Research Note

A Case of Multiple Lung Fluke (*Paragonimus westermani*) Infection of a Raccoon Dog in Japan

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Paragonimus westermani (Kerbert, 1878) Braun, 1899 is a lung fluke which is widely distributed in Southeast Asia. It has been reported that many kinds of wild mammals such as tigers, leopards, wild cats, foxes and wolves, serve as natural definitive hosts for P. westermani. The species of the hosts, however, differ according to the countries. In Japan, such mammals as dogs, cats and foxes are known as natural definitive hosts. Most recently, a few cases of natural infection of raccoon dogs, Nyctereutes procyonoides viverrinus Gray, with P. westermani-diploid type were reported (Shibahara, 1981; Yoshimura et al., 1983). Consequently, it was suspected that raccoon dogs may indeed serve as an important definitive host in the natural world in Japan, although they had previously been considered to be unsuitable as hosts. In 1985 Shibahara experimentally proved that they are suitable hosts for *P. westermani*. In the present report, a rare case of multiple infection of a wild raccoon dog with the diploid type of *P. westermani* is described.

The skinned body of a raccoon dog (adult male), which had been killed in a traffic accident in March 1983 in Tajima province of Hyogo Prefecture, an endemic area of paragonimiasis, was frozen and transported to the laboratory. After thawing, the raccoon dog was necropsied, and the pleural cavity and the lungs were carefully examined macroscopically for *Paragonimus* infection. Of the total 508 worms recovered, two hundred of the worms obtained from the worm cysts in the lungs were flattened between two slide glasses, fixed in 70% alcohol, stained with carmine, dehydrated, cleared in xylene and mounted with balsam, and were used for morphological observations and measurements. The remaining 308 worms were preserved in 10 % formalin. The eggs removed from the worm cysts in the lungs were also used for morphological observations and measurements after being preserved in 10 % formalin. The E. P. G. (eggs per gram) in the stool was counted by Stoll's method. The lungs were fixed in 10 % formalin, and then a portion was removed for histopathological examination. Paraffin sections were cut at 5 µm thickness and examined after staining with hematoxylin and eosin.

Macroscopical examination showed intense fibrous pleurisy, although the abdominal cavity was intact. The surface of the lungs was bumpy due to worm-cyst formation, and the lung size was approximately twice as large as normal (Fig. 1). Fibrous adhesions were seen between the lobes, and also between the lungs and parietal pleura. Five worms which had been migrating in the pleural cavity were found. Many worm cysts were observed on the surface or in the tissues of the lungs, from which a total of 503 worms were removed (Fig. 2). All of the 200 mounted specimens were well-matured adult worms, and the average body size of 50 specimens sampled at random measured 7.7 ± 1.4 (5.1-10.8) mm in length and $4.2 \pm$

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Fig. 1 Dorsal view of the lungs of a raccoon dog. The surface of the lungs is bumpy due to the worm cysts. In addition to thickening of the pulmonary pleura, intense fibrous adhesions are seen between the lobes.

0.8 (2.3-5.7) mm in width. The oral sucker measured 834.5±133.5 (595.0-1135.2) µm in width, ventral sucker 697.3±71.6 (546.1-837.7) μm in length and 736.9 \pm 78.6 (538.8-910.2) µm in width. The ratio of length/ width was $1.9 \pm 0.2 (1.5 - 2.3)$. Morphologically, they were characterized by the following features; the ovary had 6 lobes, the testes had 5 to 6 lobes and the seminal receptacle and seminal vesicle contained large amounts of spermatozoa. The 30 eggs removed from the worm cysts measured 78.7 ± 4.6 (71.5-90.9) μ m in length and 42.8±3.1 (32.0-48.0) μm in width. The thickness of the eggshell measured 1.3 ± 0.2 (1.1-1.7) µm. The thickening of the abopercular end was remarkable in 23 eggs (76.7 %), but slight or not at all in the remaining 7 eggs (23.3 %). The location of the maximum width of the eggs was 17 (56.7 %) in the anterior half and 13 (43.3 %) in the middle, with a preponderance of those in the anterior half. The E.P.G. in the stool was 91,480, which was the average value of five measurements.

Histopathological examination revealed that normal structure was destroyed by the worms. In all sections, in addition to fibrosis, such changes as pulmonary emphysema and edema were remarkable, especially around



Fig. 2 A large number of worms recovered from the worm cysts in the lungs. (Scale : 10 mm)



Fig. 3 Transverse section of a worm cyst, showing a worm (arrows). Hematoxylineosin (H-E) stain. $\times 13$.

the worm cysts (Fig. 3). Many eggs were also observed in the tunica media of the pulmonary arteries, parenchyma and pleura. In particular, some foci of the accumulated eggs were restricted to the parenchyma (Figs. 4A, 4B).

From the above observation and examination results of the adult worms and their eggs, the flukes obtained were identified as the diploid type of *P. westermani*.

It is well known that the raccoon dog serves as a natural definitive host of *P. ohirai* or *P. miyazakii* (Yokogawa *et al.*, 1957a; Miyamoto, 1961; Ashizawa *et al.*, 1974; Hirai *et al.*, 1978). As mentioned above, however, until recently in Japan, the raccoon dog has been considered as an unsuitable host for *P. westermani*. The reasons for this



Fig. 4 A) Tissue section, showing some foci of the accumulated eggs. H-E stain. ×10.
B) Magnification of foci of the accumulated eggs shown in Fig. A). H-E stain. ×100.

assumption were that Yokogawa et al. (1957b) reported they could find neither fully matured worms nor cyst-formation in the lungs in an experiment with a raccoon dog infected orally with metacercariae of P. westermani, and there have been no reports of natural infection of raccoon dogs with P. westermani. Recently, however, Shibahara (1985) experimentally proved that the raccoon dog is a suitable host for the two chromosomal types of *P. westermani*. This remarkable case of infection with 508 worms in one raccoon dog serves as strong evidence supporting the results of that experiment. The reason why so many worms were found in one animal may be due to a diet which included freshwater crabs infected with P. westermani -diploid type metacercariae.

As to the pathological description of paragonimiasis of a raccoon dog, few reports have been published to date. Ashizawa *et al.* (1974) reported one case of paragonimiasis ohirai in the raccoon dog. In that paper, in addition to stale lesions such as interstitial tissue proliferation, they reported a new lesion, a hemorrhagic foci, which seem to have been formed by flukes under the pulmonary pleura immediately after penetration. In the present research, such stale lesions as marked fibrosis and pulmonary emphysema were observed throughout the whole lungs. The raccoon dog, therefore, was presumed to have long been since infection with the flukes.

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短 報

日本産タヌキに見出されたウエステルマン肺吸虫の多数寄生例

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ウエステルマン肺吸虫(二倍体型)の分布地として知 られる兵庫県北部の但馬地方において1983年3月に交通 事故で死亡したタヌキ1頭(雄成獣)の剖検を行った. 肉眼検査によって肺には胸壁との高度の線維性癒着が認 められ,肺はかなり腫大していた.肺実質中には無数の 虫嚢が認められ,これらから503隻の虫体が検出された. また,胸腔内に遊離していた虫体5隻を合わせると総数 508隻の虫体が検出された.虫嚢内から得られた虫体の うち無作為に抽出して検査した200隻の虫体は,いずれ もよく成熟した成虫であり,これらの成虫および虫嚢内 虫卵はその形態的特徴からすべてウエステルマン肺吸虫 (二倍体型)と同定された. 病理組織学的検査では, 胸 膜あるいは虫嚢周辺部の増殖性変化(結合織の増生)の 他に,肺気腫および水腫などが顕著に認められると同時 に,実質中には所々に虫卵の集積巣が認められた. ま た,糞便1g中の排出虫卵数(E.P.G.)は91,480であ った.タヌキにおける肺吸虫のこのような極端な多数寄 生例は極めて珍しい事例であると同時に, タヌキが本 虫の自然終宿主として重要な役割を果しているとする Shibahara (1985)の考えを支持するものとして報告し た.