Iranian Journal of Veterinary Science and Technology

Received: 2024- Oct-29
Accepted after revision: 2025- May- 24
Published online: 2025- Jun-01

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2025.90435.1434

Concentration of blood heavy metals in terrier dogs with some common behavior problems

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ABSTRACT

Behavioral disorders in companion animals, especially dogs, are a great concern. Due to the relationship between oxidative stress and behavior problems and also heavy metals' capability of creating oxidative stress, in the current study, the effects of lead, mercury, arsenic, and cadmium on 13 common canine behavior problems (fearfulness, excessive barking, destructiveness, house soiling, inappropriate sexual behavior, coprophagia, wandering, shyness, aggression toward the owner, aggression toward familiar people, aggression toward strangers, aggression toward other dogs, and excessive activity) were evaluated. According to owners' answers to the questionnaire, 43 terrier dogs were chosen. Of these 7 dogs showed no behavior problem and 36 dogs displayed at least one behavior problem. The blood concentrations of lead, mercury, arsenic, and cadmium were measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). No significant differences in heavy metal concentrations were observed between the case and control groups. However, the cadmium concentration was significantly elevated in dogs displayed aggressive behavior toward their owners ($p < 0.048$, n=5). while arsenic level was significantly lower in dogs displaying fearfulness ($p = 0.048$, n= 25). Results of the study reported here do not support the hypothesis that "blood concentration of heavy metals may influence the occurrence or prevention of common behavioral problems in dogs". Our results suggest that there may be a direct relationship between higher levels of cadmium and aggression toward the owner and arsenic with reduced fearfulness in dogs. However, we have to consider that the behavioral effects of heavy metals are likely very complex.

Keywords

Behavior problem; Terrier dog; Cadmium; Lead; Mercury; Arsenic

Number of Figures: 0
Number of Tables: 5
Number of References:: 34
Number of Pages: 9

Abbreviations

ATP: Adenosine triphosphate
DNA: Deoxyribonucleic acid
IQ: Intelligence Quotient
ADHD: Hyperactivity/attention deficit disorder
ECG: Electrocardiogram
ICP-OESL: Inductively Coupled Plasma Optical

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Introduction

The definition of the term "behavior problems" totally depends on the owner's personal opinion. A behavior considered as problematic by one owner, can be seen as a normal behavior by another owner. In fact, any kind of behavior which is unpleasant or annoying for owners falls under the category of a behavior problem [1]. There are different criteria on which the definition and categorization of problematic behaviors are based. For example, behaviors can be categorize as either "normal" and "abnormal" or "physiological" and "pathological". Concerning the former context, "abnormal behaviors" are defined as any behavior that varies from the norm expected for a species. According to the latter type of categorization, most of the problematic behaviors root from the normal processes.Given the physiological origins of so-called "behavior problems", such behaviors may not be problematic for animals itself, but can become problematic when they conflict with owners expectations [2]. Domestic dogs are highly prone to display behaviors that owners may find inappropriate. It is estimated that over 90% of pet dogs may show at least one behavior that is not pleasant in their owners' eyes. There is a wide spectrum of such behaviors, ranging from minor issues, such as tail chasing or pulling on the lead, and major ones, such as aggression or destructive behavior. The most reported prevalent behavior disorders in typically include: fearfulness, hyperactivity, destructiveness, inappropriate elimination, Straying, Coprophagy, Excessive barking, aggression toward other dogs and humans, and sexual behavior problems [3]. Another study reported the most common behavioral problems in dogs in a different order: attention seeking, dog aggression, noise reactivity, aggression toward strangers, pica, destructiveness, aversion to strangers, excessive barking, compulsive body licking, aggression toward owners, possessiveness over toys or food, house soiling, and fearfulness during walks and tail chasing [2]. These behavioral problems can have serious consequences, including dog abandonment or relinquishment to kennels and shelters, property damage, welfare deterioration, erosion of the human-dog bond, and euthanasia [4, 5]. Possible risk factors of behavioral disorders are breed, age, time and source of acquisition, sex, and reproductive status [6]. Heavy metals can be a risk factor for behavior problems, especially when organisms are exposed to toxic metals chronically [7]. Dogs may be exposed to heavy metals through environmental pollutants such as man-made waste, and diet especially commercial foods [8]. Heavy metals can implicate variety of dangerous intracellular damages. These include oxidative stress and lipid peroxidation, disruption of cellular enzymatic system, decrease in ATP production, dysregulation and inhibition of some proteins and enzymes, DNA repair suppression, genotoxic effects, damage to cell membrane integrity, destruction of microtubules, ribosomes and endoplasmic reticulum, and mitochondria. . Furthermore, heavy metals can disturb intracellular-calcium homeostasis, inhibit cellular respiration and interference with mitosis. These negative changes can adversely affect nerval development and electric conductivity, ultimately leading to behavioral abnormalities. Consequently, neurological disorders such as learning disabilities, memory loss, decrease in the IQ, ADHD, and behavioral disorders may be developed [9-12].

An important hypothesis that has been proposed in the recent related literature is whether heavy metals have the ability to impact behavioral problems. Therefore, the current study was carried out to investigate the correlation between amounts of four toxic metals (cadmium, arsenic, lead, and mercury) in whole blood and 13 behavioral disorders in Terrier dogs. These behavioral disorders included: Fearfulness, excessive barking, destructiveness, house soiling, inappropriate sexual behavior, coprophagy, wandering, shyness, aggression toward the owner, aggression toward familiar people, aggression toward strangers, aggression toward other dogs and excessive activity.

Results

Demographic characteristics

The sex and neuter status of the dogs in the control and case groups are provided in Table 1. Out of

Table 1.
The number and the percentage of dogs based on their sex and neuter status in the control and test groups.

			Number	Percent
Sex	Control	Male	5	71.42
		Female	2	28.58
	case	Male	21	58.33
		Female	15	41.67
Neuter status	Control	Neutered	5	71.42
		None- neutered	2	28.58
	case	Neutered	13	36.11
		None- neutered	23	63.89

Blood heavy metals concentrations in dogs with or without behavior problems

Abbreviations-Cont'd

Emission Spectroscopy
SPSS: Statistical Package for the Social Science
CBCL: Child Behavior Checklist

the 43 dogs evaluated, the case group consisted of 21 males and 15 females (dog with at least one behaviour problem). The control group included 5 males and 2 females.

Distribution of behavior problems

Among the 36 dogs with behavioral problems that are listed in Table 2, the most prevalent behaviour problems were fearfulness (n = 25), house soiling (n = 18) and hyperactivity (n = 14).

Table 2.
The number and the percentage of dogs in the case group that had each of 13 behavior problems.

Behavior problems	Case Group	Number	Percent
Fearfulness	Present	25	69.44
	Absent	11	30.56
Excessive barking	Present	5	13.88
	Absent	31	86.12
Destructiveness	Present	11	30.56
	Absent	25	69.44
House Soiling	Present	18	50
	Absent	18	50
Inappropriate sexual behavior	Present	9	25
	Absent	27	75
Coprophagy	Present	4	11.11
	Absent	32	88.89
Wandering	Present	4	11.11
	Absent	32	88.89
Shyness	Present	8	22.22
	Absent	28	77.78
Aggression toward owner	Present	5	13.88
	Absent	31	86.12
Aggression toward familiar people	Present	6	16.66
	Absent	30	83.34
Aggression toward stranger people	Present	5	13.88
	Absent	31	86.12
Aggression toward dogs	Present	5	13.88
	Absent	31	86.12
Hyper activity	Present	14	38.88
	Absent	22	61.12

Heavy metals levels

Blood concentrations of four metals (lead, mercury, arsenic and cadmium) in the case and control group are present in Table 3. The median concentrations of these heavy metals showed no significant difference between the control group and the group with behavioral disorders. However, subgroups analysis that are present in Table 4, revealed two significant findings. Dogs exhibiting aggression toward their owner had significantly higher blood cadmium levels than the controls ($p < 0.05$). Also, dogs classified as fearful had lower arsenic levels compared to the control group ($p < 0.05$).

Discussion

Trace elements and toxic metals can be measured from various loci such as serum, blood, urine, or hair. Whole blood has been considered to give a better reflection of long-term dietary intake (for example selenium) or environmental exposure. In addition, toxic metals such as lead are commonly measured from whole blood, as more than 90% of lead is bound to red blood cells after absorption [13]. In this study, we aimed to explore potential associations between behavior problems and blood concentrations of heavy metals in pet dogs.

Cadmium

Our findings illustrated that cadmium level were elevated in dogs displaying aggression toward their owners compared to those without any behavior disorders. In line with our results, Terçariol *et al.* (2011) reported that rats exposed to cadmium and immobilization stress were more frequent in exhibiting several aggressive behaviors, namely total number of attacks and total duration of attack manifestations. Also, these rats had a higher composite aggression score [14]. Similarly, Godinho *et al.* (2017) experimentally poisoned mice with cadmium and caffeine. It became evident that co-exposure to cadmium and caffeine (and not just cadmium alone) caused mice to be more aggressive [15]. It seems that cadmium increases aggressiveness, both directly, possibly through interfering with serotonin function or decreasing its level in various ways [13], and indirectly, by aggravating anxiety [16,17]. Heavy metals, including cadmium, act as catalysts for biochemical reactions, regulators of gene expression, second messengers in signalling pathways and cofactors for many vital enzymes, such pathways implicated in regulating physiological, pathological and behavioral functions. Animal studies suggests it is plausible that chronic exposure to cadmium may lead to motor hyperactivity, increase in aggressive be-

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DOI: 10.22067/ijvst.2025.90435.1434

Table 3.
Amounts of median, Q1 and Q3 of metals' blood concentration in dogs with or without behavior problems.

	Lead (mg/L)	Mercury (mg/L)	Arsenic (mg/L)	Cadmium (mg/L)
Without any behavior problems (control) (n=7)	0.309 (0.182-0.391)	0.393 (0.001-0.932)	0.212 (0.175-0.335)	0.015 (0.001-0.023)
With at least one behavior problem (case) (n=36)	0.323 (0.249-0.468)	0.808 (0.710-0.888)	0.185 (0.144-0.214)	0.021 (0.016-0.025)
Blood element (ng/g) reference values from literature*	3.5±2.75 (0.28-12.37)	0.53±0.35 (0.12-1.61)	1.15±1.09 (0.04-4.30)	0.03±0.01 (0.01-0.06)

* Rosendahl S, Anturaniemi J, Vuori KA, Moore R, Hemida M, Hielm-Björkman A. Diet and dog characteristics affect major and trace elements in hair and blood of healthy dogs. Vet Res Commun. (2022) 46:261–75. doi: 10.1007/s11259-021-09854-8

Table 4.
Amounts of median, Q1 and Q3 of metals' blood concentration in the subgroups and controls of this study. Numbers marked with * have a significant difference with controls.

	Lead (mg/L)	Mercury (mg/L)	Arsenic (mg/L)	Cadmium (mg/L)
Without any behavior problems (control) (n=7)	0.309 (0.182-0.391)	0.393 (0.001-0.932)	0.212 (0.175-0.335)	0.015 (0.001-0.023)
Fearfulness (n=25)	0.322 (0.224-0.461)	0.855 (0.670-0.886)	0.178 (0.145-0.212)*	0.021 (0.014-0.025)
Excessive barking (n=5)	0.301 (0.224-0.381)	0.837 (0.699-0.867)	0.194 (0.136-0.237)	0.024 (0.017-0.027)
Destructiveness (n=11)	0.322 (0.255-0.673)	0.802 (0.766-0.894)	0.191 (0.144-0.215)	0.021 (0.017-0.024)
House soiling (n=18)	0.322 (0.267-0.456)	0.779 (0.714-0.869)	0.172 (0.126-0.215)	0.019 (0.013-0.026)
Inappropriate sexual behavior (n=9)	0.249 (0.195 -0.321)	0.837 (0.726-0.894)	0.837 (0.726-0.894)	0.020 (0.015-0.027)
Coprophagia (n=4)	0.322 (0.218-0.359)	0.759 (0.704-0.847)	0.196 (0.144-0.227)	0.017 (0.014-0.022)
Wandering (n=4)	0.328 (0.308-0.475)	0.726 (0.527-0.882)	0.184 (0.128-0.221)	0.019 (0.013-0.027)
Shyness (n=8)	0.286 (0.218-0.438)	0.822 (0.547-0.875)	0.212 (0.160-0.224)	0.021 (0.019-0.024)
Aggression toward owner (n=5)	0.328 (0.270-0.490)	0.855 (0.627-0.919)	0.221 (0.210-0.239)	0.025 (0.022-0.036)*
Aggression toward familiar people (n=6)	0.398 (0.280-0.521)	0.858 (0.744-0.964)	0.228 (0.199-0.240)	0.025 (0.019-0.035)
Aggression toward stranger people (n=5)	0.325 (0.278-0.446)	0.776 (0.703-0.855)	0.192 (0.137-0.228)	0.022 (0.014-0.026)
Aggression toward dogs (n=5)	0.345 (0.218-0.466)	0.741 (0.561-0.867)	0.189 (0.122-0.214)	0.020 (0.016-0.021)
excessive activity (n=14)	0.331 (0.252-0.429)	0.776 (0.660-0.864)	0.182 (0.150-0.203)	0.021 (0.013-0.024)

havior, impairing social memory processes, and also may alter drinking behaviour [18].

Arsenic

In the present study, no significant difference in blood arsenic concentrations was observed between the case group and the control group. This finding aligns with the study by Tolins *et al.* (2014), which also found no significant correlation between arsenic concentrations and various behavioral parameters, including ADHD prevalence, classroom behavior outcomes, behavioral scores from a validated system, answers of a self-reported behavioral test in children and behavior test in newborns [19]. Recent studies have shown that even low concentrations of arsenic exposure may impair neurological function, particularly in children [20]. Interestingly, in the present study, dogs diagnosed with fearfulness disorder had lower arsenic levels compared to the dogs without any behavioral problems. However, the importance of

this finding in the present study remains unclear, as behavioral problems may have many causes beyond heavy metals.

Regarding the potential relationship between arsenic and fear-related behavior, there are some studies in which a contextual fear conditioning test was performed on arsenic-exposed mice and rats. These studies found that freezing time decreased after arsenic exposure, showing that arsenic may be able to reduce fear responses [6, 21, 22]. Freezing behavior, defined as voluntary immobile behavior except for respiration is a well-established fear-related behavior in mice and rats and was assessed to measure fear in the previous literature [23]. A possible explanation for this mechanism of such correlation can be explained through arsenic's impact on neurological processes. Arsenic can alter DNA methylation and gene expression, which play roles in memory formation, and secondly, and impair synaptic plasticity. These changes could disrupt the consolidation of fear memory, thereby decreasing freezing time. Even in some cases, freezing time remained unaltered after arsenic exposure, whereas it was normally expected to decline over time [6].

Mercury

Our results indicated that mercury levels were not significantly related to behavioral problems. This result is consistent with the findings of Bratel *et al.* (1997), who also reported no significant correlation between mercury concentrations in blood, hair, and urine and behavior problems like depression and anxiety [24]. Contrary to our results, Lozano *et al.* (2021) reported that children with elevated hair mercury concentrations scored lower on two subscales of the Child Behavior Checklist (CBCL) and ADHD index of the Conners Parents Rating Scale-Revised: Short Form. Also, mercury concentrations in cord blood have been linked to attention problems and ADHD inattentive and hyperactive-impulsive types [25]. Further supporting this, being prenatally exposed to mercury, rats displayed hyperactivity, spatial learning impairments and adaptive behavior. Mercury poisoning is thought to cause a wide spectrum of psychological problems, such as irritability, nervousness, excessive shyness, low self-confidence, insomnia, deficits in cognitive function, attention and memory, irritability, fretfulness, aggression, anxiety, psychasthenia, alexithymia, and poor social functioning [26]. Azevedo *et al.* (2012) believe that chronic low doses of mercury have a harmful effect on vascular function by reducing Nitric Oxide bioavailability. They argue that the current mercury exposure reference values, once considered safe, should be re-evaluated and reduced [27]. One possible explanations for insignificance of our results might be the short course of study, both behavioural symptoms and heavy metal exposure.

Lead

Although, no statistically significant relationship was detected between lead levels and behavior problems in our study, a considerable number of previous researches reported plausible lead's role in developing behavioral disorders. For instance, prenatally lead-exposed children, has been associated with more intensive emotional reactions, and difficulty in emotional regulations. Moreover, lead intoxication has been correlated with aggression and depression in 7–8 year [28]. other studies have found that lead exposed children show intellectual deficits, increased risk of violent and aggressive behavior, drug abuse, criminal activity, attention deficit, and social withdrawal [9,29]. Al-Osman *et al.* (2019) stated that acute lead intoxication in children can decrease attention span and increase irritability and dullness [30]. In adults, lead exposure has been linked to major depression, panic disorders, anxiety and hostility [31]. Research on birds has also revealed that individuals from regions with high lead soil concentrations exhibit more aggressive behavior [32]. Additionally, lead poisoning is thought to be associated with ADHD particularly the Hyperactive-Impulsive subtype (ADHD-H/I) [33]. Different study conditions, such as species, age, hormonal status, neutering conditions , and sample size can contribute to the inconsistency between our data about mercury and lead impacts on behavior problems and existing literature which contradict our results Additionally, while dietary intake can also be directly related to heavy metal exposure in pets, we attempted to control this variable by selecting dogs with similar diets which can reduce the direct dietary influence on blood heavy metal concentrations.

Conclusion

The current research is the first to examine the potential relationship between behavioral problems and heavy metal concentration in pet dogs.

Materials & Methods

Ethical approval

This cross-sectional study was conducted at the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. The study protocol was ethically approved by Research Office of Ferdowsi University of Mashhad.

Dog selection

The study involved adult terrier dogs aged between 1 and 10 years, all of whom were confirmed to be in good health based on physical examination, complete blood count and serum biochemistry.

Dietary histories revealed that the dogs were fed a combination of simple home-made diet including chicken, rice, potatoes, carrots, and cheese and/or commercial dog foods. No management recommendations, behavioral modifications, or training advice were given during the study. Additionally, none of the dogs had received any drugs or supplements during 30 days before the onset of the study. All dog owners were informed about study's objectives and procedures, and informed consent was obtained from each participant.

Questionnaire

Data were obtained using a previously validated survey questionnaire [2,3], which was completed by dog owners during veterinary consultation. The questionnaire approach to collection of behavioral data, is based on the assumption that the owner usually have close and consistent interaction with their dogs and knows more about it's typical behavior. The questionnaire was used to obtain basic data about the dog's demographic information, and the dog's environment. In addition, owners were asked whether their dog had exhibited any of the 13 most common behavior problems typically seen at the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. Responses were recorded on a binary (yes/no) scale. To minimize subjectivity perception among owners of behavior problems, each individual behavior was described as objectively as possible (e.g., "Does your dog move constantly, move fast, run and jump?", for excessive activity and "Does your dog show fearful behavior such as fleeing, trembling, and panic in response to unknown noises?", for fearfulness). Owners' reports were accepted as reliable evidence of behavior problems, since owner perceptions determines whether a dog have a behavioral problem [3]. The 13 most common types of undesirable behaviors assessed were: fearfulness, excessive activity, aggression towards people (owner, familiar and unfamiliar), aggression toward other dogs, excessive barking, destructiveness, inappropriate elimination (house soiling), sexual behavioral problems, coprophagy ("eats faeces"), straying and shyness (Table 5). Participants were required to indicate whether their dog had exhibited any of these behavior problems. An open-ended "other" category was also included and allowed participants to state whether their dog had exhibited any other problem behaviors besides those mentioned.

In total, 43 healthy terrier dogs living in household settings were included in this research. Of these, 7 dogs without any reported behavioral problems served as the control group, while 36 dogs exhibiting at least one behavior disorder formed the case group. In addition, controls were compared with dogs displaying only one certain behavior problem as well. The distribution of the 13 behavioral problems evaluated in this research was as follows: fearfulness (n = 25), excessive barking (n = 5), destructiveness (n = 11), house soiling (n = 18), inappropriate sexual behavior (n = 9), coprophagia (n = 4), wandering (n = 4), shyness (n=8), aggression toward owner (n = 5), aggression toward familiar people (n = 6), aggression toward strangers (n = 5), aggression toward other dogs (n = 5) and excessive activity (n = 14).

Sample collection

Venous blood samples were obtained from the Cephalic, saphenous, and jugular veins. Between 3 to 5 ml of blood was collected from each dog using heparinized tubes.

Measurement of whole blood heavy metals

The initial volume of each blood sample was measured using graduated tubes. Samples were then diluted with 65% nitric acid and Hydrogen peroxide (Merck), in a ratio of 1:2, , to reach a final volume of 9 ml. These diluted samples were kept at room temperature

Table 5.
Binary questionnaire used in the current study.

undesired behavior	Definition
Fearfulness	Does your dog tremble, panic, and/or flee when hearing sudden noises?
Excessive activity	Does your dog move constantly, walk fast, run, jump, and rarely settle down?
Destructiveness	Does your dog damage or destroy furniture, clothes, and other objects found in your house?
House soiling	Does your dog defecate and/or urinate inside your house?
Inappropriate sexual behavior	Does your dog hump on your foot, people, or objects?
Coprophagy	Does your dog eat feces of its own, other dogs, and/or other species?
Wandering	Does your dog tend to aimlessly walk and leave your house frequently?
Shyness	Is your dog too quiet? Does your dog refuse to make contact or familiarize with others?
Aggression toward owner	Does your dog growl, bark, raise its hackles, lunge, and bite you?
Aggression toward familiar people	Does your dog growl, bark, raise its hackles, lunge, and bite when encountering familiar people (family members, relatives, and those who are being met frequently)?
Aggression toward unfamiliar people	Does your dog growl, bark, raise its hackles, lunge, and bite when encountering unfamiliar people?
Aggression toward dogs	Does your dog growl, bark, raise its hackles, lunge, and bite when encountering other dogs?

for a couple of hours. followed by incubation in a Bain-marie at 80-100°C for two hours and then, were filtered with Whatman filter paper. Heavy metal concentrations were analyzed using the SPECTRO ARCOS instrument, (model 76004555, SPECTRO, Germany) which has a detection limit of 0.001 mg/l, and the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method.

Statistical analysis

Statistical analysis was performed using SPSS software, 16th edition. For all calculations, a p-value of $p < 0.05$ was considered significant. Due to abnormal distribution of the parameters, namely lead, cadmium, arsenic, and mercury, the non-parametric Mann-Witney U test was used to analyse and compare the data between the two groups. Results were reported as the first quarter, median, and third quarter values.

Authors' Contributions

Mohammadd Heidarpour and Javad khoshnegah conceived and planned the experiments. Mohammadd Heidarpour and Javad khoshnegah and Raha Bayazi carried out the experiments. Mohammadd Heidarpour and Javad khoshnegah planned and carried out the simulations. Mohammadd Heidarpour and Javad khoshnegah and Mohammad Azizzadeh contributed to the interpretation of the results. Javad Khoshnegah took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

We would like to thank Mr Ali farahmand and Mr Hossein Barati for their technical assistance. This study was funded by FUM (Ferdowsi University of Mashhad).

Conflict of interest

The authors declare that there is no conflict of interest.

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How to cite this article
Bayazi R, Heidarpour M, Azizzadeh M, Khoshnegah J. Concentration of blood heavy metals in terrier dogs with some common behavior . Iran J Vet Sci Technol.2025; 17(2): 69-77.
DOI: https://doi.org/10.22067/ijvst.2025.90435.1434
URL:https://ijvst.um.ac.ir/article_46796.html