# Congenital infection with *Setaria digitata* and *Setaria marshalli* in the thoracic cavity of a Korean calf: a case report

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**ABSTRACT**: In March 2010, a 3.5-month-old Korean native calf was anatomized and two nematode worms were detected in the thoracic cavity. The worms were identified and classified by light and scanning electron microscopy on the basis of features at the anterior and posterior parts of the worms. The worms were female *Setaria digitata* and *Setaria marshalli* and numerous eggs which contained microfilaria were detected in the uterus of both species. The body lengths of the *S. digitata* and *S. marshalli* were 78 mm and 117 mm, respectively. Mosquitoes act as the vector for *Setaria* nematodes but these are inactivated in winter in Korea. Therefore, we concluded that this case represented setariasis with congenital infection occurring during the summer prenatal stage of life.

**Keywords**: *Setaria digitata*; *Setaria marshalli*; Korean native calf; congenital infection

*Setaria* species (Filarioidea, Nematoda) are commonly found in the abdominal cavities of ungulates. *S. digitata, S. marshalli* and *S. labiatopapillosa* are common nematodes found in cattle in the Far East and Asia (Rhee et al., 1994; Tung et al., 2003; Nakano et al., 2007; Bazargani et al., 2008). Adult worms are generally considered to be non-pathogenic although they may cause a mild fibrinous peritonitis. However, the infectious larvae, which migrate erratically into the central nervous system of abnormal hosts such as sheep, goats and horses, can cause a serious and often fatal neuropathological disorder commonly known as epizootic cerebrospinal setariosis or lumbar paralysis (Soulsby, 1982). Prenatal infection has been recorded for a number of *Setaria* species (Fujii et al., 1995; Wee et al., 1996).

In Korea, Kim et al. (1968) reported an infection rate of 5% with *S. digitata* at an abattoir in Cheju and a later study described 57% of cattle infected with *S. digitata* (Paick et al., 1976). Rhee et al. (1994) reported that 25.1% of cattle were infected with *S. digitata* and 2.9% with *S. marshalli* at an abattoir in Chonju. Wee et al. (1996) reported three cases of prenatal infection with *S. marshalli*. *S. labiatopapillosa*, meanwhile, has not been recorded in Korea. In the present study, congenital infection with *S. digitata* and *S. marshalli* is described and morphological characteristics of the nematodes are presented in detail.

## Case description

In March 2010, a 3.5-month-old Korean native calf was dissected and two nematode worms were detected in the thoracic cavity (Figure 1-1). The worms were tentatively identified under the light

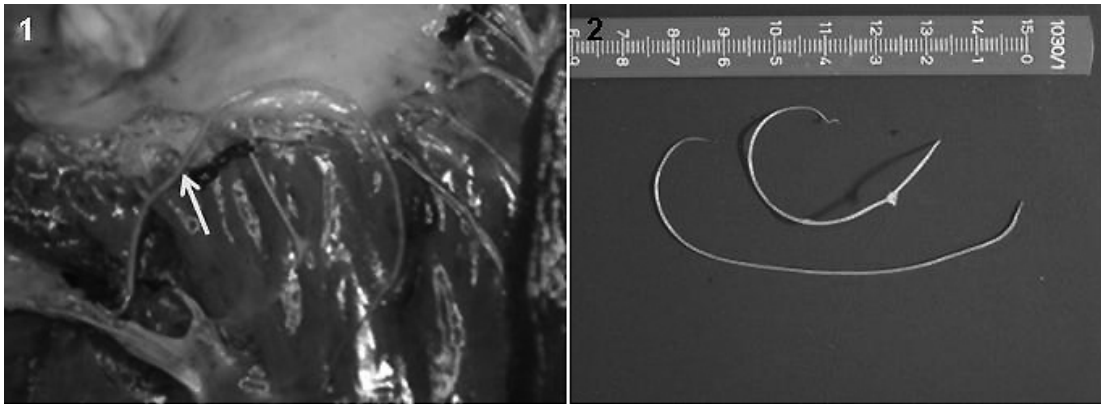


Figure 1. Gross finding of *Setaria*. 1 = *S. marshalli* found on the heart of calve (arrow). 2 = female adultworm of *S. digitata* (upper) and *S. marshalli*

microscope, before precise classification using a scanning electron microscope (SEM). For light microscopy, the worms were placed in the lactophenol solution (glycerin 20 ml, lactic acid 10 ml, phenol 10 ml, D.W. 10 ml) for 24 h. For the SEM, the parasites were washed five times with 0.2M cacodylate buffer (pH 7.3), fixed in 2.5% glutaldehyde and post fixed in 1% osmium tetroxide at 4°C. The specimens were dehydrated in a graded ethyl alcohol series, dried by $CO_2$ critical point, coated with osmium and examined by SEM (S-4800, Hitachi) at 15kV.

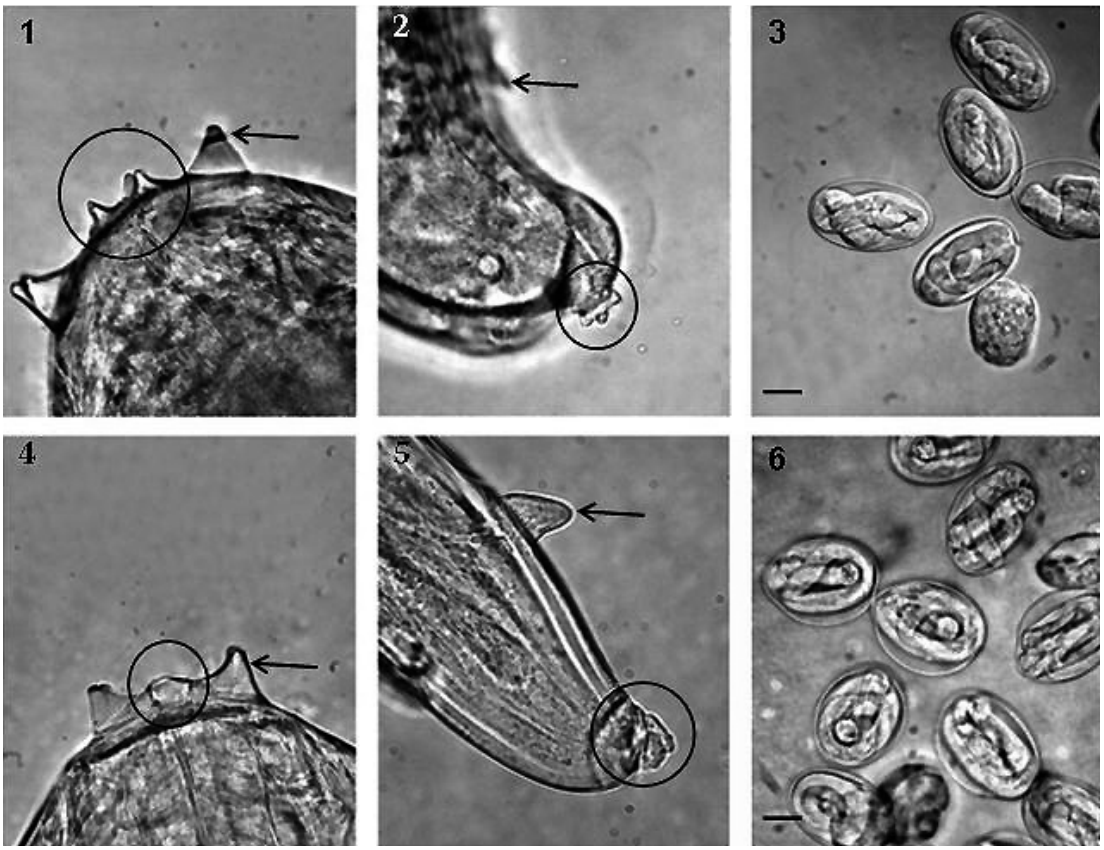


Figure 2. Light microscopy findings of female *S. marshalli* (1, 2, 3) and *S. digitata* (4, 5, 6). 1, 4 = lateral view of the head region; lateral lips (circle). 2, 5 = latero-ventral view of posterior region; lateral appendage (arrow); terminal end (circle). 3, 6 = eggs from uterus. Scale bar: 20 μm

The body lengths of *S. digitata* and *S. marshalli* were 78 and 117 mm respectively (Figure 1-2). Light microscopy revealed that the uteri of both species were filled with numerous fully developed eggs and eggs in different developmental stages. Microfilarias were detected in the fully developed eggs (Figures 2-3 and 2-6). The sizes of eggs were measured as 37.5–45.0 × 20.10–32.5 (mean 40.13 × 27.25) in *S. digitata* and 40.0–49.0 × 27.5–30.0 (mean 46.40 × 28.95) in *S. marshalli*. The structure of the dorsal and ventral projections on the peribuccal crown was clearly visible from the side view. The distance between the dorsal and ventral projection of *S. digitata* was narrow but this same distance was wider in *S. marshalli*. The female *S. marshalli* had bifid lateral lips, whereas the female *S. digitata* did not (Figures 2-1 and 2-4). A pair of lateral appendages were found at the upper terminal end in both species. The appendages of *S. digitata* were bigger than those of *S. marshalli*. The

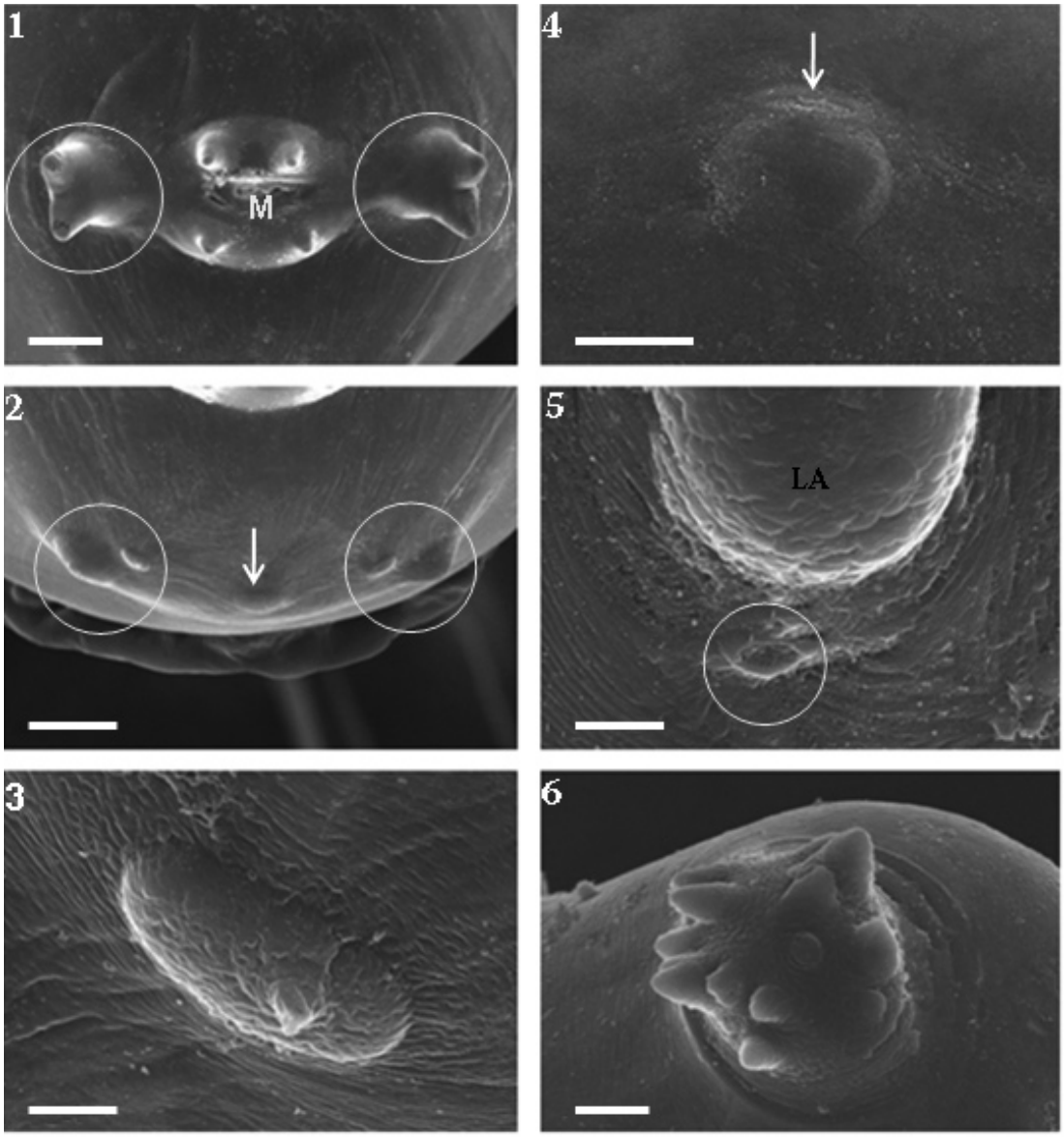


Figure 3. SEM finding of female *S. marshalli*. 1 = the slit-like mouth opening (M) flanked by lateral lips with bifurcated projection; the bifurcated ventral projections are situated ventrally (circle); scale bar: 20 µm. 2 = an amphid (arrow) located between the two pairs of submedian papillae (circle); scale bar: 20 µm. 3 = the smaller of the submedian papillae; scale bar: 2 µm. 4 = the hilly elevated amphid with is pore (arrow) at the lower bottom; scale bar: 9 µm. 5 = the plasmidal pore situated in front of the lateral appendage (LA); scale bar: 2 µm. 6 = the tail ending with the roughly furcated terminus; scale bar: 6 µm

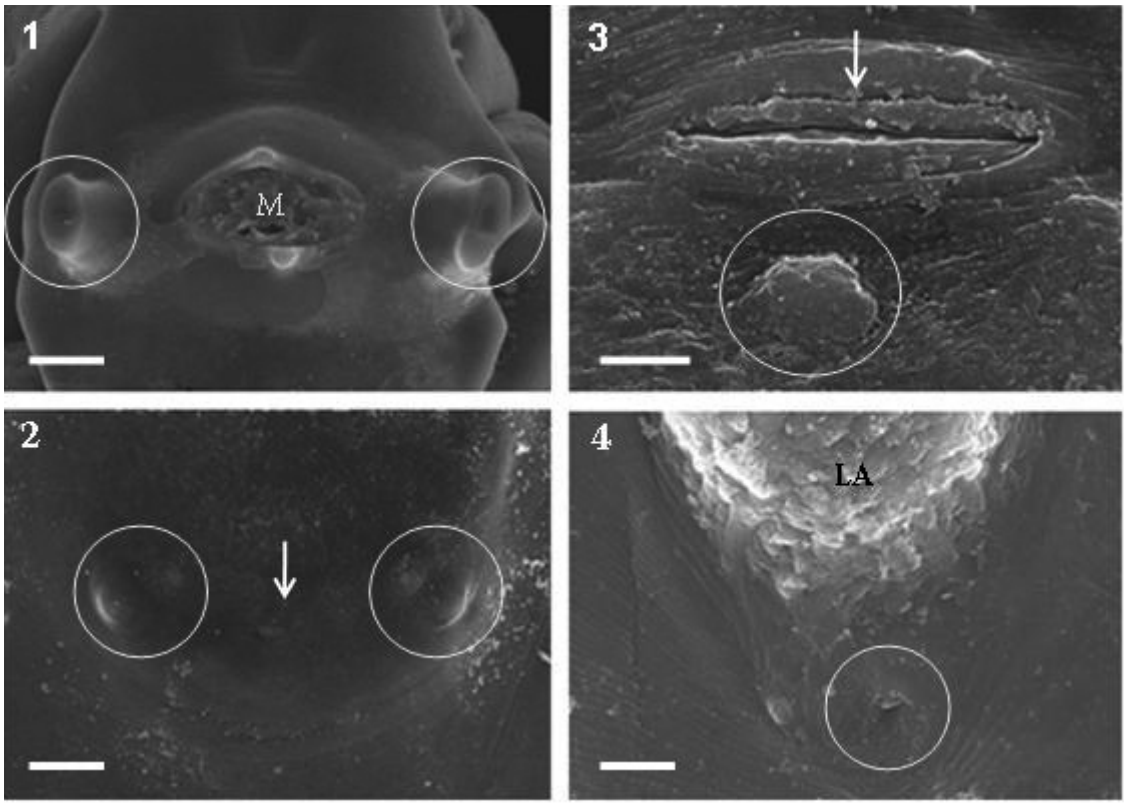


Figure 4. SEM finding of female *S. digitata*. 1 = the ovally mouth opening (M) flanked by lateral lips with nonfurcated projection; the slite bifurcated ventral projections are situated ventrally (circle); scale bar: 10 μm. 2 = an amphid (arrow) located between the two pairs of submedian papillae (circle); scale bar: 14 μm. 3 = the elevated amphid with its pore (arrow) at the lower bottom; scale bar: 1 μm. 4 = the plasmidal pore situated in front of the lateral appendage (LA); scale bar: 2.5 μm

posterior end was characterized by a tapering at the terminal end with a smooth knob in *S. digitata* and a blunted terminal end that was roughly furcated in *S. marshalli* (Figures 2-2 and 2-5).

SEM confirmed the characteristic feature of the peribuccal crown at the mouth opening in each species (Figures 3-1, 4-1). The mouth opening in *S. marshalli* was a dorsoventral slit-like form but was a long oval shape in *S. digitata*. The lateral lips were represented by one and two summits, respectively, of the mouth opening on both dorsal sides in *S. digitata* and *S. marshalli*. The position of the amphid and two pairs of submedian papillae were observed at different levels. The amphids were laterally situated, elevated and had a dorsoventral amphidal pore at the lower bottom (Figures 3-2,3,4 and 4-2,3).

A pair of lateral appendages were seen in both species with the phasmididal pore situated at the upper armpit (Figures 3-5, 4-4). The posterior end of *S. digitata* was a tapering terminus with a smooth knob. The *S. marshalli* had an obtusely ending tail with a botryoidal with many spiny projections as shown by light microscopy (Figure 3-6).

## DISCUSSION AND CONCLUSIONS

In bovids, a relatively high prevalence of *S. digitata* has been reported in Asia (Wang et al., 1990; Rhee et al., 1994; Nakano et al., 2007; Bazargani et al., 2008). In Korea, prevalence rates of *S. digitata* and *S. marshalli* have been reported and *S. digitata* was the dominant species in cattle (Rhee et al., 1994).

In this study, two parasites from the thoracic cavity of a 3.5 month old calf were morphologically identified as *S. digitata* and *S. marshalli*. In general, adults of *Setaria* spp. can be found in the peritoneal cavity. They display erratic parasitism, mainly in the pleural cavity, eye and brain spinal cord (Solusby, 1982; Shin et al., 2002; Tung et al., 2003). However, these worms were found in the thoracic cavity and

not in the peritoneal cavity. Both were classified as fully developed female adults by LM and SEM using the criteria of the structure of the lateral lip, dorsal or ventral projection, tail, terminus and embrionated eggs in the uterus. The amphid, deirid, postdeirid and phasmidal pores were typical of those reported for *S. digitata* and *S. marshalli* (Shoho and Uni, 1977; Rhee et al., 1994). An additional method of identification was the length of the worms. In our study, the length of *S. marshalli* was longer than that of *S. digitata*.

Many species of *Setaria* show no microfilarial periodicity and only low numbers of microfilariae are present in the blood. The microfilariae are taken from the blood of the infected animal by their vector, mosquitoes. The development of infective larvae occurs in the thoracic muscles of mosquitoes such as *Aedes, Culex,* and *Anopheles* spp. (Soulsby, 1982). In horses and deer the prepatent period is 7–10 months and that of *S. marshalli* is three months (Niimi et al., 1941; Soulsby, 1982).

Previous reports have documented prenatal infection with *Setaria* spp. Ishihara and Ogata (1953) observed *S. marshalli* in the peritoneal cavity of calves less than three months old, and concluded that this represented prenatal infection because of the three month prepatent period in *S. marshalli*. In a full year's epidemiological study in Japan, Fujii et al. (1995) reported that *S. marshalli* adult worms were detected in the peritoneal cavity of six bovine foetuses of 7–9 months of age during the months of October to December, but not in foetuses of any age during the January-September period. It is speculated that prenatal infection with *S. marshalli* is most common because it has not been detected in cattle older than two years and postnatal infection is rather uncommon. Wee et al. (1996) reported the prenatal infection of three neonatal calves with *S. marshalli*. Immature *Setaria* forms or those in the larval stage are capable of penetrating the placenta and migrating into the foetus, where the nematodes can complete their development. It has been believed that prenatal infection is the most common type of infection by *S. marshalli* (Kitano et al., 1994). Congenital *S. marshalli* infection has been found in calves (Kitano et al., 1994; Wee et al., 1996) and bovine foetuses (Fujii et al., 1995). However, reports on *S. digitata* prenatal infection are scant. *S. digitata* was reported in an 8-month-old bovine foetus from China (Mo et al., 1983). In a 31-day-old black-tailed deer fawn, an immature female of *S. yehi* was found in the body cavity during winter (Weinmann and Shoho, 1975). They concluded that the prenatal infection of *S. yehi* develops during the worm summer season.

In our study, two adult *S. digitata* and *S. marshalli* females were found in the thoracic cavity of a 3.5 month old calf in March 2010. There was no evidence of congenital infection, but a simple explanation may be that filarial infections occur in the summer season when mosquitoes are active. Transmission of *Setaria* to the foetus during the pregnancy of cattle and to the host or to calves during late autumn and winter is not possible in Korea because of the lack of mosquito vectors on the field. The prepatent periods of *Setaria* spp. are over three months. It is concluded, therefore, that *S. digitata* and *S. marshalli* congenital infections develop during the foetal stages in summer.

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