Serum Haptoglobin Levels in Canine Dirofilariasis

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ABSTRACT. Serum haptoglobin (Hp) concentration was determined as a hemoglobin binding capacity in normal dogs and dogs with dirofilariasis. The serum Hp level in 13 normal dogs was 114.8 ± 31.5 (S.D.) mg/dl. In 33 dogs with mild dirofilariasis, serum Hp concentration ranged widely from 0 to 349.5 mg/dl with a mean of 75.1 ± 151.4 mg/dl. In this group, the case which showed respiratory abnormalities had a higher serum Hp level. The cases in which urine or renal hemosiderin was detected showed low serum Hp concentrations. In 17 dogs with chronic serious dirofilariasis, the serum Hp level was 33.7 ± 59.8 mg/dl. In this group, only 2 cases had a slightly higher serum Hp concentration. Serum Hp level of 0 mg/dl was found in 8 cases (47.1%). In 21 dogs with dirofilarial hemoglobinuria (caval syndrome), the serum Hp concentration ranged from 9.9 to 135.0 mg/dl with a mean of 48.2 ± 29.5 mg/dl. The serum Hp level in this group was higher than in the serious group. The plasma hemoglobin concentration was higher than the serum Hp concentration in all cases in the hemoglobinuria group. From these findings, the intravascular hemolysis should exist not only in the hemoglobinuria group but also in the mild and serious groups. —**KEY WORDS:** dirofilariasis, dog, intravascular hemolysis, serum haptoglobin.

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Haptoglobin (Hp) is a serum α -glycoprotein synthesized by the liver parenchymal cells [1], which forms a stable complex with free hemoglobin (Hb) [5, 13], and also is an acute-phase reactant [11, 13, 15]. It is well recognized that the serum Hp decreased in the hemolytic states [2, 4, 6, 12, 13, 19], and increased in the inflammatory reaction [11, 13, 15].

Canine dirofilarial hemoglobinuria (caval syndrome) associates with severe intravascular hemolysis [10]. From the detection of urine or renal hemosiderin, it has been suggested that the intravascular hemolysis might exist also in mild and serious dirofilariasis [10, 21]. Besides, canine dirofilariasis complicates with inflammatory reactions associated with parasitism of adult heartworms and microfilariae [17]. However, there has been no report about the serum Hp level in canine dirofilariasis. In this

study, the serum Hp concentration was determined as an Hb binding capacity, and the factors influencing serum Hp levels in dogs with dirofilariasis were discussed.

MATERIALS AND METHODS

Animals: Seventy-one dogs with dirofilariasis were classified into 3 groups from their clinical signs and laboratory test results (Table 1). Thirty-three dogs, aged from 1.5 to 8 years old and consisting of 18 males and 15 females, were classified into the mild group. The clinical signs of the mild group were mild anemia, arrhythmia, accentuated second heart sound, cough, rough vesicular breath sound, labored respiration and so on. The serious group included 17 dogs with chronic serious dirofilariasis. The dogs in this group were aged from 4 to 10 years old, and consisted of 6 males and 11 females.

Table 1. Laboratory test results in four groups

Item	Group											
	Control			Mild			Serious			Hemoglobinuria		
	n	Mean	S.D. ^{a)}	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
Ht (%)	13	53.0	3.5	33	38.5	13.4	17	27.9	7.4	21	24.9	10.5
Hb (g/dl)	13	18.0	1.3	33	10.9	3.8	17	8.4	2.2	20	7.8	3.6
RBC ($\times 10^4/\mu\text{l}$)	13	730	65	33	582	228	17	438	131	21	361	168
WBC ($\times 10^2/\mu\text{l}$)	13	65	12	31	133	119	17	187	119	21	216	103
GOT (IU/l)	13	29.6	7.5	30	39.1	17.0	17	53.4	32.3	19	201.8	124.5
GPT (IU/l)	13	33.3	7.0	26	52.6	40.6	17	61.5	43.1	20	231.2	451.7
ALP (IU/l)	13	68.2	19.1	33	148.2	131.5	14	262.6	362.6	20	332.9	255.3
T.P (g/dl)	13	6.1	0.3	33	6.6	0.9	17	6.6	1.1	20	7.0	0.7
Albumin (g/dl)	13	3.33	0.31	31	2.88	0.45	17	2.45	0.71	19	2.34	0.51
A/G ratio	13	1.23	0.17	31	0.83	0.27	17	0.64	0.26	19	0.52	0.17
Plasma Hb (mg/dl)	13	4.2	2.5	18	5.5	5.3	16	12.0	7.4	21	196.3	157.2

a) Standard deviation.

The signs of the serious group were severe anemia, cough, rough vesicular breath sound, labored respiration and signs of cardiac dysfunction such as arrhythmia, dullness of heart sound, accentuated second heart sound and/or grade II systolic cardiac murmur. Some cases showed ascites (8 cases), subcutaneous edema (3 cases) and jaundice (2 cases). The hemoglobinuria group included 21 dogs with spontaneous dirofilarial hemoglobinuria, aged from 3 to 8 years old, and consisted of 17 males and 4 females. All the cases in this group showed the so-called "caval syndrome." Some cases also showed ascites (4 cases) and jaundice (15 cases). In the hemoglobinuria group, serum Hp concentration was determined also 20 hr after surgical heartworm removal [7] in all dogs. As the control group, 13 normal and dirofilaria-free beagles, aged from 4 or 5 years old, consisting of 6 males and 7 females, were used.

Methods: Peripheral blood was collected from experimental dogs. Blood serum was separated as soon as possible after collection, and stored at -70°C until determination. The serum Hp concentration was determined as an Hb binding capacity with

modified Yachi method [22]. In this method, Hp-Hb complex and free Hb were separated with cellulose acetate electrophoresis, and Hb was colored by peroxidase reaction. Separax-S (Jookoo Co., Ltd., Tokyo) was used for the supporting membrane. Electrophoresis was carried out in 0.05 M phosphate buffer solution (pH 6.0) at 20°C , and with a current of 0.7 mA/cm for 50 min. Under this condition, free Hb migrated towards the cathode and Hp-Hb complex towards the anode. After heating at 100°C for 15 min to fix the Hb to the membrane, coloration of Hb was made with a o-dianisidine reagent. Densitometry was made at 440 nm with a Dual Wave Length TLC Scanner (TC-901, Shimadzu Corp., Kyoto). The amount of Hb, which was added to serum to saturate the Hp, was measured by benzidine reaction. Serum Hp concentration was calculated as follows;

$$\text{Serum Hp concentration (mg/dl)} = \frac{\text{Hb concentration of sample (mg/dl)} \times \text{Hp-Hb complex (\%)}}{\text{Hp-Hb complex (\%)} + \text{Free Hb (\%)}}$$

Urine and renal hemosiderin was detected by Prussian blue reaction. Other laboratory tests were carried out by the standard

Fig. 1. Serum haptoglobin concentration in four groups.

procedures.

RESULTS

Fig. 1 shows the serum Hp concentrations in 4 groups. In the control group ($n=13$), serum Hp concentration ranged from 77.3 to 179.7 mg/dl with a mean of 114.8 ± 31.5 (S.D.) mg/dl. The normal range calculated ($\text{mean} \pm 2\text{S.D.}$) was from 51.8 to 177.8 mg/dl. In the mild group ($n=33$), serum Hp concentration ranged widely from 0 to 349.5 mg/dl with a means of 75.1 ± 151.4 mg/dl. In 6 cases (18.2%), serum Hp concentration was higher than the normal range, but lower in 18 cases (54.5%). Serum Hp level of 0 mg/dl (absent or trace level) was found in 8 cases (24.2%). Serum Hp level in cases with respiratory abnormalities such as cough, slight rough vesicular breath sound and labored respiration was significantly higher than in cases without the signs ($p < 0.01$, Table 2). There was no significant difference between the serum Hp levels in cases with or without the signs such as cardiac

disfunctions and anemia. In the serious group ($n=17$), serum Hp concentration was slightly higher in 12 cases, but lower than the normal range in 11 of the 17 cases (64.7%). Serum Hp level of 0 mg/dl was found in 8 cases (47.1%). The mean level of serum Hp level in the serious group, which was 33.7 ± 59.8 mg/dl, was significantly lower than in the control group ($p < 0.01$). In the hemoglobinuria group ($n=21$), serum Hp concentration ranged from 9.9 to 135.0 mg/dl with a mean of 48.2 ± 29.5 mg/dl. In this group, serum Hp level was slightly lower than in the control group, but higher than in the serious group. No case had a serum Hp level of 0 mg/dl. However, the plasma Hb concentration was higher than the serum Hp concentration in all cases in the hemoglobinuria group. Serum Hp level in 21 cases 20 hr after the surgical removal of heartworms was 62.5 ± 40.3 mg/dl. There was no significant difference between the serum Hp levels before and 20 hr after heartworm removal. In the serious and hemoglobinuria groups, there were no sig-

Table 2. Serum haptoglobin levels in dogs with or without the signs

Group	Sign	Serum haptoglobin level (mg/dl)						p< ^{b)}
		With the sign			Without the sign			
		n	Mean	S.D. ^{a)}	n	Mean	S.D.	
Mild	Respiratory abnormalities ^{c)}	7	221	50	26	36	48	0.01
	Cardiac dys- functions ^{d)}	25	65	98	8	99	79	NS ^{e)}
	Anemia ^{f)}	8	108	123	25	65	80	NS
Serious	Ascites	8	35	22	9	32	20	NS
	Jaundice	2	25	25	15	35	16	NS
Hemoglobinuria	Ascites	4	40	29	17	50	30	NS
	Jaundice	10	46	25	11	50	34	NS

a) Standard deviation.

b) Probability of significant difference.

c) Cough, rough vesicular breath sound and labored respiration.

d) Arrhythmia, dullness of heart sound, accentuated second sound and cardiac murmur.

e) Not significant.

f) Ht value was below 30%.

Fig. 2. Serum haptoglobin concentration in the urine or renal hemosiderin negative and positive cases of the mild group.

nificant differences between the serum Hp levels in cases with or without the signs of such as ascites and jaundice (Table 2).

Urine or renal hemosiderin was positive in 22 of 33 cases (66.7%) in the mild group, as shown in Fig. 2, and the serum Hp concentration ranged widely in both the hemosiderin positive and negative cases. However, a low serum Hp concentration (<51.8 mg/dl) was found in 16 (72.7%) of the hemosiderin positive 22 cases and 2 (18.2%) of the hemosiderin negative 11 cases. In the serious and hemoglobinuria group, urine hemosiderin was positive in all cases. As mentioned above, the serum Hp levels were lower in these 2 groups.

DISCUSSION

In the present study, the serum Hp level was considerably low in a substantial portion of dogs with mild and serious dirofilariasis. Most cases, in which urine or renal hemosiderin was detected, had a low serum

Hp concentration. These findings indicate that intravascular hemolysis may exist also in the mild and serious groups. The reason why no clear hemoglobinemia and hemoglobinuria were found was that the intravascular hemolysis might be very mild and persistent. The dogs with dirofilariasis were anemic [17]. Because of the modifying influence of underlying disorders, it is difficult to confirm the pathogenetic mechanism of anemia in dirofilariasis. However, it was suggested that the intravascular hemolysis may participate in anemia of dirofilariasis. This hemolysis may result from some physical mechanism because of the increased mechanical fragility of circulating erythrocytes [10] and abnormal blood stream associated with the existence of heartworms and lesions in the pulmonary artery. The dogs with dirofilariasis were commonly accompanied with liver disorders [10, 16, 17]. The decrease of Hp synthesis might participate in decrease of serum Hp level in cases with very severe liver disorders of the serious and hemoglobinuria groups [18].

On the other hand, one of the systemic responses to inflammation is a striking increase in the hepatic synthesis of acute-phase reactants such as Hp, ceruloplasmin, fibrinogen and the like [11, 13]. Canine dirofilariasis is commonly complicated with endoarteritis of the pulmonary arteries, interstitial fibrous inflammation of the lung, liver injuries and glomerulonephritis [9, 14, 17, 20]. These lesions might act as a factor to stimulate the Hp synthesis. In the present study, the cases displaying respiratory abnormalities had the higher serum Hp concentrations. The lung lesion might greatly stimulate the Hp synthesis in canine dirofilariasis. Further investigation, however, is necessary to determine the relationship between the serum Hp level and histo-pathological lesions.

The serum Hp decreased or disappeared in the hemolytic states [2, 4, 6, 12, 13, 19].

However, in spite of severe intravascular hemolysis and liver disorder, serum Hp in the hemoglobinuria group did not disappear, and was even higher than in the serious group without hemoglobinuria. This finding seems strange. The Hp-Hb complex was removed rapidly from circulation by RES and managed by liver parenchymal cells [3, 8]. In dirofilarial hemoglobinuria, however, removal of the complex may be slow, because the RES and liver parenchymal cells may be injured severely and saturated by excessive Hp-Hb complex resulting from severe intravascular hemolysis and severe liver injury. Serum Hp level did not change significantly 20 hr after surgical heartworm removal. However, if heartworms were removed almost completely and liver function could recover to normal, the serum Hp concentration increased in time.

The serum Hp level may well be influenced by both the increasing and decreasing factors in canine dirofilariasis.

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要 約

犬糸状虫症における血清ハプトグロビン濃度：北川 均・佐々木栄英・松井昭秀・石原勝也・内山智晴¹⁾（岐阜大学農学部家畜内科学講座，¹⁾C.S.K.実験動物研究所）——犬糸状虫症犬と健康犬の血清ハプトグロビン (Hp) 濃度をヘモグロビン結合能として測定した。健康犬13例の血清 Hp 濃度は 114.8 ± 31.5 (S. D.) mg/dl であった。犬糸状虫症軽症群33例では0から 349.5 mg/dl の広範囲に分布し，その平均値は 75.1 ± 151.4 mg/dl であった。この群の血清 Hp 濃度は呼吸器症状を示した例で高く，また，尿または腎臓のヘモジデリン陽性例では低値であった。慢性重症群17例では，その平均値は 33.7 ± 59.8 mg/dl であった。この群では2例のみがやや高い血清 Hp 濃度を示したが，8例 (47.1%) は消失ないし痕跡程度 (0mg/dl) の濃度であった。血色素尿症群21例では， 9.9 から 135.0 mg/dl の範囲にあり，その平均値は 48.2 ± 29.5 mg/dl で，慢性重症群より高値であった。また，血色素尿症群では，全例で血漿ヘモグロビン濃度が血清 Hp 濃度より高値であった。以上の所見から，血色素尿症のみならず軽症および慢性重症の犬糸状虫症でも血管内溶血が潜在していることが示唆された。