uterus (u), genital lumen (gl), ovijector (o), vaginal canal (vc). (E) Uterus full of eggs (u). (F) Latero-ventral view of posterior region; lateral appendage (arrow); terminal end (circle)

opening in *S. labiatopapillosa*. The female did not have bifid lateral lips (Fig. 3A). Dorsal and ventral projections represented by two summits were found at the upper terminal end in both nematodes (Fig. 3A, B). A pair of lateral appendages was seen with the phasmidial pore situated at the upper armpit (Fig. 3C). *Setaria labiatopapillosa* had an obtusely ending tail with botryoidal tissue with many spiny projections, as shown by SEM (Fig. 3C). Very fine longitudinal striations were observed along the entire length of the worm (Fig. 3D) under the SEM, but not under light microscope. The irregularly arranged tubercles on the cuticle of posterior end (Fig. 3E) were also observed under the SEM. A peri-esophageal collar (necklace) was seen next to the front (Fig. 3F). Details of deirid and amphid of adult female *S. labiatopapillosa* are illustrated in Fig. 4A and B. The amphid showed a "rose-like" appearance. The observations of microfilariae under SEM showed sheathed microfilariae (L1) with a rounded anterior end and a pointed posterior end (Fig. 5A–C). Moreover, a succession of horizontal bands was seen along the entire length of microfilariae.

Discussion

This is the first report on *S. labiatopapillosa* from Morocco. *Setaria* spp. have not been previously reported from neighboring countries bordering Morocco, i.e., Mauritania and Algeria. However, the presence of *S. labiatopapillosa* was reported from several sub-Saharan African countries, including Nigeria (Ogbogu et al. 1990), The Gambia (McFadzean 1955; Ndao et al. 1995), Burkina Faso (Gidel and Brengues 1972; Brengues and Gidel 1972), Senegal (Gueye et al. 1989), Chad (in a donkey) (Shoho 1976b), and Kenya (Nelson 1962). Another species, *S. equina* and its sub-species, seems to be more widespread in horses, donkeys, and zebras in sub-Saharan African countries, extending from west to east coast of the African continent, including south Africa (Shoho 1976b). At present, mosquito vectors that transmit *Setaria* spp. in Morocco are not known (Trari et al. 2003; El Joubari et al. 2014; Filali Mouatassem et al. 2019).

In our study, species other than *S. labiatopapillosa* were ruled out based on morphological features. Adult *S. labiatopapillosa* has the characteristic dorsal and ventral projections represented by two summits at the upper terminal

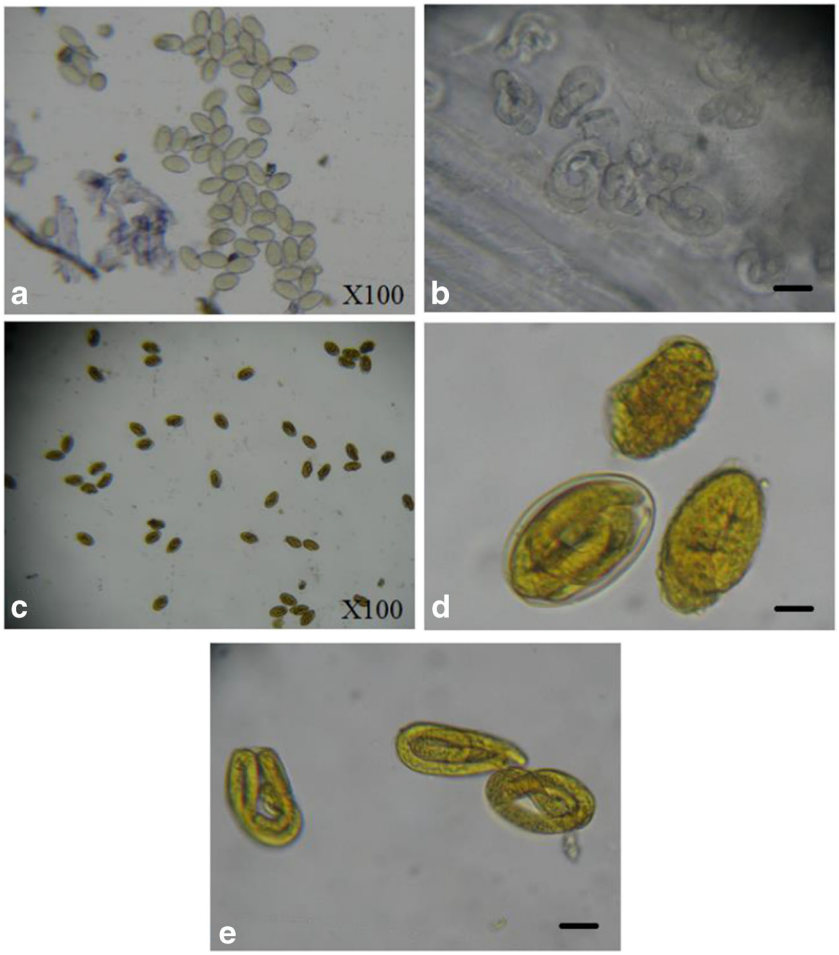

Fig. 2 Light microscopy findings of the eggs and microfilariae in the eggs. (A) Ovorectangular-shaped eggs obtained from the uterus. (B) Microfilaria in the uterus. (C–D) Eggs from the uterus containing embryonic mass inside, Lugol's iodine stain. (E) Microfilariae from the uterus, Lugol's iodine stain. Scale bar: 20 μm

end, whereas female *S. digitata* has lateral lips represented by one summit. *Setaria labiatopapillosa* is characterized by an obtusely ending tail with botryoidal tissue with many spiny projections. By contrast, the posterior end of *S. digitata* is a tapering terminus with a smooth knob. The female adult of *S. labiatopapillosa* does not have bifid lateral lips, and the distance between the dorsal and ventral projection is much smaller than in *S. marshalli* (Kim et al. 2010). In addition, amphid, deirid, postdeirid, and phasmidal pores are typically seen in *S. labiatopapillosa* (Shoho and Uni 1977).

In general, adults of *Setaria* spp. are found in the peritoneal cavity (Osipov 1972; Sundar and D'Souza 2015). However, immature *Setaria* worms or microfilariae may enter into blood circulation and may, in rare occasions, migrate erratically into the anterior chamber of the eye, abdominal cavity, pleural cavity, diaphragm, spinal cord, and brain where these worms or microfilariae can cause an inflammatory reaction (Rao 1941; Pelligrini et al. 1980; Shin et al. 2002; Tung et al. 2003; Nakano et al. 2007). Microfilariae of *Setaria* spp. and those of other filarial species, such as *Deraiophoronema evansi* (syn. *Dipetalonema evansi*), can also penetrate the placenta and migrate into the fetus, resulting in congenital infection (Fujii et al. 1995; Kim et al. 2010; Schuster et al. 2019). In this study, we found *S. labiatopapillosa* adult worms free in the thoracic cavity of a 3.5-year-old Moroccan bull. Other authors have found several worms embedded in the visceral walls of the pelvic peritoneum where inflammation occurred around the areas of worm attachment (Sundar and D'Souza 2015). Hemorrhagic inflammation was also observed in the peritoneal wall and reported in bulls. These findings are in accordance with earlier reports suggesting that these worms can also cause inflammatory reactions in their normal sites (Sarwar 1945; Chauhan and Pande 1980).

All morphological features of the anterior and posterior parts of *S. labiatopapillosa* encountered in the present study were in agreement with earlier description (Shoho and Uni 1977; Rhee et al. 1994; Nakano et al. 2007; Kim et al. 2010; Sundar and D'Souza 2015). Both nematodes were classified as fully developed female adults by using the criteria of the structure of the lateral lip, dorsal or ventral projection, tail, terminus, and embryonated eggs in the uterus. Most of the morphological findings of *S. labiatopapillosa* adult female worms and filarioids under SEM observed in our study confirmed earlier findings (Shoho and Uni 1977). Furthermore, our study on microfilariae (L1) of *S. labiatopapillosa* with SEM has shown a succession of horizontal bands limited by

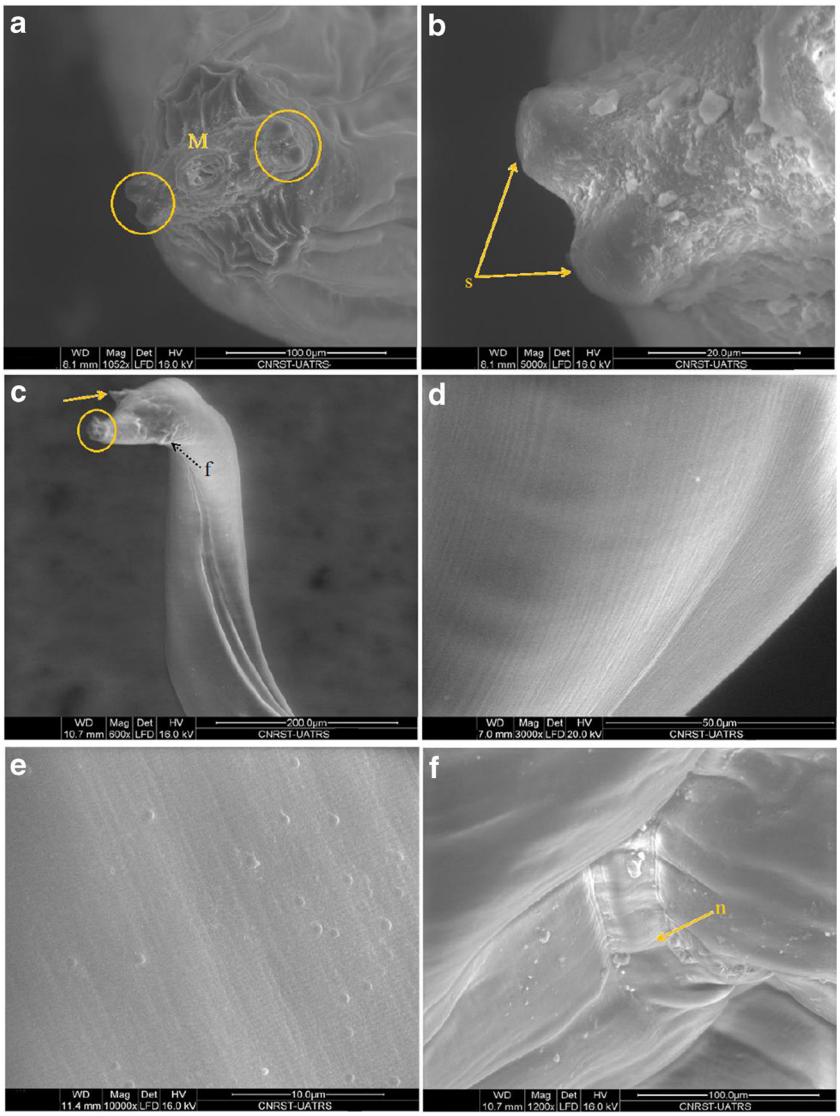

Fig. 3 Scanning electron microscopy findings of adult female *S. labiatopapillosa*. (A) SEM photograph showing the oval mouth opening (M) flanked by lateral lips with non-furcated projection; dorsal and ventral projections (circle) of female *S. labiatopapillosa*. (B) A dorsal projection represented by two summits (s). (C) Photograph showing a pair of lateral appendages (arrow) and the phasmididal pore (f); an obtusely ending tail with a botryoidal with spiny projections (circle). (D) SEM photograph showing the very fine longitudinal striations on the cuticle of *S. labiatopapillosa*. (E) SEM photograph showing the irregularly arranged tubercles on the cuticle of posterior end of *S. labiatopapillosa*. (F) A collar (necklace) was seen next to the front (n)

transverse striations along the entire length of microfilariae, similar to transverse annulation in microfilariae of *Onchocerca jakutensis* and *O. fasciata* (Bosch et al. 2016; Ghahvei et al. 2020).

Except for few "old" studies (Rao 1941; Sarwar 1946), cattle have been found to be less affected by *S. labiatopapillosa* in India. However, in a more recent Indian study, it was reported that close to 20% of the cattle

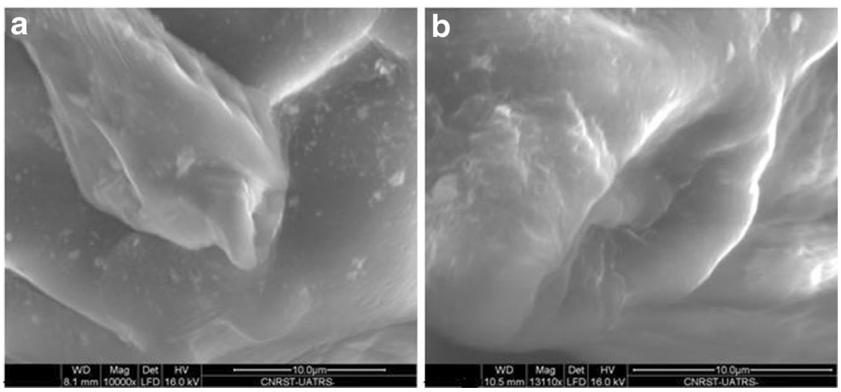

Fig. 4 Deirid and amphid of female *S. labiatopapillosa*. (A) Deirid with thorny formation, the tip of which is somewhat widened, observed in adult female *S. labiatopapillosa*. (B) The size of amphid of adult female *S. labiatopapillosa* was 16.32 μm × 5.79 μm

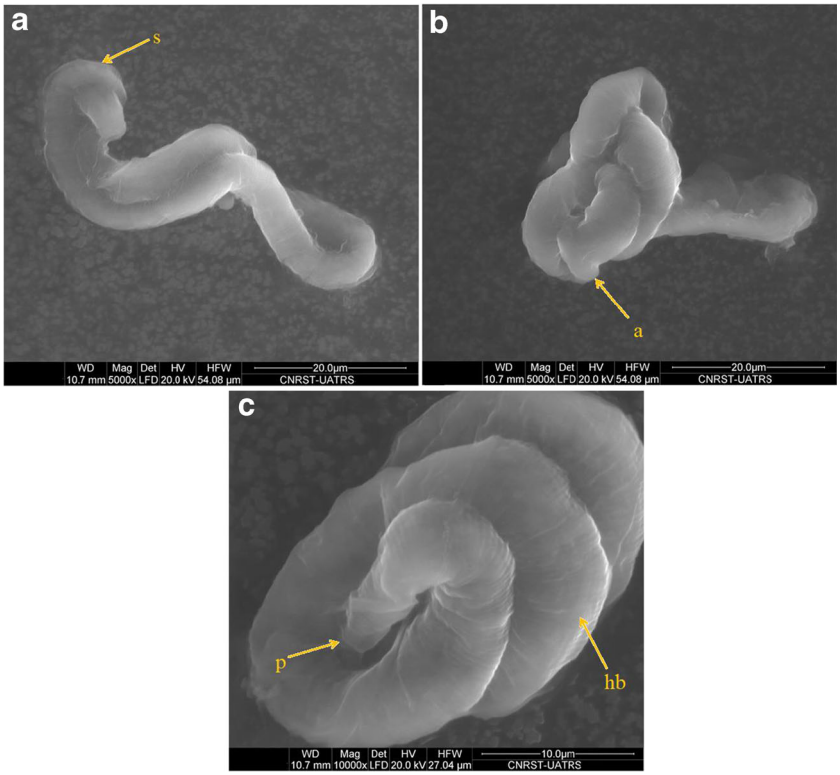

Fig. 5 SEM of microfilariae of *S. labiatopapillosa*. (A) The observations of microfilariae with SEM bearing sheathed (s) microfilariae (L1). (B) A round anterior end of microfilariae (a). (C) A pointed posterior end of microfilariae (p) and a succession of horizontal bands (hb) were seen along the entire length of microfilariae

were infected with this species (Sundar and D'Souza 2015). Reports from other countries indicated a higher infection rate: 75% in Australia (Green and Trueman 1971), 50% in France (Brengues and Gidel 1972), and 58.6% in Nigeria (Ogbogu et al. 1990). In the USA and Canada, *S. labiatopapillosa* was also reported to be the most common *Setaria* species in cattle (Becklund and Walker 1969). By contrast, only 2% (1 of 50) of the worms collected from the abdominal cavities of a cow in Japan were *S. labiatopapillosa* (Nakano et al. 2007). On the African continent, *S. labiatopapillosa* in cattle was reported from Senegal, The Gambia, Burkina Faso, Nigeria, Chad, and Kenya (McFadzean 1955; Nelson 1962; Brengues and Gidel 1972; Gidel and Brengues 1972; Shoho 1976b; Gueye et al. 1989; Ogbogu et al. 1990; Ndao et al. 1995).

In our study, *S. labiatopapillosa* was found in a bull that was born and raised in northern Morocco. Despite several visits to the slaughterhouse where the first case of *S. labiatopapillosa* was observed in Morocco in 2011, no other cow was found to be infected. One possibility that may explain this apparently isolated occurrence of setariosis in Morocco is transplacental infection, which was reported in some filarial species, including *Setaria* spp. (Fujii et al. 1995; Kim et al. 2010; Schuster et al. 2019). Microfilariae can penetrate the placenta, enter into the blood circulation of the fetus, and develop into adult worm. Adult *S. marshalli* parasites and microfilariae were detected in peritoneal cavities and in the blood of bovine fetuses, respectively, even though they were not detected in any of the mother cows (Fujii et al. 1995). Based on their observation that *S. marshalli* was not detected in cattle older than 2 years, these authors have hypothesized that while prenatal infection is common, post-natal infection of cattle occurs rarely.

At present, the origin of *S. labiatopapillosa* found in our study is unknown, and there is no evidence that *Setaria* spp. have invaded Morocco. However, further epizootiological surveillance and entomological studies are required to determine the incidence of setariasis in Moroccan cattle and aberrant animal hosts and identify the potential mosquito vectors in the country. The important implications of *Setaria* spp. for health of domesticated animals and their economic impact on the livestock and cattle industry should no longer be neglected.

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Authors' contributions This work is part of PhD thesis of RM under the guidance of his thesis director KEK. RM, KEK, and DB conceived and designed the experiments. RM performed the experiments. RM, MAL, KEK, and DB analyzed the data. RM, DB, and LB wrote the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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