

## Application of scanning electron microscopy in assessing the prevalence of some *Setaria* species in Korean cattle

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**Abstract:** The numbers of individual *Setaria* species in the peritoneal cavities of Korean cattle were estimated. The worms were tentatively identified under light microscopy, and then precisely classified by SEM on the basis of unique features at the anterior and posterior ends of the adult worms. The positive rate of *Setaria* species was 34.2% out of 1,074 Korean cattle surveyed; that of *S. digitata* was 25.1%, *S. marshalli* 2.9% and both species 6.2%, respectively. Out of a total of 1,254 worms collected, 66.8% were female and 19.1% were male *S. digitata*; 9.2% were female and 5.0% were male *S. marshalli*, respectively. The average length of *S. marshalli* was somewhat longer than that of *S. digitata*. *S. marshalli* was documented for the first time in Korea.

**Key words:** *Setaria digitata*, *Setaria marshalli*, ultrastructure, prevalence, Korean cattle

### INTRODUCTION

*Setaria* species are commonly found in the peritoneal cavity of ungulates and some *Setaria* spp. including *S. digitata*, *S. marshalli* and *S. labiatopapillosa* are very common nematodes of cattle in the Far East and Asia. Adults in the peritoneal cavity are non-pathogenic although they may cause a mild fibrinous peritonitis. The major pathogenic effect of *Setaria digitata* occurs when the immature forms migrate erratically into the central nervous system of abnormal hosts such as sheep, goats and horses (Innes and Shoho, 1952 & 1953). Light microscopy is commonly used to identify these parasites but the lack of specific distinguishing characteristics among *Setaria* spp. recognizable under light microscopy sometimes leads to misclassification. In Korea, there are no

reports on the prevalence of *Setaria* spp., with the exception of *S. digitata* (Kim *et al.*, 1968; Paick *et al.*, 1976). In addition, the ultrastructure of the worm has not been well investigated.

The purpose of the present study is to utilize scanning electron microscopy (SEM) in accurate investigation of the prevalence and classification of *Setaria* spp. from 1992 to 1993 in Korean cattle.

### MATERIALS AND METHODS

*Setaria* spp. tend to sequester into the peritoneal tissue of the host. Accordingly, as many parasites as possible were collected depending on visual inspection of the peritoneal cavity. In all, 1,074 Korean cattle aged at least 2 years were sampled at the Chonju abattoir from June, 1992 to October, 1993.

The worms were tentatively identified by light microscopy, and then a lot of worms were precisely classified by SEM. For SEM, the

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worms were washed thoroughly in PBS (pH 7.4) to free them from extraneous material. The worms were then processed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated with ethyl alcohol, followed by isoamyl acetate, then dried with liquid CO<sub>2</sub> in a critical point apparatus and finally coated with gold in an ion bombardment apparatus. These preparations were examined with a JEOL JSM-T330A scanning electron microscope, operated at 15-20 kV.

## RESULTS

### Light Microscopical Finding

**The Anterior Part:** The peribuccal crown bore a dorsal and a ventral projections, and the lateral lips were adjacent to the buccal aperture. The structure of the dorso- and ventro-projections of the peribuccal crown was clearly visible from the side view by light microscopy. Both male and female *S. digitata* had round lateral lips (Figs. 1 and 2), the female *S. marshalli* had bifid lateral lips (Fig. 3), whereas the male *S. marshalli* had not-bifid

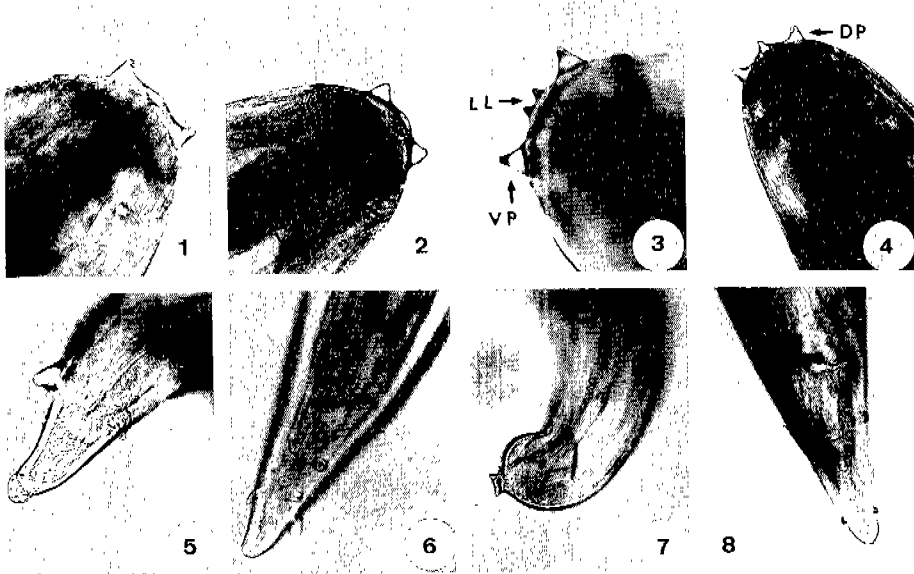
projecting lateral lips (Fig. 4). The other fine structures of *Setaria* spp. were not distinctly visible by light microscopy.

**The Posterior Part:** A pair of the lateral appendages of *Setaria* worms was found at the upper terminal end. The posterior end of the females was characterized by a tapering terminal end with a smooth knob in the case of *S. digitata* (Fig. 5) and a blunted terminal end that was roughly furcated in the case of *S. marshalli* (Fig. 7). Males had clear lateral appendages with a smooth knob or the roughly furcated terminus which are indistinct (Figs. 6 and 8).

### Scanning Electron Microscopical Finding

The fine structure of *Setaria* spp. by SEM enabled us to affirm classification for each worm.

**The Anterior Part:** The view *en face* of the head confirmed the characteristic feature of the mouth opening surrounded by the peribuccal crown for each species examined (Figs. 9 and 10 for *S. digitata* and Figs. 11 and



**Figs. 1-8.** Light micrographs showing fresh specimens of *Setaria* spp. (Figs. 1-4 for the lateral view of the head, Figs. 5 and 7 for the lateral view of the posterior end, and Figs. 6 and 8 for the ventral view of the posterior end). Magnification on all is 400 times. **Fig. 1.** Female *Setaria digitata*. **Fig. 2.** Male *S. digitata*. **Fig. 3.** Female *S. marshalli*. **Fig. 4.** Male *S. marshalli*. **Fig. 5.** Female *S. digitata*. **Fig. 6.** A pair of the large lateral appendages is located at a long distance from the tail end of male *S. digitata*. **Fig. 7.** Female *S. marshalli*. **Fig. 8.** A pair of the small lateral appendages is visible at a short distance from the posterior end of male *S. marshalli*. **Abbreviations:** DP, dorsal projection; VP, ventral projection; LL, lateral lip

12 for *S. marshalli*). The head of both male and female *S. digitata* showed uniform roundness of the buccal opening and somewhat depressed dorso- and ventro-projections of the peribuccal crown at the tips (Figs. 9 and 10). In *S. marshalli* there was a long dorsoventral oval (slit-like) buccal opening and the lateral lips were non-indented in the male and twice indented with two summits of the buccal opening on both sides in the female. The dorso- and ventro projections of the peribuccal crown also were distinctly notched in the female (Figs. 11 and 12). The position of an amphid and of two pairs of submedian papillae at different levels was clearly seen.

The amphids were laterally situated to the peribuccal crown at the convex curvature of each shoulder and were recognizable as small papillary formations under low light microscopy. They were present in all *Setaria* spp. and detailed ultrastructures were revealed by SEM as hilly elevation covered with the cuticle of characteristic mosaic and having a dorsoventrally elongated amphidial pore at the lower bottom end.

The deirids, cervical papillae, situated close to the lateral line at the level between the nerve ring and the end of the anterior oesophagus, and usually not located directly opposite one another as visualized by light microscopy, were now visualized in finer detail by SEM as a small cap-like formation situated closely along the lateral line at the dorsal side and surrounded by the fine vertical striations of the cuticle at the basement (Fig. 13).

**The Posterior Part:** The lateral appendages were paired in both sexes, with the phasmids situated just anterior to them. The phasmidial pores of *Setaria* spp. were usually unrecognizable by light microscopy, but were visible by SEM in both male and female worms, and were distinctly located at the upper junction of the lateral appendages (marked with arrow at Fig. 14).

The general arrangements of the sexual papillae of the male were distinct from the ventral view. *S. digitata* showed clear papillary arrangements; 3 pairs of the precloacal, a pair of the adcloacal, 3 pairs of the postcloacal papillae (of which the first pair was longitudinally arranged) and a central papilla

just in front of the cloaca (Fig. 15). In contrast, *S. marshalli* showed characteristic papillary arrangement; 4 pairs of the precloacal papillae, a central papilla and 3 pairs of the postcloacal papillae with 3 lateral papillae. The last one of these 3 pairs formed the lateral appendages (Fig. 16).

The postdeirid unilaterally located on the left side at about the level of the posterior to the retracted position of the left spicule was recognizable in similar formation to the deirid by light microscopy. Its detailed fine structure was best revealed by SEM. It was so distinct that the cuticular vertical striations could be seen to converge at the base of the postdeirid, while they encircle the deirid (Figs. 13 and 19).

The central papilla (marked with arrow) just anterior to the cloaca was dorsoventrally elongated in both species, appearing as a small papilla by light microscopy (Figs. 15 and 16). The ventral bands, lugae, were shown to be composed of fine longitudinal microstriations among males (Fig. 20).

The posterior end of the female was equipped with a knob at the case of *S. digitata* (Fig. 17) and roughly furcated in *S. marshalli* (Fig. 18) as shown by light microscopy (Figs. 5 and 7 for *S. digitata* and *S. marshalli*).

**Length of *Setaria* spp.:** The mean length and standard deviation of the worms detected, based on 50 determinations, are shown Table 1. The females on the average were almost twice as long as their corresponding males. The average length of *S. marshalli* was somewhat longer than that of *S. digitata*.

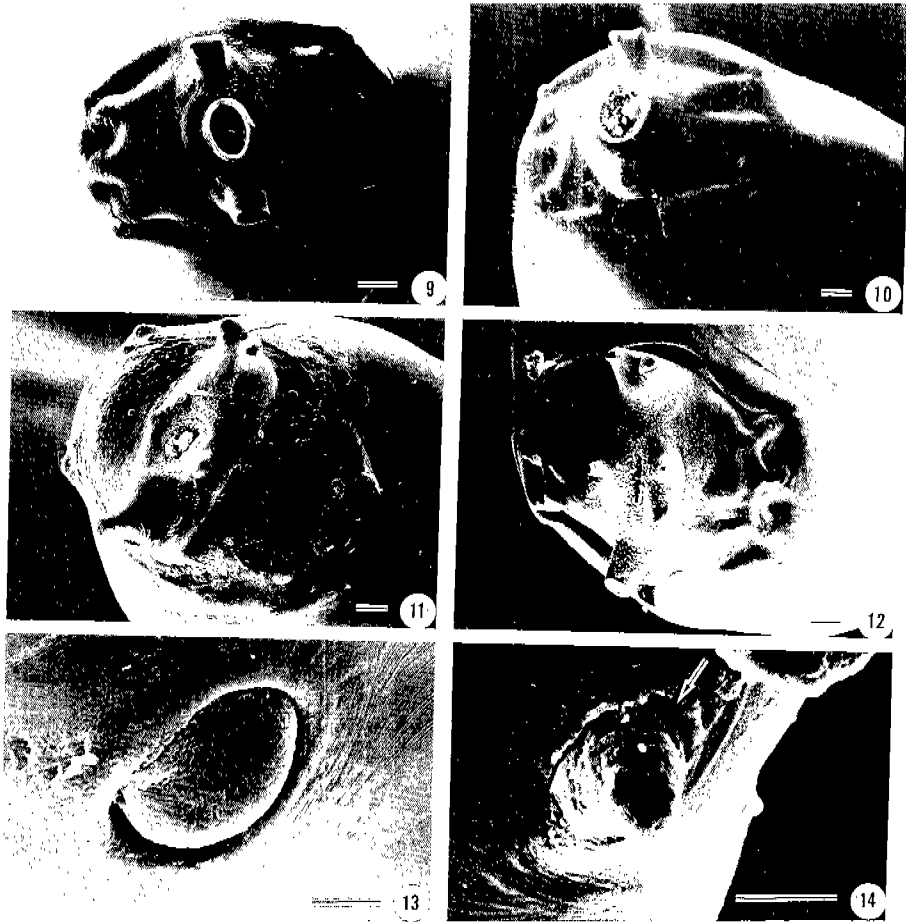
**Prevalence of *Setaria* spp.**

Of 1,074 Korean cattle sampled, 367 (34.2%) were positive for *Setaria* spp., of which 269 (25.1%) were confirmed to be *S. digitata*, 31 (2.9%) *S. marshalli* and 67 (6.2%) were positive

**Table 1.** Comparison of the lengths ( $n = 50$ ) of *Setaria* spp. in relation to sex

Species	Male ( $\mu\text{m}$ )		Female ( $\mu\text{m}$ )	
	Mean	SD	Mean	SD
<i>S. digitata</i>	4769.30	389.62	7368.09*	1229.39
<i>S. marshalli</i>	5068.56	261.07	9150.00*	1449.59

\* $P < 0.05$  (SAS t-test) compared with the values for males.



**Figs. 9-20.** Scanning electron micrographs showing different organs of *Setaria* spp. (Figs. 9-12 for the view *en face* of the head) **Fig. 9.** Male *Setaria digitata*, bar = 10  $\mu$ m. **Fig. 10.** Female *S. digitata*, bar = 10  $\mu$ m. **Fig. 11.** Male *S. marshalli*, bar = 10  $\mu$ m. **Fig. 12.** Female *S. marshalli*, bar = 10  $\mu$ m. **Fig. 13.** The view *en face* of a deirid of female *S. digitata*, bar = 5  $\mu$ m. **Fig. 14.** The lateral appendage enlarged and the phasmidial pore (marked with arrow) are distinctly visible for female *S. marshalli*, bar = 5  $\mu$ m.

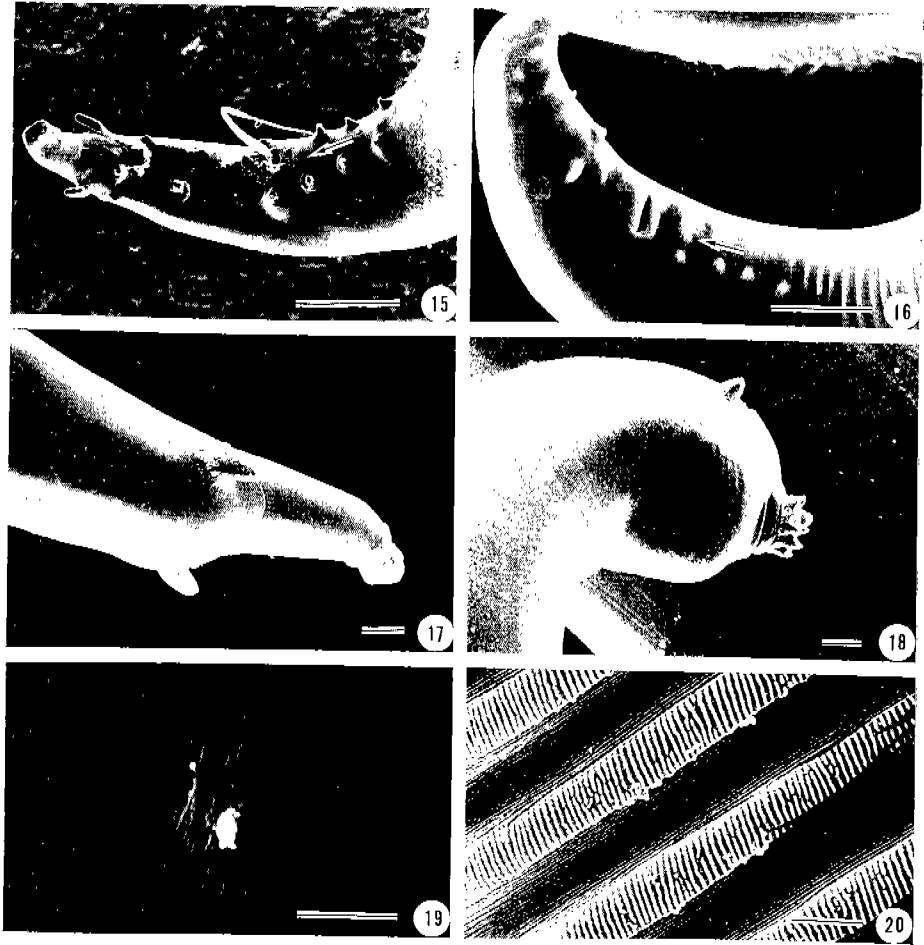
for both species. In analyzing 1,254 worms isolated from this survey, 239 (19.1%) and 837 (66.8%) were male and female *S. digitata*, and 63 (5.0%) and 115 (9.2%) were male and female *S. marshalli*, respectively.

#### DISCUSSION

A number of species of the genus *Setaria* have been reported from unguates (Baylis, 1936; Becklund and Walker, 1969; Shoho, 1976) and identification of *Setaria* spp. by light microscopy often led to misidentification of the species. The SEM has the advantage of high resolution of the ultrastructure of *Setaria* spp., thereby facilitating definitive species

identification as briefly discussed in literature (Shoho and Uni, 1977; Almeida *et al.*, 1991).

In this investigation, *S. digitata* and *S. marshalli* were precisely classified by SEM using the criteria of the structure of the lateral lip, dorsal or ventral projection, tail and terminus of the female and the numbers and arrangement of sexual papilla of the male of the adult worms. The length of the worms was an additional key to the identification of the species of *Setaria*. In addition, the amphid, the deirid, the postdeirid, the ventral band and the phasmidial pore were typical of *Setaria* species, confirming previously published reports (Shoho and Uni, 1977; Almeida *et al.*, 1991). These data present the first attempt to use SEM as a



**Fig. 15.** The ventral view of the posterior end of male *S. digitata*, bar = 50  $\mu$ m. **Fig. 16.** The ventral view of the posterior end of male *S. marshalli*, bar = 50  $\mu$ m. **Fig. 17.** The tapering terminal end with a smooth knob for female *S. digitata*, bar = 10  $\mu$ m. **Fig. 18.** The obtusely ending tail with the roughly furcated terminus for female *S. marshalli*, bar = 10  $\mu$ m. **Fig. 19.** The postdeirid, at the base of which cuticular vertical striations intermit, with the furcated needle-like formation for female *S. digitata*, bar = 5  $\mu$ m. **Fig. 20.** The ventral bands of the tail of male *S. marshalli*, bar = 5  $\mu$ m.

criterion for classification and prevalence survey of *Setaria* spp. in Korean cattle.

It is suggested that infection rate of *Setaria* spp., in fact, may be much higher since the investigation period was limited to the short slaughtering time. Of special interest, is the fact that since the last report (Paick *et al.* 1976), approximately 18 years ago, the prevalence in the same district seems to have dropped from 47% at that time to less than 35% as reported in our current survey. The factors contributing to this decline are not known but one could speculate that the changes are partly attributable to improved

animal husbandry techniques in a changing agricultural environment in Korea. The validity of this hypothesis needs more stringent epidemiological testing.

Although the life cycle of *S. marshalli*, which has until now been an unrecorded species in Korea, involves transmission by an intermediate host (mosquito) and transplacental passage in the definitive host, 178 (14.2%) out of all the worms collected were found in animals in the age group of 2 years or older. Accordingly, the significance of this parasite in particular age groups warrants more attention than is currently given in this country.

### ACKNOWLEDGEMENTS

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=국문초록=

### 주사전자현미경을 적용한 한우의 *Setaria* spp. 감염상황

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1992년 6월부터 1993년 10월까지 전주도축장에 출하되는 2세 이상의 한우 1,074두의 복강으로부터 *Setaria*속 선충류 2종을 검출하여 그 형태를 관찰, 감염상황을 조사한 결과를 요약하면 다음과 같다. 이들 사상충은 *S. digitata*와 *S. marshalli*이었으며, 성충의 두부와 미부의 형태적 특징을 광학 및 주사전자현미경으로 관찰하여 분류할 수 있었으며 특히, *S. marshalli*는 국내 미기록종이다. 또한, 전북지방 한우의 *Setaria*속 감염율은 34.2%이었으며, 이 중에서 *S. digitata* 25.1%, *S. marshalli* 2.9%, 중복감염 6.2%로 나타났다. 한편, 검출된 *Setaria*의 총체총수는 1,254마리이었으며, 그 중 *S. digitata* 암컷 66.8%, 수컷 19.1%, *S. marshalli* 암컷 9.2%, 수컷 5.0%로 나타났다. 그리고, 그 크기에 있어서 *S. digitata* 암컷  $7368.09 \pm 1229.39 \mu\text{m}$ , 수컷  $4769.30 \pm 389.62 \mu\text{m}$ , *S. marshalli* 암컷  $9150.00 \pm 1449.59 \mu\text{m}$ , 수컷  $5068.56 \pm 261.07 \mu\text{m}$ 이었다.

(기생충학잡지 32(1): 1-6, 1994년 3월)