NOTE *Parasitology*

Large-Group Infection of Boar-Hunting Dogs with *Paragonimus westermani* in Miyazaki Prefecture, Japan, with Special Reference to a Case of Sudden Death Due to Bilateral Pneumothorax

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ABSTRACT. We recently found a group of 5 boar-hunting dogs infected with *Paragonimus westermani*. As wild boars are known to be the potential paratenic hosts for this parasite, boar-hunting dogs have obviously a high risk of infection by this parasite. In the present study, therefore, we investigated 20 dogs of another group kept by a hunter in Miyazaki Prefecture, Japan, in order to determine whether paragonimosis is a common problem among boar-hunting dogs. The results showed that *P. westermani* eggs were present in the feces of 10 out of 20 dogs, while 17 dogs were seropositive on ELISA. Taken together with our previous results, it appears that paragonimosis is a serious problem in boar-hunting dogs. The possible risks of infected dogs acting as a source for maintaining the *P. westermani* life cycle are also discussed.

KEY WORDS: canine, *Paragonimus westermani*, praziquantel, wild boar.

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We recently found a small group of boar-hunting dogs infected with *Paragonimus westermani* (all 5 dogs examined were seropositive and egg-positive) in Miyazaki Prefecture, Japan [4]. Boar hunting is an important economic activity for people living in mountainous areas throughout the southwestern Japan including Miyazaki Pref. Teams of specially trained dogs have long been used for wild boar hunting. The number of registered hunters in Miyazaki Pref. is approximately 6,000, and the number of boar-hunting dogs is estimated to exceed 10,000.

Although wild boar is known to be a potential paratenic host for both diploid and triploid types of *P. westermani* [2, 5–7, 14, 15], so far only triploid type has been reported in Miyazaki pref. As more than 10,000 wild boars per year were hunted in Miyazaki Pref., we speculated that there are more hidden paragonimosis cases among boar-hunting dogs. In addition, human cases of *P. westermani* infection have recently re-emerged in Japan [9–11], particularly in southern parts of Kyushu, including Miyazaki Pref. [16], where the majority of these human paragonimosis cases are thought to be the result of ingesting raw/undercooked boar meat [9–11, 17]. However, in Miyazaki Pref. if paragonimosis were endemic among boar-hunting dogs, it would represent a serious problem for the dogs, as well as a serious public health issue for the residents in the area. In order to clarify whether paragonimosis is a common problem in boar-hunting dogs, we examined another group of 20 boar-hunting dogs kept in Miyazaki City. The results showed that 10 of the 20 dogs were infected with *P. westermani* by fecal egg examination, and 17 of the 20 dogs were serologically positive for *P. westermani* on ELISA. In addition, two dogs died during the research period, and one of these was shown to have bilateral pneumothorax due to *P. westermani* infection.

A group of 20 boar-hunting dogs housed in Tano-cho, in the western part of Miyazaki City, Japan, was examined for paragonimosis. They included 12 males and 8 females, and their ages ranged from 6 months to 8 years. All dogs were mixed-breeds and the majorities were of Plott hound background.

Fecal samples were collected manually from the rectum of each dog. The presence of *Paragonimus* and other parasite eggs was examined by the direct wet mount method and the formalin/ether sedimentation method (MGL method).

P. westermani-specific IgG antibody titers were measured by microplate ELISA, as described previously for humans [8], with slight modification. Briefly, wells of a 96-well microtiter plate (Nunc, Roskilde, Denmark) were coated with 50 μl of antigens containing crude somatic extracts of adult *P. westermani* (10 μg/ml) by incubation at 4°C overnight. Wells were washed with phosphate buffered saline containing 0.05% Tween 20, blocked with blocking buffer (1% casein in 20 mM Tris HCl, pH 7.6), and then incubated with 50 μl of sera from the dogs diluted at 1:1000 at 37°C for 1 hr. After washing, 50 μl of peroxidase-labeled rabbit anti-canine IgG1 (Bethyl Laboratories Inc., TX, U.S.A.) diluted at 1:2,000 was added to each well, followed by incubation at 37°C for 1 hr. ABTS Peroxidase Substrate (one component type; Kirkegaard & Perry Laboratories Inc., MD, U.S.A.) was added to each well, followed by incu-

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Table 1. Profiles of the dogs examined in this study

No.	Age	Sex	BW (kg)	WBC (/ml)	Eosino (%)	*Pw* eggs in feces	Anti-*Pw* antibody (OD at 405 nm)
1	8	M	21.5	8700	2.4	No	1.524
2	5	M	30.5	8200	9.2	No	1.508
3	8	F	20.5	13100	22.9	No	0.020
4	6 m	F	ND	18400	25.0	No	0.532
5	6 m	M	11.5	17100	14.8	No	0.512
6	4	M	21.5	14900	7.8	No	1.486
7	2	M	24	10400	8.8	No	1.570
8	5	M	ND	12500	1.7	Yes	1.634
9	6	F	18	1100	7.3	Yes	1.533
10	2	F	ND	12100	4.0	No	1.506
11	2	F	18.0	10900	5.5	Yes	1.580
12	3	F	19.0	13300	6.0	Yes	1.493
13	6	F	13.0	9600	13.5	Yes	0.031
14	2	M	15.5	8900	9.7	Yes	1.570
15	2	M	16.5	10600	10.4	No	0.224
16	2	M	ND	7600	5.9	No	0.064
17	4	M	ND	14800	6.5	Yes	1.548
18	unknown	F	19.5	11900	14.9	Yes	1.591
19	6 m	M	18.5	14700	12.8	Yes	1.588
20	6 m	M	14.5	14100	6.5	Yes	1.658

bation at 37°C for 15 min. The reaction was stopped by adding 50 μl of 1% sodium dodecyl sulphate to the wells and the optical density (OD) was read at 405 nm in an ELISA reader (Emax: Molecular Devices Co., CA, U.S.A.). The cut-off value (OD=0.065) for the present microplate ELISA data was arbitrarily defined as the mean OD value plus three standard deviations (SD) of the sera from 10 non-boar-hunting dogs kept at home and had no obvious parasitic diseases.

In late August 2003, we examined 20 boar-hunting dogs kept as a group. The profiles and basic laboratory data, including fecal egg examination and ELISA for paragonimosis, are summarized in Table 1. Among the 20 dogs, 10 dogs (50%) were positive for *Paragonimus* eggs by fecal examination, and 17 dogs (85%) were seropositive by ELISA; 14 were strongly positive, 3 were weakly positive and 3 were negative. One egg-positive but seronegative dog (No. 13) was thought to have passed eggs as a result of coprophagia.

While we were analyzing this data, one of the dogs (No. 17) was brought to our veterinary teaching hospital exhibiting CPAOA (cardiopulmonary arrest on arrival) on March 4th, 2004. Resuscitation was not performed, as he was confirmed dead on examination. His body was immediately necropsied in the Laboratory of Veterinary Pathology of our school. According to the owner's statement, he was apparently healthy until suddenly deteriorating; however, another dog (No. 10) also died suddenly on March 1st, 2004, only three days prior to the death of dog No. 17.

On necropsy, the direct cause of death of the dog No. 17 was shown to be bilateral pneumothorax, as both lungs collapsed and the diaphragm was depressed downwards. Macroscopically both lungs were congested, and approximately 10 nodular/cavity lesions of 1–2 cm in diameter were sparsely seen on the surface of both lungs (Fig. 1A). A small hole was observed on the pleural surface of at least one of the lesions in both lungs (Fig. 1B), which caused pneumothorax. Almost all lesions contained 1–2 worms, and a total of 19 worms were recovered from the lungs (Fig. 1C). These worms were identified as adult *P. westermani*

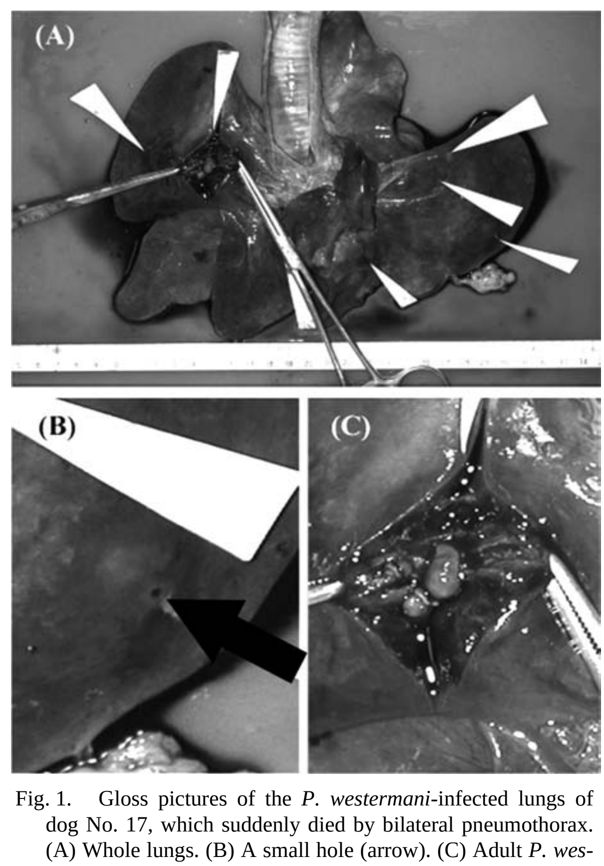

Fig. 1. Gloss pictures of the *P. westermani*-infected lungs of dog No. 17, which suddenly died by bilateral pneumothorax. (A) Whole lungs. (B) A small hole (arrow). (C) Adult *P. westermani* in a nodule.

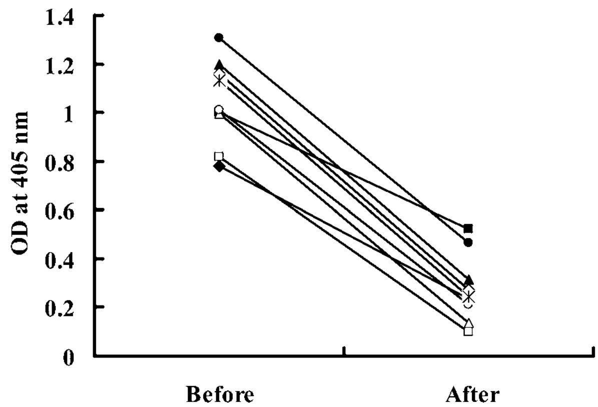

Fig. 2. Antibody titers (OD at 405 nm) of *P. westermani*-infected boar hunting dogs immediately before and at 4 months after praziquantel (78 mg/kg/day × 2 days) treatment.

by morphology, which were also supported by DNA sequences (data not shown).

Immediately after encountering the sudden death of the dog No. 17, we went to the owner's home to inspect the remaining dogs. At this time, the dog No. 11 already died at 3 days prior to the sudden death of No. 17, and 4 dogs were transferred to other owners. Of remaining 14 dogs, 9 seropositive dogs were treated with an oral dose of praziquantel (Biltricide® for human use; 600 mg/tablet) at 78 mg/kg/day for two days. Specific serum antibody titers against *P. westermani* immediately before and at 4 months after therapy were examined by microplate ELISA. As shown in Fig. 2, antibody titers in the seropositive dogs decreased at 4 months after treatment.

The present results confirm and expand our previous observation that paragonimosis is a serious problem in boar-hunting dogs in Miyazaki Pref., Japan. Wild boars are known to be the potential paratenic hosts for *P. westermani* [2, 5–7, 14, 15] and recent seroepidemiological studies have revealed that the infection rate of wild boars hunted in Miyazaki Pref. was over 75% [2]. Furthermore, about 70% of human cases of *P. westermani* infection in Japan are thought to be due to ingesting raw or undercooked wild boar meat [10–12]. Thus, the high prevalence of paragonimosis among boar-hunting dogs should have been predicted much earlier. Wild boar meat is an important source of *P. westermani* infection in both humans and boar-hunting dogs.

In an experimental study, dogs and cats were shown to act as definitive hosts for *P. westermani*, as adult worms can be recovered from the lungs with a high infection/recovery rate [7]. Nevertheless, when compared to the extensive epidemiological survey for *P. westermani* in humans and freshwater crustaceans [7], the works have been scarcely done on the role of dogs and cats in the maintenance of *P. westermani* life cycle in Japan, except for a few case reports [1, 3]. The results of our studies with the high prevalence of paragonimosis among boar-hunting dogs strongly suggest that the dogs serve as a definitive host for *P. westermani* to maintain its natural life cycle in Japan. More extensive seroepidemiological surveys on paragonimosis in boar-hunting dogs are now underway in our laboratory.

During this study, we encountered one confirmed case and one suspected case of sudden death due to infection by *P. westermani*. The cause of the death of the confirmed case was bilateral pneumothorax. Paragonimosis is generally a mild, chronic non-fatal disease that rarely results death. Although pneumothorax is commonly seen in human paragonimosis in Japan [9, 11, 17], it is mostly unilateral and not as serious as seen in dog No. 17. Although death due to paragonimosis is extremely rare in human cases, migration of *Paragonimus* worms into the CNS (central nervous system) sometimes causes deleterious effects with poor prognosis [7, 12].

In the present study, 9 dogs were treated with praziquantel at a dose of 78 mg/kg/day for 2 days. At this dose, human paragonimosis has been effectively cured with an almost 100% cure rate [13, 16]. Efficacy of the treatment was demonstrated by a significant decrease in the antibody titer in the paired sera on microplate ELISA. Earlier diagnosis and treatment are recommended to avoid unexpected death due to such curable diseases.

In conclusion, the present results clearly indicate that paragonimosis due to *P. westermani* infection is endemic among boar-hunting dogs. As paragonimosis is a zoonotic disease and is re-emerging in southern Kyushu, including Miyazaki Pref. [9–11, 17], mass screening and chemotherapy for paragonimosis in boar-hunting dogs in Miyazaki Pref. is necessary in order to maintain the public health.

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