Relationship between Pulmonary Arterial Pressure and Pulmonary Thromboembolism Associated with Dead Worms in Canine Heartworm Disease

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ABSTRACT. To examine effects of thromboemboli due to dead worms on pulmonary arterial pressure (PAP), 20 to 50 dead heartworms were inserted into the pulmonary arteries of 4 heartworm uninfected dogs (uninfected group) and 11 dogs infected with heartworms (infected group). In the uninfected group, the mean PAP rose 1 week after worm insertion (10.9 to 166. mmHg), but it recovered by the 4th week. Clinical signs, hemodynamics and blood gas findings also deteriorated at the 1st week, but recovered at the 4th week. Angiographic and pathological findings indicated that blood flow recovered through the spaces between thromboemboli and vessel walls at the 4th week. The infected dogs were divided into three groups. In the infected-I group (5 dogs), the intimal lesions of the pulmonary arteries were slight, and clinical and laboratory findings showed changes similar to those of the uninfected group. In the infected-II group (4 dogs), the pulmonary arterial lesions were severe and the mean PAP was higher (25.7 mmHg) than in the uninfected group before worm insertion. An increase in PAP (34.1 mmHg) and worsening of clinical and laboratory findings were noticed till the 4th week. Thromboemboli adhered extensively to the vessel walls. Two dogs in the infected-III group died of severe dyspnea on the 9th and 10th day, and the mean PAP rose remarkably at the 1st week (from 19.4 to 28.2 mmHg). Severe pulmonary parenchymal lesions with edema or perforation were observed. From the above results, it was clarified that effects of dead worms on PAP and clinical signs depended on the severity of pulmonary arterial lesions before worm insertion. These results may explain the clinical diversity in spontaneous canine heartworm disease.—KEY WORDS: Dirofilaria immitis, dog, heartworm, pulmonary hypertension, thromboembolism.

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In pulmonary heartworm disease, it is well known that pulmonary hypertension is one of the most important causes inducing clinical signs [14, 20, 26]. Many factors such as intimal lesion [20], parenchymal lesions [26], live adult worms [10, 12, 24] and thromboembolism [14, 15, 20, 26] are considered to cause in pulmonary arterial pressure (PAP) to increase in heartworm disease. The authors have considered that thromboembolism associated with naturally dead worms is the most important factor in pulmonary hypertension in spontaneous heartworm disease, because fresh thromboemboli containing many dead worms were noticed in the pulmonary arteries of cases showing serious signs such as caval syndrome [13] and pulmonary heartworm disease with ascites, and thromboemboli had the highest multiple coefficient correlation with PAP among cardiopulmonary lesions [25]. Knight [15] also observed that pulmonary arterial pressure (PAP) rose considerably due to the natural death of worms. Results as to the effects of dead worms after adulticidal treatment have varied among investigators; some reported favorable results of adulticidal treatment without particular clinical problems [5, 17–20], whereas others revealed some clinical troubles such as dyspnea or even death [6–8]. Although the mechanism inducing such a variety of results has not been reported, prior cardiovascular lesions were considered to be contributing factors [14]. There are few reports on the effects of worm death from both the standpoint of hemodynamics and pathological changes. Rawlings *et al.* [17, 18] showed that radiographic findings improved and PAP fell 7 and 12 month after adulticidal therapy. Atwell *et al.* [2, 3] investigated the relationship between dead worms and pulmonary arterial lesion, but not between PAP and the lesions.

The present report describes the relationship between changes in PAP and lesions after dead heartworm insertion (worm insertion) in order to clarify the effects of dead worms.

MATERIALS AND METHODS

Fifteen mongrel dogs, presumed to be 2 to 8 years old and weighing 7 to 15 kg, were used. Natural heartworm infection was confirmed by echocardiography at the beginning of the experiment and by necropsy at its end. Twenty to 50 dead female worms per dog (2 to 6 /kg) were surgically inserted

into the pulmonary arteries through the jugular vein. Live worms in the pulmonary arteries sometimes migrate to the tricuspid orifice and cause regurgitation of blood flow after dead worm insertion [11]. Therefore, in dogs infected with heartworms, dead worms were inserted after the removal of live worms. Flexible alligator forceps [9] were used for dead worm insertion and live worm removal.

Dead worms for insertion into the pulmonary arteries were prepared by the following procedure. Live worms were obtained by necropsy from dogs with natural infection, and stored in a refrigerator (−30°C) until insertion. Then, these dead worms were washed with saline containing 5 IU/m*l* of heparin and used for insertion.

Clinical observations, pulmonary angiography and measurements of hemodynamics and blood gas were carried out before, and 1 and 4 weeks after worm insertion. Hemodynamics measurements, echocardiography, pulmonary arterial angiography, and histopathological examinations were performed by the same methods described in our previous reports [10, 13]. Arterial blood was collected from the femoral artery by puncture and blood gas was analyzed with 168 pH/Blood Gas Analyzer (Corning Medical and Scientific, Halstead, England). Blood gas data were corrected to body temperature of the dog and blood hemoglobin concentration.

All dogs were euthanatized with overdosing of anesthesia at the end of the experiment for necropsy. Paired *t* test was used for statistical comparison of the data before and after dead heartworm insertion, and Student *t* test was used for comparison of the data between groups.

RESULTS

Experimental dogs were retrospectively divided into 4 groups after the experiment (Table 1). Changes in clinical signs are shown in Table 2. Slight depression, anorexia and moderate coughing were noticed about 1 week after worm insertion, but these symptoms disappeared within the 4th week in most cases in the uninfected and infected-I groups. In the infected-II group, slight or moderate dyspnea, cyanosis and accented second heart sound, as well as depression, anorexia and coughing at the 1st week, were noticed in all cases. These signs tended to worsen, and subcutaneous edema of legs was also seen in 2 of 4 cases at the 4th week. Furthermore, in the infected-III group, severe clinical signs were noticed since 1 day after worm insertion and worsened gradually. Dogs died on the 9th (dog No. 1809) and 10th (dog No. 1884) day.

Changes in hemodynamics findings are shown in Table 3. In the uninfected group, the mean PAP valve (mean±SD) rose moderately from pre-insertion to 1 week after worm insertion (10.8±1.6 to 16.6±3.6 mmHg), but fell to about the pre-

Table 1. Profile of experimental dogs and number of heartworms

Group	Dog				Number of heartworms			
	No.	Age (y)	Body weight (kg)	Sex	Removed	Remaining	Inserted	No. of insertion/kg of body weight
Uninfected	1788	3	15	Female	0	0	30	2.0
	1794	2	11	Male	0	0	30	2.7
	1802	2	9	Female	0	0	30	3.3
	1828	3	11	Male	0	0	50	4.5
Infected-I	1772	6	11	Male	47	3	30	2.7
	1810	3	6	Female	41	0	30	5.0
	1868	5	9	Male	36	2	30	3.3
	1889	4	8	Female	14	3	50	6.2
	1909	4	11	Male	35	7	30	2.7
Infected-II	1784	8	11	Male	3	0	24	2.1
	1826	6	14	Male	41	3	30	2.1
	1850	7	11	Male	47	0	30	2.7
	1861	8	10	Male	29	2	28	2.8
Infected-III	1809	4	7	Female	7	1	20	2.8
	1884	3	10	Male	18	5	30	3.0

Table 2. Changes in clinical signs after dead heartworm insertion into pulmonary arteries

Group	Dog	Depression			Anorexia			Coughing			Dyspnea			Cyanosis			Subcutaneous edema		
	No.	Pre[a]	1w[b]	4w[c]	Pre	1w	4w	Pre	1w	4w	Pre	1w	4w	Pre	1w	4w	Pre	1w	4w
Uninfected	1788	−	+	+	−	+	+	−	++	+	−	−	−	−	−	−	−	−	−
	1794	−	+	−	−	+	−	−	++	+	−	−	−	−	−	−	−	−	−
	1802	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	1828	−	+	−	−	+	−	−	++	−	−	+	−	−	+	−	−	−	−
Infected-I	1772	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	1810	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	1868	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	1889	−	+	−	−	−	−	−	++	−	−	+	−	−	−	−	−	−	−
	1909	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−
Infected-II	1784	−	++	++	−	+	+	−	+	+	−	+	++	−	++	+++	−	−	++
	1826	−	++	+	−	+	+	−	+	++	−	++	++	−	+	+	−	−	−
	1850	−	++	++	−	+	+	−	+++	+++	−	+	+	−	+	++	−	−	+
	1861	−	+	++	−	−	+	−	++	++	−	+	+	−	++	−	−	−	−
Infected-III	1809	−	++	ND[d]	−	+++	ND	−	++	ND	−	+++	ND	−	+++	ND	−	−	ND
	1884	−	+++	ND	−	+++	ND	−	+++	ND	−	+++	ND	−	++	ND	−	−	ND

a) Before insertion of heartworms. b) One week after heartworm insertion. c) Four weeks after heartworm insertion.
d) Not done.
Grade of clinical sign: −...normal, +...mild, ++...moderate, +++...severe.

Table 3. Changes in right-heart hemodynamic values after dead heartworm insertion into pulmonary arteries

Item	Group	No. of	Pre[a]	Post-1w[b]	Post-4w[c]
		dogs	Mean±SD	Mean±SD	Mean±SD
Diameter of pulmonary arterial trunk (mm)	Uninfected	4	14.8±0.8	20.0±2.4*	16.5±1.1
	Infected-I	5	15.6±1.5	21.2±4.2*	18.2±3.4
	Infected-II	4	23.2±3.2#	27.0±3.6*	28.0±2.5*
	Infected-III	2	17.0±1.0	29.0±1.0	ND[e]
Mean PAP[d] (mmHg)	Uninfected	4	10.8±1.8	16.6±3.6*	13.4±2.1
	Infected-I	5	15.0±2.4	19.3±4.1*	15.0±1.4
	Infected-II	4	25.7±29.9#	29.9±12.3	34.1±17.7
	Infected-III	2	19.4±4.7	28.2±5.5	ND[e]
Right ventricular EDP[f] (mmHg)	Uninfected	4	0.85±1.21	2.00±1.45	0.83±1.18
	Infected-I	5	0.40±0.77	2.48±1.72	1.22±1.44
	Infected-II	4	1.03±1.54	5.78±2.00	5.54±1.70
	Infected-III	2	1.00±0.20	7.30±0.50*	ND
Cardiac output (l/min)	Uninfected	4	3.08±0.72	2.93±0.74	2.65±0.72**
	Infected-I	5	2.67±0.45	2.37±0.39	2.03±0.35*
	Infected-II	4	3.04±0.50	2.74±0.38	2.28±0.20*
	Infected-III	2	2.43±0.15	2.06±0.41	ND
Total pulmonary resistance (dyne·sec·cm^{-5}·kg)	Uninfected	4	3390±536	5299±1199*	4138±688
	Infected-I	5	4126±1419	5839±1682**	4444±1241
	Infected-II	4	7927±2999#	10302±4230	12390±4953*
	Infected-III	2	5715±2242	7735±2651	ND

a) Before worm insertion. b) One week after worm insertion.
c) Four weeks after worm insertion. d) Pulmonary arterial pressure.
e) Not done. f) End diastolic pressure.
*: Significant difference from pre-worm insertion value ($P<0.05$).
**: Significant difference from pre-worm insertion value ($P<0.01$).
#: Significant difference from uninfected group ($P<0.05$).

insertion level at the 4th week. There was no correlation between the grade of PAP rising at the 1st week and number of worms inserted. The pulmonary arterial diameter (PAD), right ventricular and end diastolic pressure (RV-EDP) and total pulmonary resistance (TPUR) changed in parallel with PAP. In the infected-I group, these parameters showed a similar pattern to that of the uninfected group. In the infected-II group, the mean PAP was higher than in the uninfected group before worm insertion (25.7±29.9 mmHg), and rose continuously for 4 weeks after worm insertion. PAD and RV-

Table 4. Changes in blood gases after dead heartworm insertion into pulmonary arteries

Item	Group	No. of	Pre[a]	Post-1w[b]	Post-4w[c]
		dogs	Mean±SD	Mean±SD	Mean±SD
PaO_2 (mmHg)	Uninfected	4	97.0±1.3	82.3±13.0	89.4±6.8
	Infected-I	5	94.9±4.5	79.4±12.6*	85.8±4.5*
	Infected-II	4	86.6±8.4	71.4±2.2*	63.9±9.2**
	Infected-III	2	88.0±1.1	63.4±2.5*	ND[d]
$PaCO_2$ (mmHg)	Uninfected	4	33.0±1.6	36.8±1.3	33.6±4.7
	Infected-I	5	34.2±2.9	35.3±3.8	32.6±1.9
	Infected-II	4	30.3±2.9	31.0±5.7	29.5±2.2
	Infected-III	2	32.6±2.3	27.1±0.3*	ND
$AaDO_2$[c] (mmHg)	Uninfected	4	12.2±1.8	29.4±14.3	18.1±7.7
	Infected-I	5	12.0±6.0	26.4±8.0*	21.4±6.5
	Infected-II	4	25.3±10.0	39.7±8.6*	49.0±11.2**
	Infected-III	2	21.0±3.9	52.9±3.3**	ND
Pulmonary shunt rate (%)	Uninfected	4	4.4±1.8	22.3±14.1	3.2±1.2
	Infected-I	5	4.0±2.1	16.3±10.2*	9.4±4.4*
	Infected-II	4	7.5±4.7	18.4±6.4*	26.3±10.0**
	Infected-III	2	7.0±0.4	24.7±3.3*	ND

a) Before worm insertion. b) One week after worm insertion. c) Four weeks after worm insertion. d) Not done. e) Alveolar-arterial oxygen difference.
*: Significant difference from pre-worm insertion value ($P<0.05$).
**: Significant difference from pre-worm insertion value ($P<0.01$).

Table 5. Changes in pulmonary arterial angiograms

Group	Dog	Dilation			Stricture			Obstruction			Recanalization		
	No.	Pre[a]	1[b]	4[c]	Pre	1	4	Pre	1	4	Pre	1	4
Uninfected	1788	−	++	+	−	−	−	−	+	−	−	−	−
	1794	−	−	++	−	−	−	−	++	+	−	−	+++
	1802	−	−	+	−	−	−	−	+	+	−	+	+
	1828	−	+	+	−	−	−	−	+	−	−	+	+++
Infected-I	1772	+	+	+	−	−	−	+	++	++	−	−	+
	1810	+	++	++	−	−	−	−	++	+	−	+	++
	1868	+	+	+	−	−	−	−	+	+	−	−	++
	1889	−	+	+	−	−	−	−	+	−	−	−	++
	1909	+	++	++	+	+	+	−	++	+	−	−	++
Infected-II	1788	++	++	++	+++	+++	+++	++	++	+++	−	−	−
	1826	+++	+++	++	++	++	++	+	+	++	−	−	−
	1850	++	++	++	++	++	++	+++	+++	+++	−	−	−
	1861	+++	+++	+++	+++	+++	+++	+++	+++	+++	−	−	−
Infected-III	1809	+	++	ND[d]	−	−	ND	−	+++	ND	−	−	ND
	1844	+	+++	ND	−	−	ND	+	+++	ND	−	−	ND

a) Before worm insertion. b) One week after worm insertion. c) Four weeks after worm insertion. d) Not done. Severity of lesion: −...no change, +...mild, ++...moderate, +++...severe.

EDP increased moderately at the 1st week, and TPUR increased continuously till the 4th week. In the infected-III group, PAP, PAD and RV-EDP increased remarkably at the 1st week. Cardiac output tended to decrease at the 4th week in all groups.

Blood gas findings are shown in Table 4. In the uninfected and infected-I groups, arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), alveolar-arterial oxygen difference ($AaDO_2$) and pulmonary shunt rate became somewhat worse at the 1st week and tended to recover at

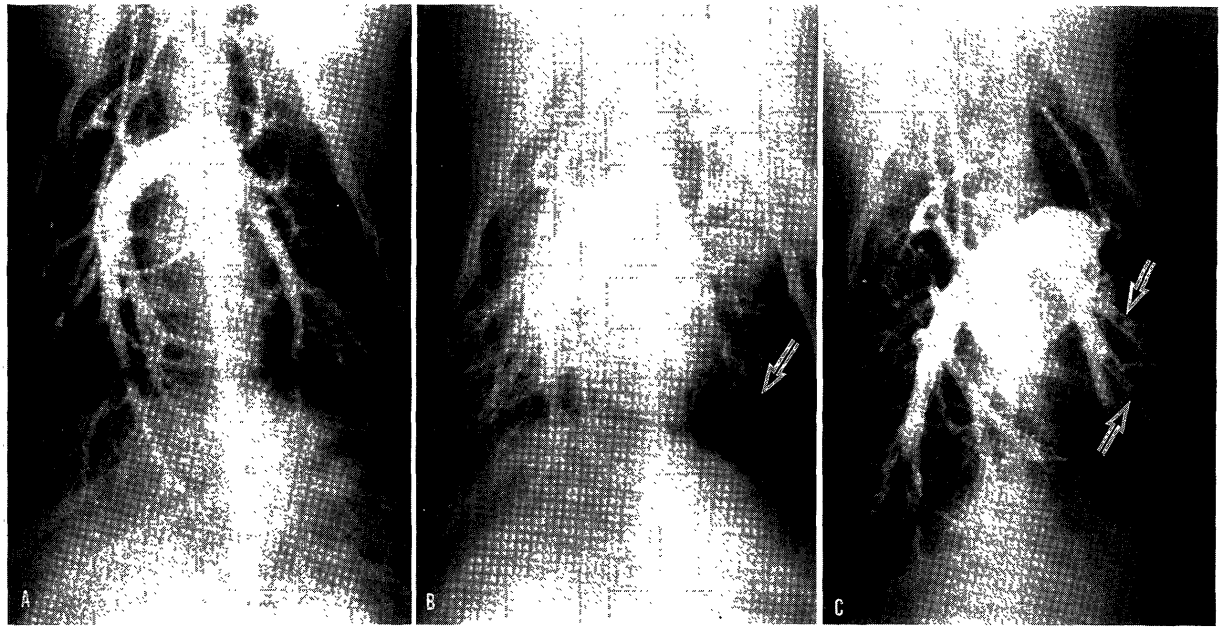

Fig. 1. Pulmonary angiogram in the uninfected group (dog No. 1794) before (A), 1 week (B) and 4 weeks (C) after worm insertion. Dilation of the main pulmonary arteries and obstruction of lobar arteries in left caudal lobe (arrow) were noticed 1 week after worm insertion, and recanalizations (arrows) around obstruction and peripheral blood flows were seen 4 weeks after worm insertion.

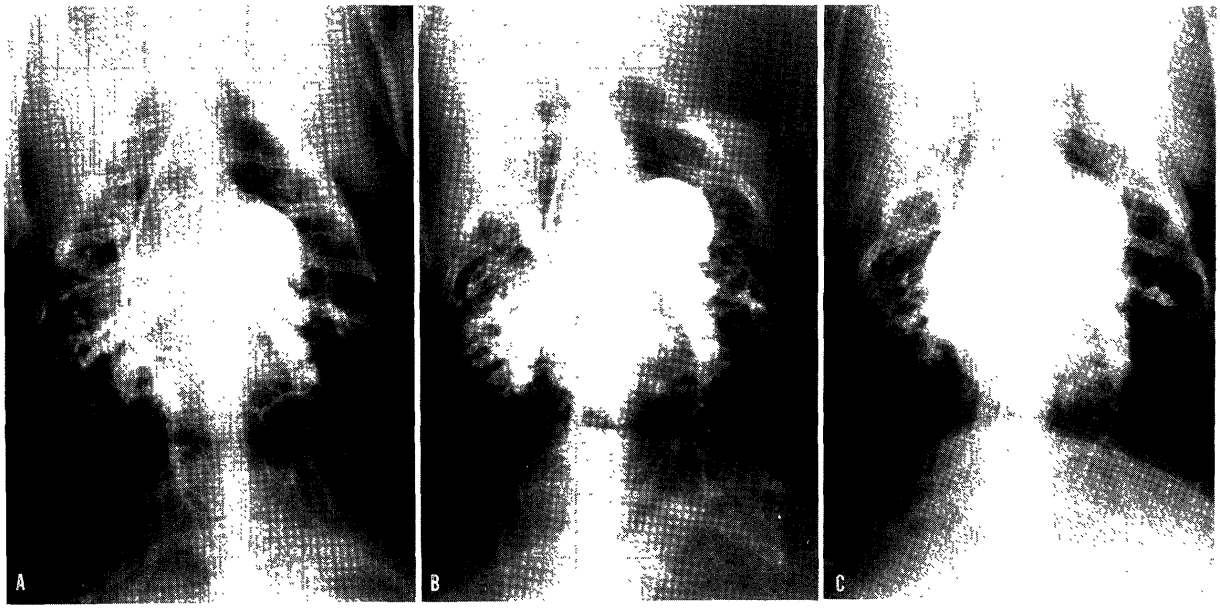

Fig. 2. Pulmonary angiogram in the infected-II group (dog No. 1784) before (A), 1 week (B) and 4 weeks (C) after worm insertion. Dilation, stricture and obstruction of the pulmonary arteries were noticed even before worm insertion. Obstruction became progressively worse till 4 weeks after worm insertion.

the 4th week. In the infected-II group, these parameters had been moderately worse before worm insertion, and deteriorated remarkably at the 4th week. In the infected group-III, blood gas findings were also moderately worse before worm insertion and became much worse at the 1st week.

The angiographic findings are summarized in Table 5. In almost all cases of the uninfected and infected-I groups, the dilation of the main and lobar pulmonary arteries and obstruction of blood flow recognized in each lobe, were especially marked in the caudal lobes at the 1st week. A little blood flow was, however, recognized around obstructed substances at the 4th week (Fig. 1). In the infected-II group, slight or serious dilation, stricture and obstruction of the pulmonary arteries were noticed in almost all cases before worm insertion, and obstruction of the pulmonary arteries grew worse 1 to 4 weeks after worm insertion, but recanalization was not seen (Fig. 2). In the infected-III group, severe obstructions were found in almost all lobes and peripheral blood flow was virtually unobservable at the 1st week.

The histological findings showed a slight endothelial proliferations in the main and lobar pulmonary arteries in all cases of the infected-I group, but not in the uninfected group. Reddish-brown or grayish thrombi with columnar shapes surrounded dead worms densely arranged in bundles along the arteries in both groups. These lesions were noticed in the main and large lobar pulmonary arteries in each lobe, and were especially numerous in the caudal lobes. The thrombi adhered to vessel walls along one side, and narrow spaces between no adhering part of the thrombi and vessel walls were seen. The thrombi fibrocytes extended from one side of the vessel subendothelium and surrounded the dead worms (Fig. 3). There were small consolidations with hemorrhage in the parenchyma around some thromboemboli in all cases in both groups. In the infected-II group, severe intimal proliferations with rugous, grainy, pebbly, or villous form were noticed in almost all cases. Dead worms were arranged in disorderly fashion in the main and lobar arteries of each lobe. Thrombi around the worms adhered extensively to vessel walls and no spaces between the thrombi and vessel wall were detected (Fig. 4). Organized thromboemboli contained some worm fragments, which might have developed prior this experiment, were also found mainly in the peripheral arteries in the caudal lobe. Consolidations and hemorrhages in the parenchyma

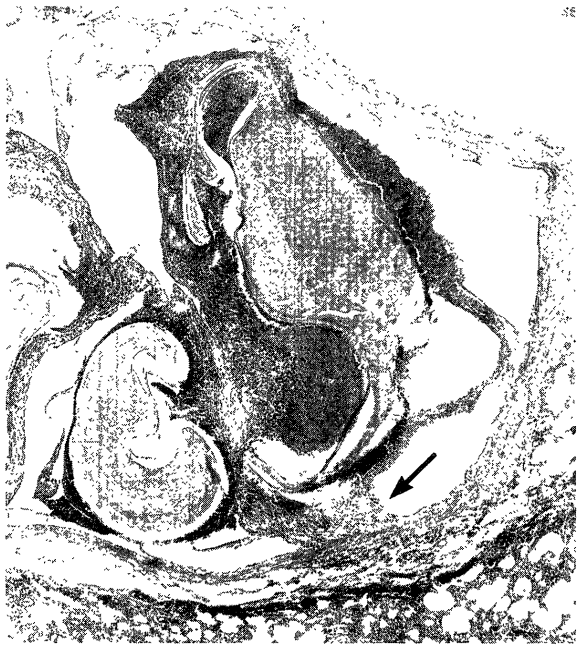

Fig. 3. Microscopic findings of thrombus around dead worms in the lobular pulmonary artery in the infected-I group (dog No. 1772). The fibrocytes extended from one side (arrow) of the vessel subendothelium and surrounded the dead worms. Gaps between thrombus and vessel wall were noticed. × 150.

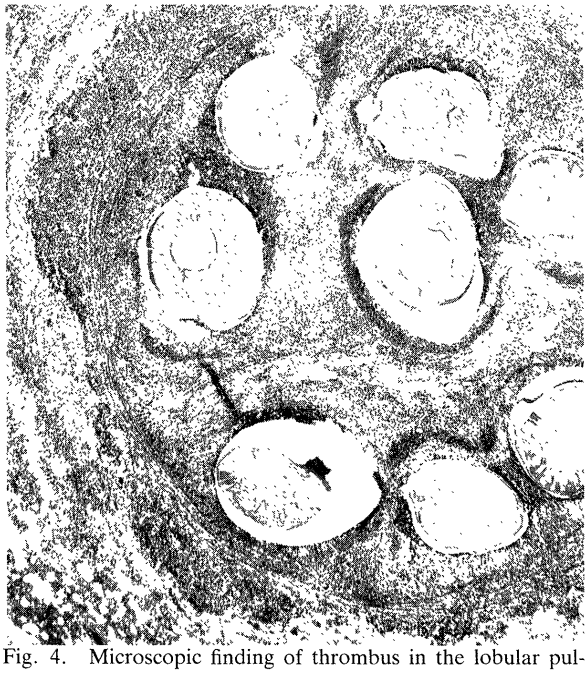

Fig. 4. Microscopic finding of thrombus in the lobular pulmonary artery in the infected-II group (dog No. 1784). Thrombus adhered entirely to vessel walls, and severe cell infiltrations were seen in the thrombus and artery wall and alveolar wall around the artery. × 110.

were severer than those of the infected-I group. In the infected-III group, thromboemboli were more proximate to lobar arteries in all lobes and adhered extensively to the vessel walls. Severe pneumonic lesions with either edema (dog No. 1809) or perforation (dog No. 1844) were noticed.

DISCUSSION

In the uninfected and infected-I groups, clinical, hemodynamic, angiographic and blood gas findings became worse at the 1st week, but they improved and recanalization was noticed at the 4th week. On the other hand, these findings gradually became worse till 4 weeks after worm insertion, and recanalization was not seen in the infected-II group. It was considered that the prior severity of the pulmonary arterial lesions was involved in these differences.

Intimal proliferation and thromboemboli are usually noticed as the pulmonary arterial lesions in heartworm disease [14, 20]. Intimal proliferative lesions are induced by endothelial injury due to mechanical irritation of live heartworms, and the severity of intimal lesion depends on the duration of infection and the number of live worms harbored [14, 20]. It was reported that thromboemboli were formed by dead worms and fragments of intimal lesions [1–3, 14, 20]. Most of the live worms generally exist in caudal lobes [4, 14], so the pulmonary arterial lesions are more frequent there, and serve to impede blood flow and undermine vessel compliance [14, 20]. Intimal lesions are found 90 to 130 days after infective larvae, but the period of 3 to 5 years is commonly required for developing of cor-pulmonale [14].

From these evidences, the following processes in rising PAP were considered to take place in the infected-I and -II groups. In the uninfected and infected-I groups having slight or no pulmonary lesions, dead worms were carried into peripheral arteries in the caudal lobes by strong blood flow [4, 14], then thrombi developed around the dead worms and PAP rose due to blood flow obstruction or vessel contraction about 1 week after worm insertion. Thereafter, however, the space between the thrombi and vessel wall might have developed by dilation of blood vessels, because vessels remained elastic and adhered to thrombi in only limited areas, and/or by shrinking of thrombi due to fibrosis. Then blood flow was recanalized through the space. On the other hand, in the infected-II group already having severe lesions in the pulmonary arteries and impeded blood flow to caudal lobes before worm insertion, the dead worms inserted were also carried into other lobes in addition to caudal lobes. Then thromboemboli around worms might have adhered progressively over the surface of the vessel, because the vessel was already injured and lost its compliance by prior lesions [20]. Blood flow was therefore obstructed over a greater area, and clinical, hemodynamics and blood gas findings reflected this serious deterioration.

Many attempts have been made to clarify the mechanism of pulmonary hypertension in the pulmonary embolism in man, who shows severe symptoms and high mortality [16, 21, 23]. However, the mechanism is still obscure. Since mechanical obstruction of the pulmonary arteries is not considered only a causative agent for producing the pulmonary hypertension, it was suggested that the prior cardiopulmonary disease, particularly emboli in the pulmonary arteries was the most important factor in patients with acute pulmonary embolism [16, 21, 23]. These findings agreed with results of this study; the grade of rising PAP was not dependent on the number of worms inserted, but on the severity of pulmonary arterial lesions.

In the canine heartworm disease, thromboembolism associated with dead worms is considered one of the most important factors of pulmonary hypertension [14, 15, 20], although it has not been confirmed experimentally. Rawlings *et al.* [17–20] reported effects of adulticidal treatment showed no serious clinical problems. These results were suspected to depend on the slight pulmonary arterial lesion of experimental dogs, because the dogs were used 12 months after inoculation of infective larvae. Carlisle [8] reported the development of clinical problems after adulticidal treatment, such as signs to congestive heart failure or even death, coincided roughly with clinical signs before treatment in 228 spontaneous cases. These observations also supported the results of this study.

Two dogs in the infected III group died of severe complications of parenchymal lesions with edema or perforation 9 and 10 days after worm insertion, respectively. Perforation of the pulmonary parenchyma was reported after adulticidal treatment in heartworm disease [22]. It was considered that pulmonary perforation and edema were induced by circulation failure due to sudden obstruction of the pulmonary arteries [22].

From the above results, the effects of worm death on PAP and clinical signs are different due to the prior severity of pulmonary arterial lesions. These results will be useful to the explain clinical diversity in spontaneous canine heartworm disease. Further studies are needed concerning the detail precise changes in hemodynamics and morphology of thromboemboli in the early stage in cases with slight lesions and in the longer term in cases with severe lesions.

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