

Sudden Death in Calves Infected with *Strongyloides papillosus*

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Abstract

Parasitological examination was conducted in 10 cases of sudden death which occurred in calves raised in the southern part of Japan where sawdust litter confinement pens were used. Extensive infection of the small intestine with *Strongyloides papillosus* (SPL) was observed in every necropsied case. Inspection of the pens where the fatal cases were detected revealed the presence of a number of infective larvae of SPL in sawdust litter, suggesting that this material was conducive to the growth of the larvae and that *per cutem* infection of calves with the larvae was promoted by the use of this management system. The scabs, which were occasionally found at coronary bands in the field cases, were considered to result from *per cutem* infection with the larvae. The autopsy revealed the presence of some migratory larvae in various tissues. An attempt was made to reproduce the sudden death symptoms by experimentally infecting calves with the larvae. Ten calves which were infected *per cutem* with more than 320,000 larvae per 100 kg of body weight died suddenly from 10.8 to 27.4 days after the infection. Clinical and parasitological findings in these experimental cases were similar to those of the field cases. Outbreaks of the disease in the farms ceased following the administration of thiabendazole or ivermectin.

Discipline: Animal health

Additional key words: infection, new disease, outbreak, sawdust

Introduction

Each year during the hot season from July to October, since 1978, a calf-raising farmer in Kagoshima Prefecture, in the southern part of Japan, had reported the loss of several of his calves by sudden death which occurred in certain pens. The calves, 2 to 5 months old, fell suddenly without any premonitory clinical signs and died within a few minutes, bellowing in agony. The calves were raised by pen confinement on sawdust litter, which was foul and moist to some extent due to the deposition of feces and urine. Additional cases of sudden death were detected in many other farms in 14 prefectures thereafter⁹⁾.

Pathological, bacteriological and virological investigations on the necropsied cases did not reveal

significant pathological changes which might have induced the sudden death⁴⁾, nor any bacteria or viruses³⁾. We carried out parasitological investigations on the field cases in 1987 and found that the sudden death of the infected calves was associated with extensive infection with *Strongyloides papillosus* (SPL).

The present paper reviews the parasitological investigations on the field cases¹²⁾, as well as the successful reproduction of sudden death symptoms by experimental infection of calves with a high dose of infective larvae of SPL^{11,13)}.

Materials and methods

1) Parasitological examination

The intestinal tract of the necropsied cases was examined for the presence of adult worms. The

number of adult worms of SPL in the small intestine was counted according to the technique described by Skerman and Hillard⁷⁾. The rectal feces were examined for the egg counts of SPL. For the experimentally infected cases, rectal feces collected every morning during the course of infection and at the time of the necropsy were examined. The number of eggs per gram feces (EPG) was determined according to the modified McMaster's technique⁸⁾.

The number of migratory larvae in various tissues, such as the brain, myocardium, lung, eye orbit, tongue and muscles from various parts of the body, was counted by the modified Baermann's technique. Briefly, the number of larvae, which emerged into physiological saline in a test tube out of thin sections of 10 g of the tissues was counted.

The number of infective larvae of SPL in the sawdust litter of the pen with fatal cases at farm YMK was counted by collecting the larvae from 1 g of litter, staining the larvae with Lugol's solution and identifying the SPL infective larvae among the other kinds of soil nematoda under a microscope.

2) *Experimental infection of calves with the infective larvae*

Fifteen parasite-free Holstein-Friesian calves, weighing 45.5 to 85.6 kg, were divided into 6 groups, which consisted of 2 calves, respectively, except for one group (group B) of 5 calves. The calves of the 6 groups (A to F) were infected *per cutem* once with 100,000, 320,000, 1,000,000, 3,200,000, 10,000,000 and 32,000,000 larvae per 100 kg of body weight, respectively. The Himeji strain of SPL¹⁰⁾, which was initially isolated from field cases in Hyogo Prefecture in 1988, was used for the experimental infection. The strain had been passaged once in a calf, 23 times in rabbits and once in a calf before use in the present experimental infection.

The inoculum for experimental infection was prepared as follows: The suspension in natural water of the feces containing eggs of SPL was filtered through an absorbent cotton layer in order to recover the eggs on the cotton layer. The layer was incubated for hatching of the eggs and growth of the rhabditiform larvae to infective ones at 25°C for 3 to 4 days. The larvae were collected in a certain amount of natural water and the larval suspensions prepared to obtain a given number of infective larvae. They were filtered through a new cotton layer so that the

cotton layers contained a given number of infective larvae.

The calves were infected *per cutem* with the larvae by fixing the cotton layers around the region of ankle joints of both forefeet for 5 hr. Penetration of almost all the larvae into the skin was confirmed by counting the number of the larvae remaining on the cotton layers.

Results

1) *Parasitological findings in the necropsied field cases*

The findings are summarized in Table 1. Ten calves were subjected to a necropsy. The scabs, which appeared to result from *per cutem* penetration of the infective larvae, remained at the coronary bands in the necropsied cases (Plate 1). Pneumonic lesions of limited extent were found in 6 cases. The EPG of SPL in the rectal feces ranged between 52,000 and 411,000. No adults worms or eggs of other kinds of helminths than SPL were detected in the intestinal tract. The number of adults worms of SPL in the small intestine ranged between 19,000 and 141,500. A maximum of 95 migratory larvae was detected in 10 g of the tissues examined.

2) *Relation between calf-raising conditions and disease outbreak*

Seasonal outbreaks of the disease are presented in Fig. 1. They occurred mainly during the hot season between July and October, when the atmospheric temperature ranged between about 20 to 35°C. In each of the farms, SND, YMK and YNO, approximately 300 calves of Holstein-Friesian breed for beef production were raised. Management/rearing of calves in sawdust litter pens was used in all the cases. Groups of 5 to 7 calves were raised in a narrow pen about 5 m² in size set up in the barns. The sawdust litter in these pens was foul and moist to some extent due to the deposition of feces and urine. The litter was warmed up by the atmospheric temperature. Thirty to 98 infective larvae of SPL, which were growing in the litter, were detected in 1 g of the litter samples collected at farm YMK during the hot season. The farmers reported that they saw a few calves run about in the pens or shake their legs frequently a few weeks prior to the occurrence of sudden death. They noticed also that some of them

Table 1. Parasitological findings in calves with sudden death

Farm-case no.	SND-1	YMK-1	YNO-1	YNO-2	UNO-3	YNO-4	YNO-5	YNO-6
Age at death (days)	144	119	56	67	76	69	51	51
Rectal EPG of SPL ^{a)}	116,000	73,000	99,800	172,000	63,800	89,000	78,000	116,200
No. of adult worms ^{b)}	41,000	141,500	74,500	48,000	50,800	19,000	70,400	54,000
No. of migratory larvae ^{c)} in:								
Brain	0	0	0	0	0	0	0	0
Myocardium	0	0	1	1	0	0	0	0
Lung without pneumonia	0	0	10	95	24	8	13	1
Lung with pneumonia	0	- ^{d)}	2	7	1	-	2	-
Eye orbit	0	0	0	8	5	0	1	4
Tongue	0	0	0	0	0	0	0	0
Mandibular muscle	0	1	0	1	0	0	0	0
Trunk muscle ^{e)}	0	0	0	5	0	14	4	0
Extremities muscle of:								
Upperarm and thigh ^{f)}	0	16.7	1.8	8.3	3.3	2.5	3.8	0.3
Ankle ^{f)}	1.3	28.5	4.3	2.3	4.8	1.5	11.0	1.8

Farm-case no.	YNO-7	YNO-8
Age at death (days)	69	56
Rectal EPG of SPL ^{a)}	411,000	52,000
No. of adult worms ^{b)}	117,400	23,100
No. of migratory larvae ^{c)} in:		
Brain	0	0
Myocardium	1	0
Lung without pneumonia	4	2
Lung with pneumonia	0	-
Eye orbit	24	4
Tongue	0	0
Mandibular muscle	1	0
Trunk muscle ^{e)}	1	0
Extremities muscle of:		
Upperarm and thigh ^{f)}	6.5	0
Ankle ^{f)}	4.0	0.5

showed footrot.

3) Reproduction of sudden death symptoms by experimental infection

Immediately after exposure to the infective larvae, the calves started to show symptoms of pain or irritation in the part inoculated with the larvae, for about 30 min and no other symptoms were observed thereafter. Sudden death occurred 10.8 to 27.4 days after infection, in 10 calves to which more than 320,000 infective larvae per 100 kg of body weight had been inoculated (Table 2). The calves died within 4 min after the first development of symptoms of agony.

A shorter incubation period between the initiation of experimental infection and the onset of sudden

- a): Rectal EPG of SPL at the time of necropsy.
 b): Number of adults of SPL in the small intestine.
 c): Number of migratory larvae in 10 g of the tissues.
 d): -: Absence of pneumonic lesions.
 e): A portion of the abdominal muscle was collected for examination.
 f): Average number of larvae obtained by examination on 4 extremities.

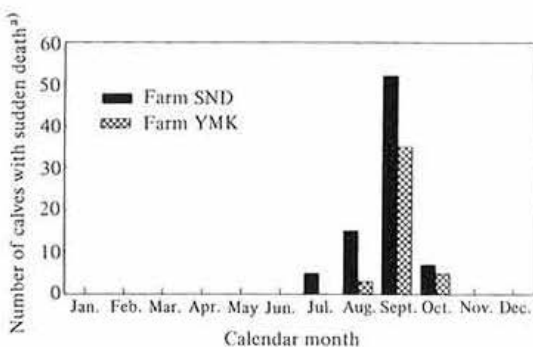


Fig. 1. Seasonal outbreaks of sudden death in farms SND and YMK

a): Total number of cases in 9 years, 1978-1986.

death was observed in the calves which had received a higher dose of infective larvae (Table 2).

The autopsy did not reveal any macroscopic lesions which may have induced sudden death in various organs including the brain and heart, although pneumonic lesions of limited extent and mild hyperemia of the mucosa of the intestinal tract were observed in some cases.



Plate 1. Scabs observed at the coronary bands in a calf with sudden death

Table 3 shows the parasitological findings of the experimentally infected calves. The maximum EPG in the rectal feces from animals with experimental sudden death ranged between 17,600 and 201,800, while the number of adult worms in the small intestine at the time of the necropsy ranged between 20,630 and 1,089,000. No other helminths than SPL were detected. Migratory larvae of SPL were detected in the lung, eye orbit and various muscles of the heavily infected calves. In the brain and myocardium, on the other hand, no or very few larvae were detected. The largest number of migratory larvae was detected in the muscles of the fore ankles which were exposed to infective larvae.

4) Prevention of sudden death by anthelmintic medication

The occurrence of sudden death at farms SND, YMK and YNO ceased following the administration to calves of a regular dose of thiabendazole or ivermectin (Table 4).

Table 2. Reproduction of sudden death by experimental infection with SPL.

Calf no.	Number of infective larvae inoculated ^{a)}	Reaction	Incubation period ^{b)} (days)
A-1	100,000	Survived	-
A-2	100,000	Survived	-
B-1	320,000	Survived	-
B-2	320,000	Survived	-
B-3	320,000	Died ^{c)}	25.8
B-4	320,000	Sudden death	27.4
B-5	320,000	Sudden death	16.8
C-1	1,000,000	Sudden death	14.8
C-2	1,000,000	Sudden death	14.8
D-1	3,200,000	Sudden death	13.3
D-2	3,200,000	Sudden death	14.2
E-1	10,000,000	Sudden death	11.0
E-2	10,000,000	Sudden death	11.1
F-1	32,000,000	Sudden death	11.6
F-2	32,000,000	Sudden death	10.8

a): Number of infective larvae inoculated to 100 kg of body weight of calves.

b): Interval (days) between experimental infection and the onset of death.

c): Died of emaciation with diarrhoea.

Table 3. Parasitological findings in the calves experimentally infected with SPL

Group-case no.	B-1 ^{a)}	B-2 ^{b)}	B-3	B-4	B-5	C-1	C-2	D-1	D-2	E-1
Maximum EPG of SPL ^{b)}	27,600	69,600	49,400	73,000	62,600	107,000	59,600	201,800	104,400	39,200
Rectal EPG of SPL ^{c)}	0	0	12,400	4,000	42,400	107,000	68,800	192,800	146,200	112,400
No. of adult worms ^{d)}	0	0	24,500	20,630	22,860	105,140	136,000	565,200	299,400	403,130
No. of migratory larvae ^{e)} in:										
Brain	0	0	0	0	0	0	0	0	2	NT ^{f)}
Myocardium	0	0	0	0	0	0	0	0	0	0
Lung without pneumonia	0	0	0	0	0	0	0	7	16	18
Lung with pneumonia	0	0	0	0	0	2	- ^{g)}	-	-	-
Eye orbit	0	0	0	0	0	0	0	2	4	2
Tongue	0	0	0	0	0	0	0	0	0	0
Mandibular muscle	0	0	0	0	0	0	0	16	0	0
Trunk muscle ^{h)}	0	0	0	0	0	0	0	6	4	ND
Extremities muscle of:										
Upperarm ⁱ⁾	0	0	0	0	0	0	3.5	42.0	40.0	116.0
Ankle ^{j)}	0.5	0.5	0	0	0	3.0	357.0	17.5	48.5	265.5

Group-case no.	E-2	F-1	F-2
Maximum EPG of SPL ^{b)}	17,600	46,000	50,400
Rectal EPG of SPL ^{c)}	48,800	271,600	50,400
No. of adult worms ^{d)}	585,000	210,380	1,089,000
No. of migratory larvae ^{e)} in:			
Brain	0	0	0
Myocardium	0	0	0
Lung without pneumonia	10	10	607
Lung with pneumonia	41	5	518
Eye orbit	3	0	73
Tongue	0	0	0
Mandibular muscle	0	0	619
Trunk muscle ^{h)}	1	2	16
Extremities muscle of:			
Upperarm ⁱ⁾	161.0	5.0	712.5
Ankle ^{j)}	167.5	601.5	7,457.0

Discussion

SPL has generally been considered to be a helminth of minor importance in calves^{2,5,6)}, although some fatal cases due to severe diarrhea were reported in association with experimental SPL infection^{1,4,16)}. The occurrence of sudden death in association with SPL infection in calves had not been documented before our first report in some field cases¹²⁾. Recent successful experimental reproduction of the disease¹¹⁾ confirmed our previous findings.

Several conditions should be met before extensive infection of calves with infective larvae occurs in a certain calf-raising farm as follows: (1) a calf or calves which excrete(s) SPL eggs in the feces should

a): Necropsied 34 days after infection for parasitological examination.

b): The maximum EPG of SPL in rectal feces collected every morning during the course of infection.

c), d), e), g), h): See Table 1.

f): Not tested.

i): Average number of larvae obtained by examination of the upperarms of both forefeet.

j): Average number of obtained by examination of the ankles of both forefeet; this part was exposed to infective larvae of SPL.

Table 4. Number of calves with sudden death before and after the administration of anthelmintic drugs

Farms	Number of calves with sudden death	
	Before medication	After medication
SND	93	1
YMK	43	0
YNO	14	1
Total	150	2

first be present in the herd, (2) the litter in the pen should be moist due to the excreta of calves, warmed up by a high atmospheric temperature suitable for hatching of the eggs and growth of the rhabditiform larvae to infective ones, and (3) the pen should be narrow enough to prevent calves from leaving the pen due to painful or irritating *per cutem* infection with the infective larvae.

Sawdust is the most common component of litter in calf-raising farms in Japan. As shown in Plate 2,

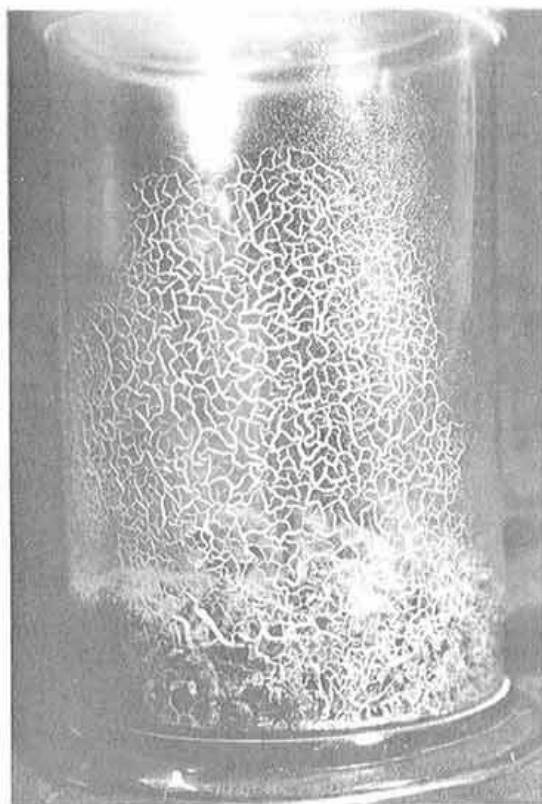


Plate 2. A large number of infective larvae of SPL which are creeping up the inside walls of a glass cylinder containing the feces and sawdust at the bottom

sawdust mixed with the feces can be conducive to the hatching of the eggs of SPL and growth of the rhabditiform larvae to infective ones in a moistened chamber. In addition, the period of outbreak of the disease, from July to October, corresponds to the hot and humid season of the year. Also, narrow pens with a large number of calves can easily be found in these farms. These unfavorable conditions increase the risk of calf's infection with a large number of infective larvae and the occurrence of sudden death. Routine fecal examination should be performed and medication administered for the prevention of this disease.

Pathogenesis of the sudden death observed has not been clarified, although a causal relation between extensive SPL infection and the occurrence of sudden death was revealed. Kubo⁴⁾ and Watase¹⁵⁾ observed lesions of atypical interstitial pneumonia in the lungs

of calves that died suddenly. However, 4 animals out of 10 and 4 out of 13 experimental cases in our investigations did not show any macroscopic pneumonic lesions. It is suggested that pneumonia is not the direct cause of sudden death and that a factor related to SPL is responsible for the malfunction of the heart. An attempt to elucidate the pathogenesis is in progress.

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References

- 1) Davis, L. R., Herlich, H. & Bowman, G. W. (1960): Studies on experimental concurrent infections of dairy calves with coccidia and nematodes. III. *Eimeria* spp. and the threadworm, *Strongyloides papillosus*. *Am. J. Vet. Res.*, **21**, 181-187.
- 2) Georgi, J. R. & Georgi, M. E. (1990): Parasitology for veterinarians. (5th ed.) W. S. Saunders Co., Philadelphia.
- 3) Hase, M. et al. (1983): An unknown disease in calves at intensive raising calves — pathological, biochemical and bacteriological examinations. *Proc. Meeting Kagoshima Pref. Vet. Diagnostitian*, **16**, 2-5 [In Japanese].
- 4) Kubo, M. (1984): Sudden death, 'Pökkuri byo', in cattle. *J. Clin. Vet. Med.*, **2**(8), 21-26 [In Japanese].
- 5) Lepage, G. (1968): Veterinary parasitology. (2nd ed.) Oliver & Boyd, Edinburgh.
- 6) Levine, N. D. (1980): Nematoda parasites of domestic animals and of man. (2nd ed.) Burgess Publ. Co., Minneapolis.
- 7) Skerman, K. D. & Hillard, J. J. (1966): A handbook for studies of helminth parasites of ruminants. FAO, Rome.
- 8) Taira, N. (1988): O-ring technique. In *Buiatrics*. (2nd ed.) eds. Shimizu, T. et al., Kindai Publ. Co., Tokyo, 89-90 [In Japanese].
- 9) Taira, N. (1991): Strongyloidiasis in calves. *Bull. Nat. Inst. Anim. Health*, **96**, 425-428 [In Japanese].
- 10) Taira, N., Minami, T. & Smitanon, J. (1991): Dynamics of faecal egg counts in rabbits experimentally infected with *Strongyloides papillosus*. *Vet. Parasitol.*, **39**, 333-336.
- 11) Taira, N. et al. (1992): Sudden death of calves by experimental infection with *Strongyloides papillosus*. I. Parasitological observations. *Vet. Parasitol.*, **42**, 247-256.
- 12) Taira, N. & Ura, S. (1991): Sudden death in calves associated with *Strongyloides papillosus* infection. *Vet.*

- Parasitol.*, **39**, 313-319.
- 13) Ura, S. et al. (1992): Sudden death of calves by experimental infection with *Strongyloides papillosus*. II. Clinical observations and critical moments of the disease analyzed by a video tape. *Vet. Parasitol.*, **44**, 107-110.
 - 14) Vegors, H. H. (1954): Experimental infection of calves with *Strongyloides papillosus*. *Am. J. Vet. Res.*, **15**, 429-433.
 - 15) Watase, H. (1988): Atypical interstitial pneumonia. In *Buiatrics*. (2nd ed.) eds. Shimizu, T. et al., Kindai Publ. Co., Tokyo, 423-425 [In Japanese].
 - 16) Woodhouse, C. A. (1948): Observations on pathogenicity of *Strongyloides* parasites in ruminants. *J. Amer. Vet. Med. Assoc.*, **113**, 354-356.

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