FULL PAPER *Parasitology*

Cerebrospinal Setariosis with *Setaria marshalli* and *Setaria digitata* infection in Cattle

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ABSTRACT. *Setaria digitata* and *S. marshalli* larvae were observed in the cerebrospinal cavity of 2 paralyzed cattle in Taiwan. The 2 affected cattle showed quadriplegia and lumbar paralysis, respectively. At necropsy, which was performed 7 days after the 7-month-old cattle became quadriplegic, three and nineteen *S. marshalli* larvae as well as two female adult worms were found in the cranial cavity, spinal cavity and peritoneal cavity of the cattle, respectively. Necropsy on the other 8-month-old cattle was also performed 3 days after it showed lumbar paralysis, and ten *S. digitata* larvae were found in the spinal cavity. In both cattle, many mononuclear inflammatory cells mixed with a few eosinophils were seen accumulated in the connective tissue around the root of the spinal nerves. Infiltration of eosinophils and mononuclear inflammatory cells into the epidura and arachnoidea of the brain were also observed. The major inflammatory cell was lymphocytes, but neutrophils and eosinophils were also present. The number of cells in the cerebrospinal fluid collected initially from the two affected cattle were 105/0.01 m*l* and 143/ 0.01 m*l*, respectively. This is the first report of cerebrospinal setariosis in cattle associated with *S. marshalli*.

KEY WORDS: bovine cerebrospinal setariosis, cerebrospinal fluid, *Setaria digitata*, *Setaria marshalli*, Taiwan.

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Setaria species are filarial parasites that are commonly found in the peritoneal cavity of cattle and other ungulates. The parasites are generally considered to be nonpathogenic in their natural hosts, but transmission of infective larvae through mosquito and other arthropod vectors to non-permissive hosts such as goats, sheep, or horses, can result in serious and often fatal neuropathological disorder commonly referred to as cerebrospinal nematodiasis [1, 11]. Moreover, *Setaria cervi* (Rudolphi, 1819) [2], *S. digitata* (Linstow, 1906) [18, 19], *S. labiatopapillosa* (Alessandrini, 1848) [3, 14], *S. marshalli* (Boulenger, 1921) [8, 16, 18, 19], and *S. leichungwingi* (Chen, 1935) [7] had been reported to parasitize in the peritoneal cavity of cattle without producing clinical signs. However, larvae of *S. cervi*, which normally parasitized in the deer peritoneal and thoracic cavities as adult worms, had been found in the cerebrospinal cavity of deer resulting in lumbar paralysis of the host [6, 15, 17]. Our literature search showed that no cases of cerebrospinal setariosis with *S. marshalli* infection in cattle had ever been reported. We report herein the discovery of *S. marshalli* and *S. digitata* larvae from the cerebrospinal cavity of 2 paralyzed cattle in Taichung, central Taiwan. Pathological findings and inflammatory cell counts of cerebrospinal fluid (CSF) of the affected cattle are also presented.

MATERIALS AND METHODS

Case history: In August and September of 2001, a 7-month-old (case 1) and an 8-month-old (case 2) Holstein-Friesian strain cattle were found to have quadriplegia and lumbar paralysis, respectively, in Taichung, central Taiwan. The cattle were injected with xylazine hydrochloride (0.1 mg/ Kg B.W./i.m.) supplemented with ketamine hydrochloride (1 mg/ Kg B.W./i.m.) as anesthesia to obtain their CSF and blood. The CSF in case 1 was collected by lumbar puncture on days 1, 3, and 7 after the onset of quadriplegy. The CSF in case 2 was collected 3 days after the onset of paralysis. Necropsy of cattle in cases 1 and 2 were performed on days 7 and 3 after the onset of clinical signs, respectively. A portion of the brain and spinal cord were collected for histopathological processing and the rest of central nervous system organs examined for the presence of the worms.

Examination of Blood and CSF: Ten μL of CSF was smeared onto a glass slide followed by staining with Giemsa stain. The total number of cells on the slide glass and the different inflammatory cell types were counted. Peripheral blood leukocyte count was done manually by lysing in 0.1 N HCl and then counting the cells in a hemocytometer [4]. Blood smear was stained with Giemsa stain for differential cell count.

Worm measurements and scanning electron microscopy: Filarial worms collected from the cattle were fixed in 10 % phosphate-buffered formalin. Measurement of the worms was carried out using a drawing tube (camera lucida) attached to a light microscope. For scanning electron microscopy, the larvae were fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in an ethyl alcohol series, treated with isoamyl acetate (for replacement of alcohol), dried in liquid CO_2 in a critical point dryer (LADD research industries) and finally coated by gold-sputter in an ion bombardment apparatus (BIO-RAD microsience division SC 502). The prepared specimens were then observed with TOPOCON ABT-150S scanning electron microscope, operating at 15 kV.

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Histopathology: Tissue samples of the paralysed cattle brain and spinal cord were fixed in 10% phosphate-buffered formalin, dehydrated in an alcohol series, embedded in paraffin, and sectioned at 5 μm for histopathological examination. The histological sections were stained with haematoxylin and eosin (H & E) or Luxol Fast Blue-Cresyl Echt Violet (LFB-CEV) stain for identification of the myelin sheath.

RESULTS

Based on the morphological descriptions published previously [10, 15–19], the collected worms were identified as either *S. marshalli* or *S. digitata*.

Morphology of larvae: In case 1, two 4th stage female larvae (2.2 and 2.3 cm in length; 273.0 and 280.8 μm in width; Figs. 1–4) and one 5th stage larvae in the cranial cavity, nineteen 5th stage larvae in the spinal cavity (9 in cervical, 8 in thoracic and 2 in lumbar region.), and two female adult worms in the peritoneal cavity of the cattle were found (Figs. 5–8, 15 & 16). With the exception of the two 4th stage larvae, all the other larvae and adult worms were identified as that of *S. marshalli*. No microfilariae were observed in either the uterus of the female worms or in the peripheral blood of the cattle. The morphometric measurements of the *S. marshalli* larvae collected are shown in Table 1.

The two 4th stage female larvae have a pair of indistinct spinous teeth at the apex of the anterior end of the worm body (Figs. 1 & 3). A pair of lateral caudal appendages and terminal knob could clearly be observed at the posterior end (Figs. 2 & 4). The 5th stage female *S. marshalli* larva was identified by the presence of a pair of distinct bifid lateral lips at the apex (Figs. 5 & 7), whereas the 5th stage male larva has a verticle triangular lateral lip (Fig. 15). The posterior end of the 5th stage female *S. marshalli* larva resembles that of the 4th stage, except for the rough surface knob (Figs. 6 & 8). Fifth stage male *S. marshalli* larva does not possess any clearly defined lateral appendage (Fig. 16).

In case 2, two 4th stage female larvae (2.4 and 2.5 cm in length; 304.2 and 312.0 μm in width; with similar morphological features shown in Figs. 1–4) and eight 5th stage larvae in the spinal cavity (1 in cervical, 7 in thoracic and 2 in lumbar regions) were collected. The 5th stage larvae were identified as that of *S. digitata* (Figs. 9–14). The morphometric measurements of the *S. digitata* larvae collected are shown in Table 2.

The dorsal and ventral bifid projections of the peribuccal crown as well as the round lateral lip in both of the male and female 5th stage larvae of *S. digitata* were clearly visible (Figs. 9, 11 & 13). The posterior end of the 5th stage female *S. digitata* was visible like that of the 5th stage female *S. marshalli* (Figs. 10 & 12). Fifth stage male *S. digitata* larva has a pair of clear lateral appendage (Fig. 14).

Cell count in CSF and in blood of cattle with cerebrospinal setariosis: The total number of inflammatory cell count as well as the differential cell count in the CSF and peripheral blood of both cattle are shown in Table 3. There was an increase in the total number of inflammatory cells (including eosinophils, lymphocytes, neutrophils and monocytes) in the CSF of case 1. In both cases, the major cellular components of the CSF was lymphocytes followed by monocytes and eosinophils.

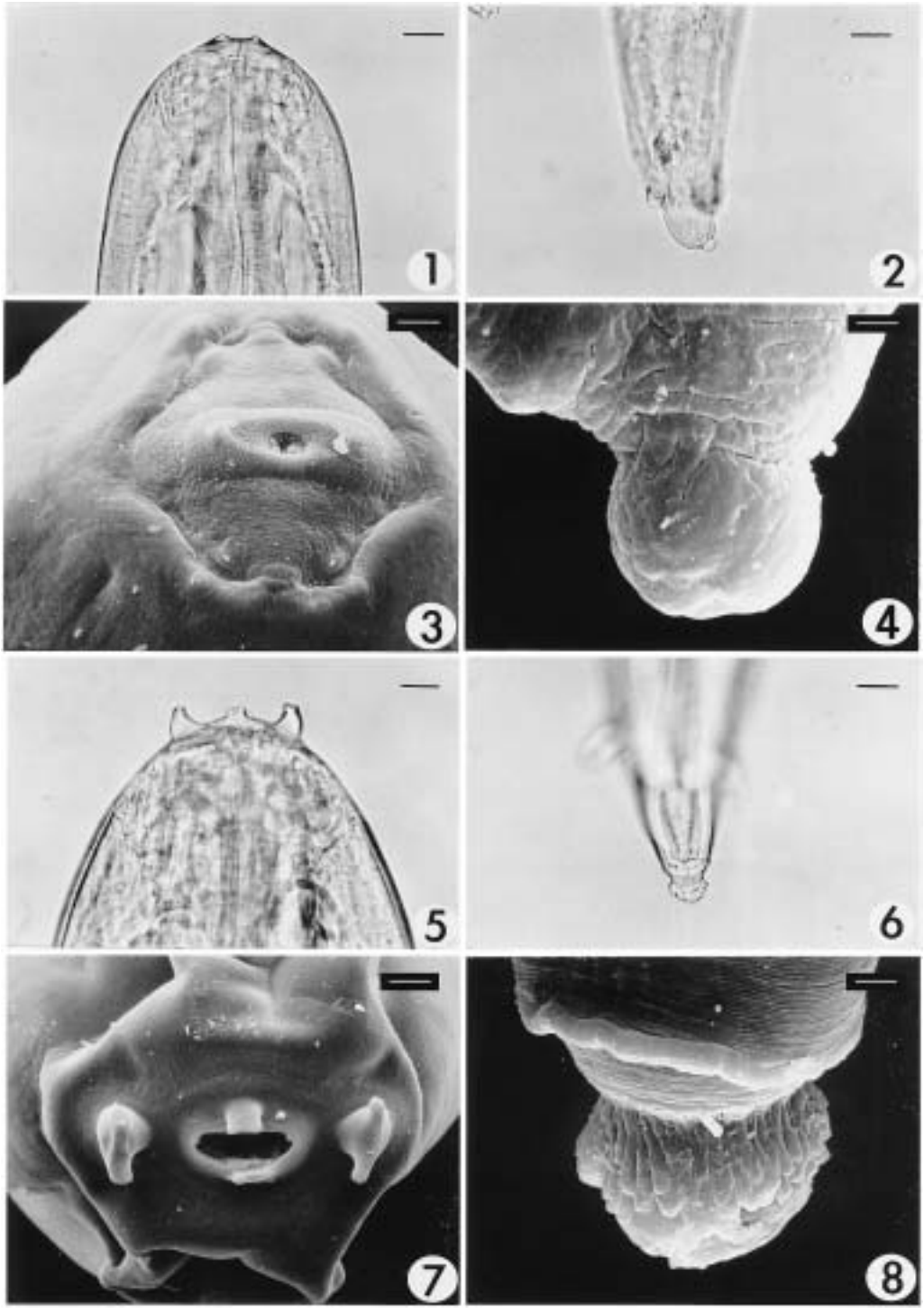
Histopathological findings: Histopathological pictures of both cattle were almost the same. In both cases, many mononuclear cells (the major one being lymphocytes) and a few eosinophils were seen to have infiltrated into the meninges of the cerebellum (Fig. 17). Similar inflammatory picture were observed in the adipose and connective tissue around the roots of the cervical, thoracic, and lumbar spinal nerves, the dura, arachnoidea, and the epidural space. Perivascular cuffing by lymphocytes and their infiltration around the blood vessels and nerve roots in the extra-epidura of the spinal cord were also observed (Fig. 18). Furthermore, some multinucleate giant cells, plasma cells, and macrophages with brown pigments were seen in the extra-epidural cellular infiltrations of the spinal cord (Figs. 19). Slight to severe demyelination of the roots of cervical, thoracic, and lumbar spinal nerves could be observed (Figs. 20 & 21). Cross-section of a 5th stage larva was observed in the extra-epidural space in case 2 (Fig. 22).

DISCUSSION

Three species of *Setaria*, namely, *S. cervi*, *S. digitata* and *S. marshalli* had been reported in Taiwan [11, 16, 17]. However, only *S. cervi* had been reported to cause lumbar paralysis in deer [17], and *S. digitata* in goat [11]. Our study confirmed the occurrence of cerebrospinal setariosis in cattle in Taiwan for the first time. The prevalence of *S. digitata* infection in cattle slaughtered in Taichung had been reported to be 46.2% (79/171) in Holstein-Friesian strain cattle, 22.6% (17/75) in buffalo and 56.4% (31/55) in the local yellow cattle, respectively [16]. In view of the comparatively high prevalence of *Setaria* infection in cattle in Taiwan, cerebrospinal setariosis in cattle might become an important disease in the future in Taiwan.

It is difficult to differentiate between the 4th stage female larvae of *S. digitata* and *S. marshalli* based only on the morphology of their head and tail ends. However, it is quite easy to identify the 5th stage larvae of the two species. The female 5th stage *S. marshalli* larvae had prominent characteristic lip-like projections (lateral lip) between the dorsal and ventral teeth, which resembled that of the female adult worm. The male 5th stage *S. marshalli* larvae had upright triangular-shaped lateral teeth, like those in the male adult worms [16, 18]. Thus, the lip-like projections between the dorsal and ventral teeth process at the anterior end could be used as a criterion for differentiating the 5th stage larvae of *S. digitata*, *S. marshalli* and *S. cervi* [16, 17]. *S. cervi* has four large prominent elevations, each situated at equi-distance in the four different corners [17].

We postulated that the site of predilection of *Setaria* larvae within the central nervous system might be related the



Figs. 1–8. Anterior and posterior ends of *Setaria* spp. larvae collected from the cerebrospinal cavity of 2 calves in Taichung, central Taiwan. Note the differences in the prominences surrounding the mouth and a knob-like structure at the tail. Figs. 1–4: Fourth stage *Setaria* sp. larva; Figs. 5–8: Fifth stage *S. marshalli* female larva; Figs. 1 & 5: Anterior part of larva, lateral view; Figs. 2 & 6: Posterior part of larva; Figs. 1, 2, 5, 6: Bar=25 μm; Figs. 3 & 7: The *en face* view of the head (SEM); Fig. 3: Bar=5.56 μm; Fig. 7: Bar=8.0 μm; Figs. 4 & 8: Knob-like structure at the tail (SEM); Fig. 4: Bar=1.0 μm; Fig. 8: Bar=2.0 μm.

Table 1. Morphometric measurements of the *Setaria marshalli* from cattle

Case 1	No. of worms	Length (cm)		Width (μm)	
		Range	Average (M ± SD)	Range	Average (M ± SD)
Fifth stage larvae					
Female	12	3.2–4.8	4.0 ± 0.6	312.0–405.6	366.6 ± 38.7
Male	8	2.7–3.4	3.0 ± 0.3	296.4–327.6	309.1 ± 10.1
Female adult worms	2	9.2, 9.3		686.4, 639.6	

Table 2. Morphometric measurements of the *Setaria digitata* larvae from cattle

Case 2	No. of worms	Length (cm)		Width (μm)	
		Range	Average (M ± SD)	Range	Average (M ± SD)
Fifth stage larvae					
Female	4	3.0–3.9	3.3 ± 0.4	335.4–390.0	337.4 ± 36.8
Male	4	2.3–2.8	2.5 ± 0.2	249.6–273.0	263.3 ± 11.7

Table 3. Inflammatory cell count in cerebrospinal fluid and blood of cattle with cerebrospinal setariosis

	Case 1						Case 2	
Fluid examined	CSF*			Blood			CSF*	Blood
Date examined	8/3	8/6	8/9	8/3	8/6	8/9	9/1	9/1
Total no. of inflammatory cell ($\times 10^2$/m*l*)	105	416	1323	95	120	101	143	125
Differential count								
Neutrophils (%)	0	0	3	46	37	73	2	61
Lymphocytes (%)	94	96	89	51	60	27	78	37
Eosinophils (%)	3	2	2	0	0	0	4	1
Monocytes (%)	3	2	6	3	3	0	16	1

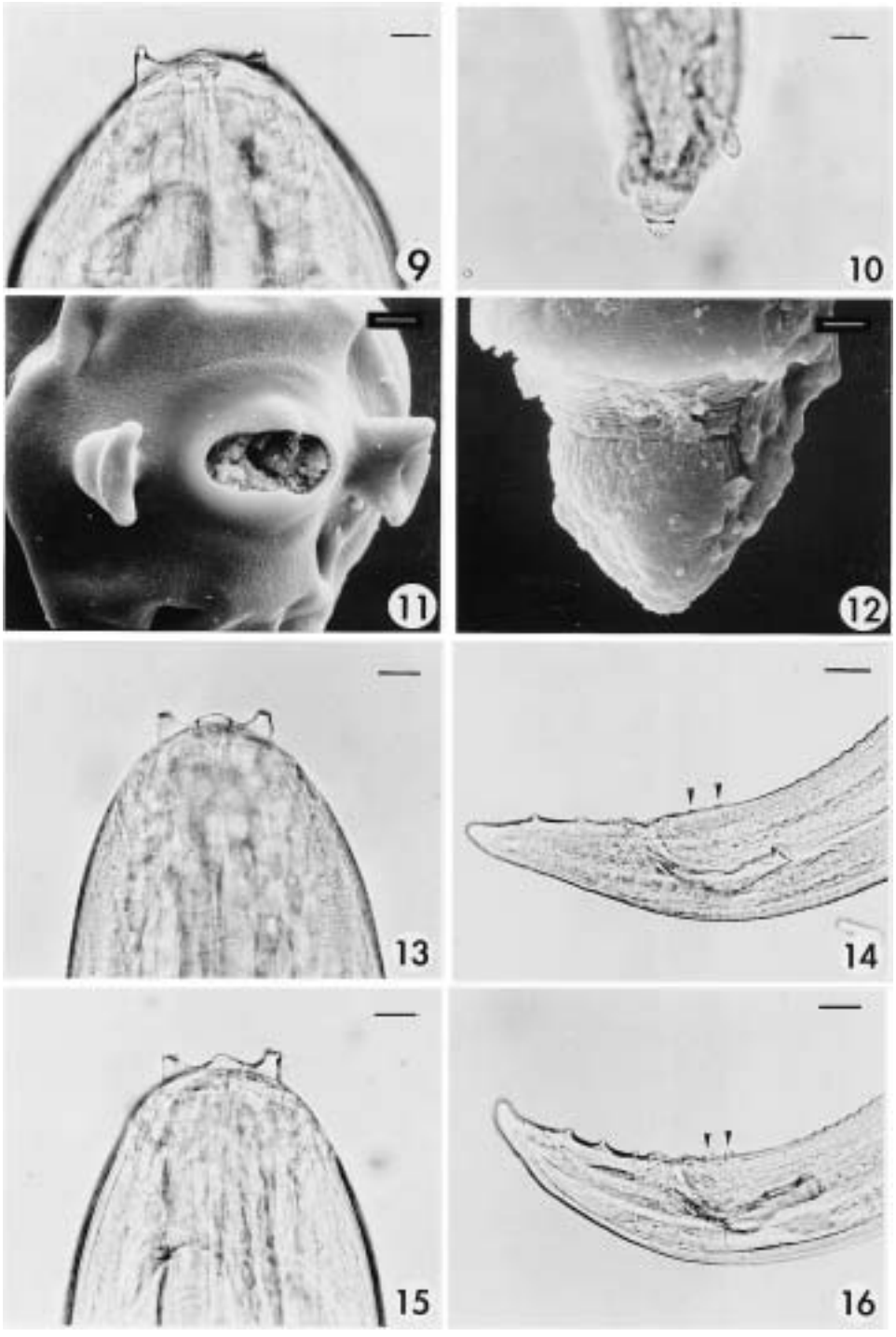
* CSF: cerebrospinal fluid.

clinical sign observed. In case 1 of our study, in which the cattle was quadriplegic, that is, all four limbs were paralyzed, most of the larvae were found parasitizing in the anterior portion of the spinal cord and also in the brain. On the contrary, in case 2 of our study, the cattle showed only hind limb paralysis. This might be associated with the *S. digitata* larvae being concentrated in the thoracic and lumbar region of the spinal cord and no larva being found in the brain. Nevertheless, further clinico-pathological studies will be needed to further confirm our postulation.

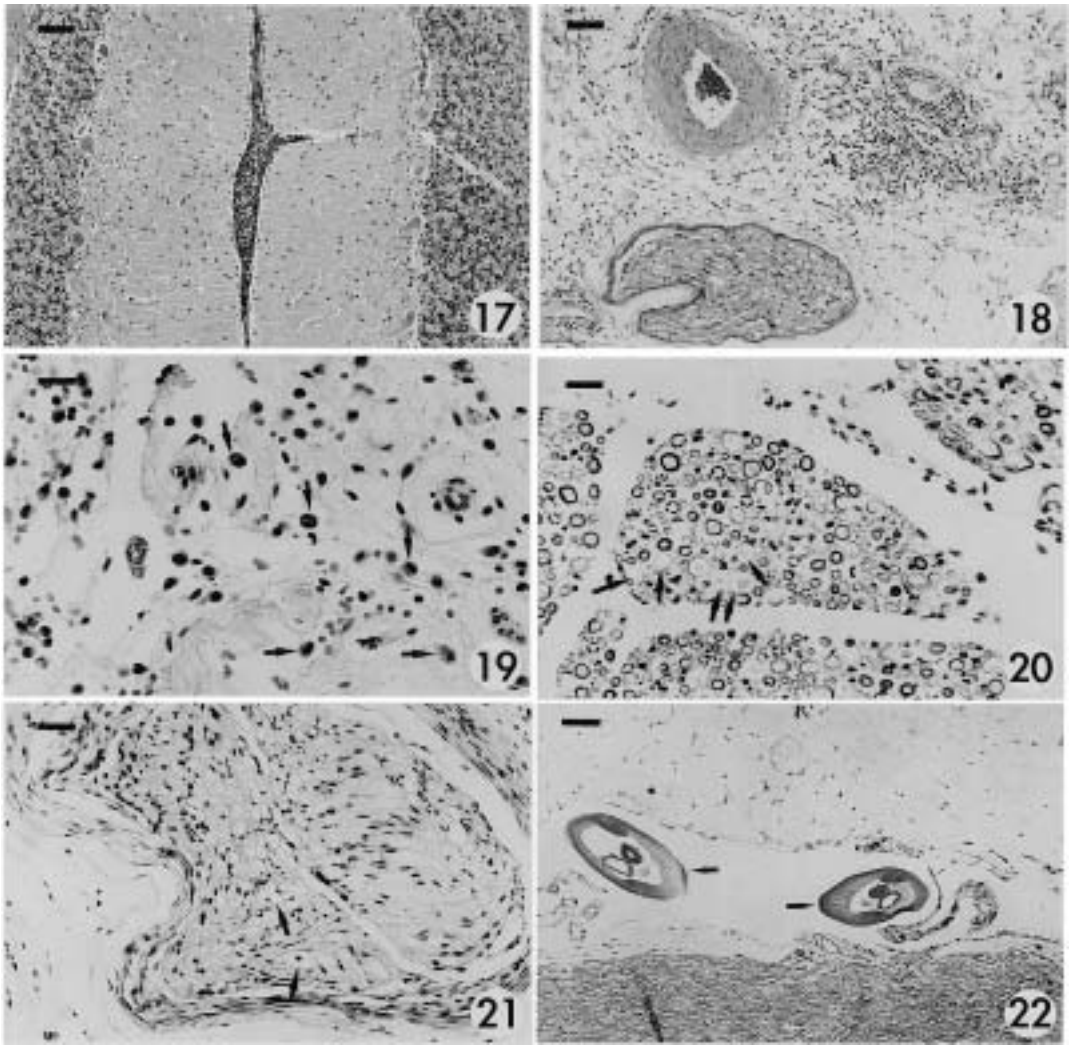
The histopathology of the cerebrospinal lesions of the 2 cattle in our study resembled those reported for deer infected with *S. cervi* [6, 17]. They were principally chronic, patchy leptomeningitis, granulomatous ependymitis, and focal, nonpurulent encephalomyelitis. Lymphocytes and plasma cells were more numerous than eosinophils in the inflammatory lesion. The granulation tissue was composed of histiocytes, lymphocytes, and multinucleate giant cells. However, these histopathological pictures were different from that reported for sheep with lumbar paralysis attributed to setariosis, in which, eosinophilic cuffings in the white matter and fissures of the cerebellum were observed [1].

Heterotopic parasitism, such as *S. digitata* on the epicardium of the cardiac ventricle [9] and in urinary bladder [20] of cattle resulting in the formation of granuloma had been reported in Japan. Recently, ocular infection by *S. digitata* in cattle was reported by Ohtake, *et al.* in Japan [10] and Shin, *et al.* in South Korea [13]. In our study, since *S. digitata* and *S. marshalli* larvae were found in the cerebrospinal cavity, it might probably not be a simple case of heterotopic parasitism. It might be possible that the larvae of *S. digitata* first migrated to the cerebrospinal cavity and after becoming mature there, will only migrate to the peritoneal cavity. This proposal is supported by the observation that there has been no report of the finding of 4th stage *S. digitata* larvae in the peritoneal cavity of cattle. Nevertheless, further study on the migration route of *S. digitata* worm in cattle is warranted.

The possibility of bacterial and viral infection causing quadriplegia and lumbar paralysis in the two cattle of our study could be ruled out because the major cellular components of the CSF were both lymphocytes and eosinophils. Generally, during bacterial infection, the major type of cell



Figs. 9–16. *Setaria* spp. larvae collected from the cerebrospinal cavity of 2 calves in Taichung, central Taiwan. Note the differences in the prominences surrounding the mouth and the tail ending. Figs. 9–12: Fifth stage *S. digitata* female larva; Fig. 9: Anterior part of larva, lateral view; Fig.10: Posterior part of larva; Fig. 11: The *en face* view of the head (SEM), Bar=5.0 μm; Fig. 12: Tail end (SEM), Bar=1.67 μm; Figs. 9, 10, 13, 14, 15, 16: Bar=25 μm; Figs. 13 & 15: Anterior part of larva, lateral view; Figs. 14 & 16: Posterior part of larva; Figs. 13 & 14: Fifth stage *S. digitata* male larva; Figs. 15 & 16: Fifth stage *S. marshalli* male larva; The pair of arrows show the difference of the distance between the two papillae that can be used to differentiate the species between the male larvae.



Figs. 17–22. Histopathological findings of cattle with cerebrospinal setariosis. Fig. 17: Many mononuclear cells, the major one being lymphocytes, in meninges of the cerebellum. H&E. Bar=80 μm; Fig. 18: Lymphocytic infiltration around the vessels and nerve roots in extra-epidura. H&E. Bar=80 μm; Fig. 19: Macrophages with brown pigment (arrow) in extra-epidura of spinal cord. H&E. Bar=25 μm; Fig. 20: Slight demyelination of the roots of cervical spinal nerves (arrow). Luxol Fast Blue-Cresyl Echt Violet stain. Bar=45 μm; Fig. 21: Severe demyelination of the roots of lumbar spinal nerves in extra-epidura (arrow). Luxol Fast Blue-Cresyl Echt Violet stain. Bar=45 μm; Fig. 22: Cross-sections of 5th stage larva of *S. digitata* larvae (arrow) in extra-epidura, in case 2. H&E. Bar=80 μm.

found in the CSF is neutrophil, whereas in viral infection, it is usually lymphocytes and few neutrophils [5]. Moreover, besides quadriplegia and lumbar paralysis, the two cattle had no fever, and could eat and drink normally. Such physiological conditions are seldom seen in most bacterial and viral infections.

Paralysis or incoordination of the limbs in ruminants with cerebrospinal setariosis might be directly caused by the demyelination of the nerves that extend from the spinal cord. Demyelination of the CNS nerves had been attributed to the action of matrix metalloproteinases (MMPs) that were secreted by the infiltrating inflammatory cells [12]. Our observation of CNS nerve demyelination in the 2 bovine cerebrospinal setariosis cases correlates well with the notion that the demyelination might be caused by the MMPs. However, there is also a possibility that certain form of demyelination protease might be produced by the *Setaria* larvae. Thus, further study on the mechanism relating to the clinical manifestation in setariosis is needed.

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