Studies on the Concentrations of Cd, Pb, Hg and Cr in Dog Serum in Korea

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ABSTRACT : Heavy metal pollution has become a serious health concern in recent years. Dogs are a very good indicator of the pollution load on the environment. They share people's environment and are exposed to the action of the same pollutants. This study estimated the heavy metal contents in the serum of dogs in domestic districts, and assessed the age, sex, feeding habits, living area, breeding environment and smoking habit of the owners. The findings suggest that dogs can be used to monitor the environmental quality of heavy metals. The mean concentrations of heavy metals in the dog serum from 204 samples (108 male and 96 female) were 0.22±0.01 μg/ml, 0.24±0.04 μg/ml, 0.61±0.08 μg/ml, and 0.50±0.06 μg/ml (for Cd, Hg, Pb, and Cr), respectively. Concentrations of Pb, Cd, Hg, and Cr in the dog serum were higher in Yeongnam including Ulsan, and Seoul higher than those of Chungchong and Honam, especially Pb concentration, which was significantly higher ($p<0.01$). Concentrations of Cd, Hg, Pb, and Cr in serum, were increased by age ($p<0.05$). When commercial pet food was provided to dogs, Cd and Cr concentrations were significantly higher in dog serum than dogs fed a human diet ($p<0.01$ in Cd and $p<0.05$ in Cr). Heavy metal concentrations of dogs owned by smoking owners, were higher than non-smoking owners although there was no significant difference. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1623-1627)***

Key Words : Dog Serum, Heavy Metal Ion, Age, Sex, Feed, Area, Breeding Environment

INTRODUCTION

Continuous exposure to low levels of heavy metals may result in bioaccumulation and subsequent health concerns in human and other animals directly and indirectly (Alonso et al., 2000). It is recognized that heavy metals may exercise a definite influence on the control of biological functions, affecting hormone systems and growth of different body tissues (Teresa et al., 1997). Many heavy metals accumulate in one or more of the body organs (liver, kidney, bone and brain) with differing half-lives (King, 1990). Furthermore, these heavy metals apart from acute or chronic poisoning can be transferred to the next generation and have potential toxicity for public health (Iyengar et al., 2000). As we know, after heavy metals absorbed in body through various channels by the blood stream accumulated in high affinitive organs and metabolized, excreted via feces and urine. It is also excreted in sweat and deposited in hairs (Oostdam et al., 1999). The measurement of heavy metals in the blood may reflect recent absorption of individuals (Esben et al., 2004).

The use of animals as sentinels for a number of environmental hazards has a long history, beginning with miners' canaries (Hayes et al., 1982). Zook et al. (1978) convincingly suggested urban dogs as an indicator of lead level in the city environment. Hamir et al. (1986) presented data on lead levels in dogs from Victoria (Australia). Their normal blood lead concentrations are similar in city dogs living in close symbiosis with human (Kucera et al., 1988), although pets may be even more exposed than their owners to some contaminants, such as soil or house dust. Also, several heavy metals are added to animal diets for various purposes, resulting in a high concentration of heavy metals in animal waste (Ko et al., 2004). Animals respond to many toxic insults in ways analogous to humans, and they can develop similar environmentally induced diseases by the same pathogenic mechanisms. Because animals typically have shorter, physiologically compressed life spans when compared with people, latency periods for the development of some diseases are shorter in animals (Backer et al., 2001). Only a few attempt have so far been made to study the content of heavy metals in companion animals (Philippe et al., 1995; Kozak et al., 2002). Therefore, it seemed reasonable to use household pets to determine the concentration of Cd, Pb, Hg, and Cr distributed environmentally. However, only a few attempts have so far been made to determine the concentration of heavy metals in serum from dogs, no studies have ever tried to take into consideration age, sex, food habits, living area, breeding environment and smoking habit of pet owner.

This study measured the heavy metal concentrations in dog serum in Korea and so as to examine the suitability of dogs to monitor the environmental quality of heavy metals.

MATERIALS AND METHODS

Collection of samples

Dogs' blood (5 ml) was collected from apparently healthy dogs with no history of occupational exposure to Cd,

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Table 1. Sample distribution by area, age, and sex

Areas	Ages (years)	Male	Female	Total
Seoul	<1	5	9	14
	1-2	11	7	18
	>2	9	7	16
Yeongnam	<1	7	9	16
	1-2	11	7	18
	>2	9	7	16
Chungchong	<1	9	9	18
	1-2	11	7	18
	>2	9	7	16
Honam	<1	11	11	22
	1-2	7	9	16
	>2	9	7	16
Total		108	96	204

Table 2. Operating conditions and data acquisition parameters of ICP-MS (HP 4500)

Analytical parameters	Conditions
Rf power (W)	1,200
Argon gas flow rates	
Plasma	16.0 L/min
Auxiliary	1.1 L/min
Carrier	1.0 L/min
Torch	Quartz, 2.5 mm
Nebulizer	Peek, Babington - type
Spray chamber	Glass, double pass
Sampler an skimmer cones	Nickel
Data acquisition	
Acquisition parameters	Quantitative
Points/mass	12
Integration time/mass	0.1 sec
Total acquisition time/replicate	7.17
Replicates	3
Total acquisition time/sample	21.5 sec

Pb, Hg, and Cr from different localities of Korea. After the skin was cleaned with 70% ethanol, whole blood was collected by vein puncture, and immediately centrifuged at 800 g for 15 mins. The supernatant serum was transferred to an Eppendorf tube (Becton Dickinson. USA) and frozen at -70°C until analysis.

Reagents and laboratory ware

Preparation of standards was carried out under clean conditions using deionized water of specific resistivity of 16-18 MΩ (ELGA Ltd., UK), Analytical standards of Cd, Pb, Hg, and Cr (Sigma Co., USA) were prepared from solutions (50% nitric acid, Merck Co., Germany) with a nominal concentration of 50, 100, 200 and 1,000 mg/L. All chemicals and reagents used in the present study were of ultrapure reagent grade.

Sample preparation

Dog serums were into the vessel of microwave digestion system (MDS, Qlab 6000, Questron Co., USA), washed briefly with acetone, deionized water, again with acetone and then dried at 105°C (Clarke, 1974). The samples were left overnight to dry and digested the next day. For the analysis of Cd, Pb, Hg, and Cr samples (1 ml serum) were digested by weighing the sample directly into the Teflon vessel of MDS, adding 10 ml 20% HNO_3 (v/v) and then placing them into the microwave Teflon vessel of MDS, which was placed into the microwave oven and digested with MDS. On completion of the digestion program, the Teflon vessel was placed in a bath of cold water for 1 h before the vial was opened. The Teflon vessel was shaken thoroughly for 5 mins, filtered through a 0.45 μm membrane filter (Millipore Co., USA).

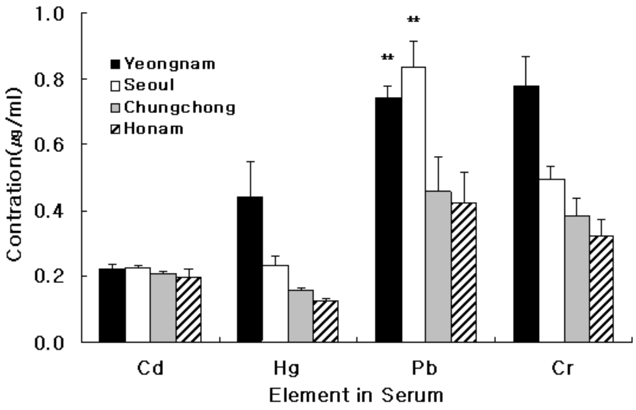

Figure 1. Metal concentration in dog serum by area.
** Significantly different between the indicated groups (p<0.01).

Instrumentation

Cd, Hg, Pb and Cr were determined by ICP-MS (Hewlett-Packard 4500, USA). The operation conditions are outlined in Table 2.

Statistical analysis

Statistical analyses were performed by use of SPSS (version 10). All data were expressed as mean±SD and the level of significance was determined at $p<0.05$ and $p<0.01$.

RESULTS

Effect of areas on metal concentrations in dog serum

The Cd concentration in dog serum showed similar level regardless of region however, Hg and Cr content was highest in Yeongnam, followed by Seoul, Chungchong and Honam. However Pb level was the highest in Seoul area. This result demonstrated that industrialized area, Yeongnam, showed high heavy metals concentrations in dog serum, on the contrary, those levels were low in rural area, Honam (Figure 1).

Effect of sex on metal concentrations in dog serum

In dog serum, the mean Cd concentration was 0.21±0.01 μg/ml in males and 0.21±0.01 μg/g in females. The mean

Table 3. Metal concentrations in dog serum by sex (μg/ml)

Element	Sex		Mean
	Male (108)	Female (96)	(204)
Cd	0.21±0.01	0.21±0.01	0.21±0.01
Hg	1.02±0.86	1.17±0.12	1.10±0.49
Pb	0.66±0.24	0.69±0.14	0.68±0.19
Cr	0.65±0.19	0.66±0.12	0.66±0.15

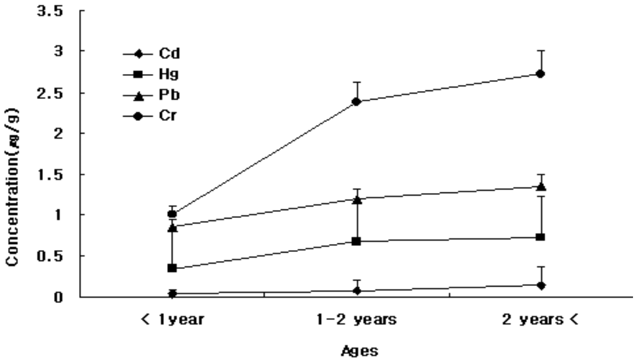

Figure 2. Metal concentrations in dog serum by age.

Hg concentration was 1.02±0.86 μg/g and 1.173±0.12 μg/g. The mean Pb concentration was 0.66±0.24 μg/g and 0.69±0.14 μg/g. The mean Cr concentration was 0.65±0.19 μg/g and 0.66±0.12 μg/g. But there were no statistically significant differences (Table 3).

Effect of each age on metal concentrations in dog serum

In dog serum, the mean Cd concentration was 0.21±0.02 μg/g for below one year, 0.22±0.02 μg/g for 1-2 years and 0.23±0.01 for over 2 years, respectively. The mean Hg concentration was 0.71±0.21 μg/g, 1.16±0.14 μg/g, and 1.69±0.01 μg/g, respectively. The mean Pb concentration was 0.85±0.09 μg/g, 1.21±0.08 μg/g, and 1.35±0.02 μg/g, respectively. The mean Cr concentration was 0.56±0.02 μg/g, 0.61±0.21 μg/g, and 0.83±0.02 μg/g, respectively. The concentration of heavy metals tended to increase by age but in this study there were no statistically significant differences in metal concentrations in dog serum among the groups (Figure 2).

Effect of smoking habit of owner on metal concentrations in dog serum

In dog serum, it shows a comparison between dog serum of smoking habit owner and that of nonsmoking habit owner in relation to the concentrations of heavy metals. Cd were 0.23±0.01 μg/g and 0.21±0.01 μg/g, Hg were 1.07±0.07 μg/g and 0.88±0.09 μg/g, Pb were 0.68±0.11 μg/g and 0.65±0.01 μg/g, Cr were 0.74±0.04 μg/g and 0.50±0.07 μg/g, respectively. Slightly higher levels of heavy metals are in dog serum of smoking habit owner, but there were no statistically significant differences (Table 4).

Table 4. Metal concentrations in dogs serum by smoking habit of owner (μg/ml)

Element	Smoking habit of owner		Mean
	Smoking habit owner (100)	Non-smoking habit owner (104)	(204)
Cd	0.23±0.01	0.21±0.01	0.22±0.01
Hg	1.07±0.07	0.88±0.09	0.98±0.08
Pb	0.68±0.11	0.65±0.01	0.66±0.06
Cr	0.74±0.04	0.50±0.07	0.62±0.06

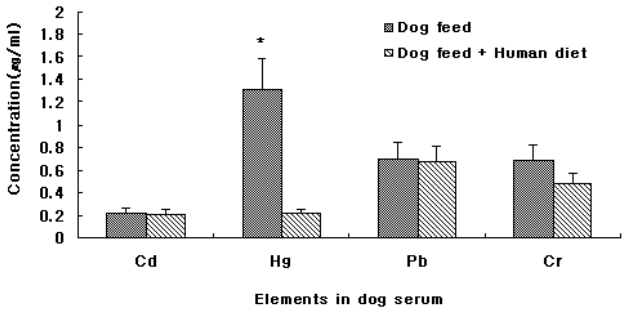

Figure 3. Metal concentration in dog serum by feed styles.
* Significantly different between the indicated groups ($p<0.05$).

Effect of feed types on metal concentrations in dog serum

In serum, the mean Cd concentration was 0.22±0.02 μg/g for dog feed and 0.21±0.01 μg/g for dog feed+human diet. The mean Hg concentration was 1.12±0.11 μg/g and 0.22±0.08 μg/g. The mean Pb concentration was 0.70±0.15 μg/g and 0.67±0.12 μg/g. The mean Cr concentration was 0.68±0.09 μg/g and 0.48±0.13 μg/g, respectively. Serum of dogs fed on dog feed+human diet had significantly higher levels of Hg than that of the other ($p<0.01$) (Figure 3).

Effect of breeding environment on metal concentrations in dog serum

In dog serum, the mean Cd concentration was 0.21±0.01 μg/ml for indoor, 0.21±0.01 μg/ml for outdoor (cement) and 0.21±0.01 μg/ml for outdoor (sand). The mean Hg concentration was 0.63±0.01 μg/ml, 1.59±0.05 μg/ml, and 4.69±0.07 μg/ml. Outdoor (sand) had significantly higher levels of Hg in their serum than the others ($p<0.05$). The mean Pb concentration was 0.69±0.05 μg/ml, 0.64±0.01 μg/ml, and 0.61±0.03 μg/ml. The mean Cr concentration was 0.53±0.04 μg/ml, 0.55±0.01 μg/ml, and 1.86±0.06 μg/ml, respectively. Outdoor (sand) had significantly higher levels of Cr in their serum than the others ($p<0.01$) (Table 5).

DISCUSSION

In recent years, authoritative international organizations such as E.P.A., Ministry of Health Labor and Welfare in

Table 5. Metal concentrations in dog serum by breeding environment (μg/ml)

Element	Breeding environment			Mean
	Indoor (140)	Outdoor-cement (40)	Outdoor-sand (24)	(204)
Cd	0.21±0.01	0.21±0.01	0.21±0.01	0.21±0.01
Hg*	0.63±0.01	1.59±0.05	4.69±0.07	2.30±0.04
Pb	0.69±0.05	0.64±0.01	0.61±0.03	0.64±0.03
Cr**	0.53±0.04	0.55±0.01	1.86±0.06	0.98±0.03

* Significantly different between the indicated groups ($p<0.05$).
** Significantly different between the indicated groups ($p<0.01$).

Japan, World Wildlife Fund designated Cd, Pb and Hg etc. as materials disturbing the endocrine system.

In other previous studies (Mortada et al., 2001), the determination of Cd, Pb and Hg levels in blood, urine, hair, and nails have proven to be of considerable use for the diagnosis of metal over exposure. Thus, reference levels of these heavy metals in such biological samples would seem to be of crucial value for the interpretation of the results. Though a large number of studies have been made on concentration of hairs and blood in human, little is known about that of hairs and serum in dogs. Therefore, it is essential to identify the distribution ranges of Cd, Pb, Hg, and Cr in dog serum in our environment and to study the effects of some predictors such as sex, species, age, food habits, living area, smoking habit of pet owner and breeding status on such ranges.

Information on the levels of Pb in biological tissues in dogs is scarce. The levels of heavy metal in dogs are particularly interesting, since dogs share a similar living environment with humans. In another previous study (Muller et al., 1994), the determination of Pb levels in dog hairs have been carried out. Our study documented the values of 0.74±0.04 μg/ml in Yeongnam, 0.83±0.08 μg/ml in Seoul, 0.46±0.11 μg/ml in Chungchong, and 0.42±0.10 μg/ml in Honam for serum as reference range for Pb in dogs. There were statistically significant differences in Pb concentrations in dog serum among the groups ($p<0.01$). No studies have ever tried to measure Pb in dog serum in Korea. But Koh et al. (1986) compared blood Pb levels in dogs from a lead-mining (1.05 μmol/g), lead-smelting (0.80 μmol/g), urban (0.38 μmol/g) and rural island (0.32 μmol/g) environment. Ward et al. (1977) described results of a 1976 survey in New Zealand of 1,142 domestic animals, including 271 dogs. The mean lead concentrations of dogs in New Zealand were reported as 0.23 μg/ml, with a range of 0.08-0.36 μg/ml. Rural sheepdogs had a mean blood lead value roughly half that of urban dogs (0.15 vs. 0.27 μg/ml), and this difference was reported as being highly significant. Ostrowski et al. (1987) described results of blood lead values in dogs from a rural area in 1987, the mean lead concentrations of dogs were reported as 0.05 μg/ml.

Cigarette smoking is an important predictor for heavy metal exposure, especially of Cd and Pb (Bucket et al., 1990). Blood-Pb levels in human could be positively correlated to smoking (Oostdam et al., 1999). The influence of dietary and environmental sources of Cd exposure appears to be minor compared to the contribution from smoking. Also second-hand smoking in the home is very harmful (Iyengar et al., 2000). Our study illustrated that dogs which are affected by indirect smoking had higher levels of hair-Cd, hair-Pb, serum-Cd and serum-Pb. These findings support that indirect smoking contributes to Cd and Pb concentration in dogs. But no significant differences in the levels of heavy metals have been found between dog serum in smoking habit owner and non-smoking habit owner ($p<0.05$).

It was concluded that levels of heavy metals in dog serum are affected by geographic factors. Especially, Pb levels in dog serum were significantly different ($p<0.01$) and dog serum levels of Cd, Hg, Pb, and Cr were increased by age. Sex was not associated with levels of heavy metal.

Also, the correlation coefficients between dog serum in heavy metals were studied. A similar study for Pb in blood and hairs was found for a population in Germany (Foo et al., 1993; Barbara et al., 2000), observed that the hair Pb was significantly ($p<0.0001$) correlated to blood Pb ($r = 0.85$) in human. In addition to this, there are studies concerning correlation between trace element concentrations in serum (Folin et al., 1991). And Hodkins point to high correlation coefficients for Pb concentrations in blood and hair: in children ($r = 0.85$ for $p<0.1$), and in adults ($r = 0.72$ for $p<0.1$), respectively (Hodkins et al., 1991). Further investigations are needed to assess the sensitivity and reliability of using dog in sentinel animal to identify and predict risks adverse human health effects from exposure to environmental contaminant.

Considering this, a conclusion may be drawn that high positive correlations were observed between dog serum in Pb and Hg. The results indicate that dog serum is good indicator of exposure to levels of Hg and Pb reciprocally.

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