Polish Journal of Veterinary Sciences Vol. 27, No. 2 (2024), 301–304

DOI 10.24425/pjvs.2024.149359

Short communication

Effect of sex on the levels of total arsenic (As) and As(III) in dog urine: a preliminary study

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Abstract

The aim of the study was to analyze differences in the concentration of total arsenic (As) and As(III) in urine depending on the sex of mixed-breed dogs. Therefore, a research hypothesis was put forward that sex is a variable determining the degree and efficiency of urinary arsenic excretion. Two study groups were established: female (group 1) and male (group 2) mixed-breed dogs of similar body weight (9-13 kg) and aged 8-11 years. Urine samples were collected using a device designed specially for this purpose (utility model registered at the Patent Office of the Republic of Poland, no. WUP 13/2023). Samples were wet-digested following the protocol presented in the PN-EN 13805:2014 standard and analysed using an EcaFlow 150 GLP coulometer integrated with an E-53 Au electrode and EcaCell. Arsenic content in dog food was also measured to verify the effect of this variable. Results were analyzed using Statistica 13.1 software. Sex had a significant effect on the urinary arsenic excretion. The levels of total As and As(III) were significantly higher in urine from male dogs (18.45 and 2.92 [μg L^{-1}]; $p \leq 0.05$) with compared to urine from female dogs (13.43 and 1.67 [μg L^{-1}], respectively).

Keywords: total arsenic, arsenic(III), dogs, sex, urine

Introduction

Most arsenic (As) compounds are easily soluble in water, and the primary sources of exposure to this element include drinking water, ingested food and inhalation (Chen et al. 2020, Mukherjee and Gopalakrishnan 2023). Arsenic is one of the most toxic xenobiotics. Chronic exposure to arsenic has many different negative effects on health, including non-cancerous skin lesions, and increased risks of cancers of the skin, bladder, and lung (Ghosh et al. 2008). In addition, arsenic shows a negative influence on female and male reproduction. Anomalies caused by arsenic reported from studies in humans and animals include reduced sperm count and motility (Chen et al. 2020, Machado-Neves 2022, Mukherjee

and Gopalakrishnan 2023). Factors such as the duration of exposure to arsenic, species, age, sex, diet, health status and genetic makeup are of key importance for the effective elimination of this toxic element and largely determine the severity of poisoning (Timbrell 2008, Wei et al. 2017).

The aim of the study was to analyze differences in the concentration of total As and As(III) in urine depending on the sex of mixed-breed dogs. Therefore, a research hypothesis was put forward that sex is a variable determining the degree and efficiency of urinary As excretion.

Materials and Methods

Study animals

Based on an interview with a local veterinarian all dogs were clinically healthy. Animals were divided into two study groups: six female (group 1) and six male (group 2) mixed-breed dogs of similar body weight (9-13 kg) and aged 8-11 years. All dogs received the same type of commercially available food (its brand name was intentionally undisclosed). Dogs used in the study lived in an Animal Shelter located in Kuyavian--Pomeranian province, central Poland. Study groups only differed in terms of sex, while other parameters, such as age, body weight, diet and location of animals were identical. Moreover, all individuals were mixed-breed.

Preparation of samples for analysis

Three samples of urine, about 30 ml from each individual, were collected in zip-closure plastic bags at 2-day intervals and frozen for further analysis. Eighteen samples of urine were collected from each study group: (6 individuals x 3 samplings) x 2 study groups. The total number of collected samples was 36 (n=18/per study group). The urine sampling kit was designed by the authors of this study, and the utility model was registered at the Patent Office of the Republic of Poland, no. WUP 13/2023. The designed sampling kit allowed for the stress-free collection of urine during daily dog walks. The sampling procedure was non-invasive and had no negative impact on animal welfare.

Preparation of samples for determination of total As

A 2 ml sample of filtered urine and 2 ml of concentrated nitric acid (HNO_3) were transferred to a vessel of the EthosPlus digestion system (Milestone, Italy) and wet-digested in accordance with the PN-EN 13805:2014 standard.

Preparation of samples for determination of As(III)

A 5 ml sample of clear, filtered urine was transferred to a 50 ml flask. Hydrochloric acid (HCl) concentration 0.1 [mol L^{-1}] was added up to the mark. Samples prepared according to this protocol were analysed.

Assessment of total As and As(III) levels in urine

The levels of total As and As(III) in urine were measured using an EcaFlow 150 GLP coulometer (Istran, Slovakia) integrated with an E-53 Au electrode and EcaCell. R-007 was used as the electrolyte to flush the system. Arsenic concentrations were calculated based on the calibration curve, which was prepared using the certified reference standard of As, concentration 1000 [µg mL^{-1}] As(III) (SPEXCertiPrep). Analytical results were expressed in [µg L^{-1}] of urine. Laboratory control procedures were performed for each series of measurements. The concentrations of different chemical forms of arsenic measured in the urine were higher than the limit of detection (LODs).

Assessment of total As and As(III) levels in commercial dog food

In order to supplement primary results, the concentrations of total As and As(III) in dog food was also determined using the EcaFlow 150 GLP coulometer and the same reagents as in the protocol for urinalysis. The results of six measurements for six samples were converted to 1000g of food and expressed in [mg kg^{-1}] of dry matter (dm).

Statistical analysis

Results were analysed using Statistica 13.1 software (StatSoft, Poland). Three results from dependent repetitions obtained from each individual were averaged, and then the average values were used for statistical analysis. All data from measurements were analysed for the normality of distribution and homogeneity of variances using the Shapiro-Wilk test and the Levene test. Analysis revealed that the requirements for normal distribution were met only for the As(III) data. Therefore, the significance of differences between the concentrations of As(III) was analysed with Student's t-test. The significance of differences between the levels of total As was analysed with the non-parametric Mann-Whitney U test. Spearman's correlation coefficient for the analysed forms of As was also calculated.

Table 1. Levels of total arsenic (As) and As(III) in urine from male and female dogs.

	Group 1 ♀		Group 2 ♂	
	Total As [μg L^{-1}] n=6	As(III) [μg L^{-1}] n=6	Total As [μg L^{-1}] n=6	As(III) [μg L^{-1}] n=6
$\bar{x}$	13.43[a]	1.67[a]	18.45[b]	2.92[b]
Me	14.75	1.70	18.60	3.0
SD	4.33	0.42	4.54	0.68
CV	3.05	1.45	5.69	2.72

[a, b] – means marked with different letters in different groups, separately for total As and As(III) indicate significant differences at $p \leq 0.05$, $\bar{x}$ – mean, Me – median, CV – coefficient of variation, n – number of animals, Group 1: female dogs, Group 2: male dogs

Table 2. Sperman's rank correlation coefficient for the analysed parameters in both dog groups together.

Parameter	As(III)
Total A*s*	0.888*

Correlation coefficient (r_{xy}) significant at $p \leq 0.05$ *

Results and Discussion

Levels of total As in urine is a commonly used biomarker of exposure of living organisms to As (Chang et al. 2019, Liao et al. 2022). In this study the levels of total As and As(III) were significantly higher in urine from male dogs (18.45 and 2.92 [μg L^{-1}], $p \leq 0.05$) with compared to urine from female dogs (13.43 and 1.67 [μg L^{-1}], respectively) (Table 1). Coefficients of variation (CV) for both forms of arsenic are presented in Table 1.

To investigate the relationship between the two forms of arsenic, Spearman's rank correlation coefficient was calculated for both study groups together, and relevant statistics are presented in Table 2.

As shown in the table above, there was a statistically significant, positive correlation (r_{xy}=0.89, $p \leq 0.05$) between total As and As(III).

To supplement findings, the content of total As and As(III) in dog food was also analysed. The content of these forms of As was 0.25±0.01 mg kg^{-1} dm for total As and 0.082±0.002 mg kg^{-1} dm for As(III). The level of As in tap water was below the LODs.

Regardless of the route of exposure to As, some of this xenobiotic accumulates in tissues, and some is eliminated with faeces and urine. Analysis of urine samples is the most reliable and non-invasive way to assess exposure to As. Therefore in most studies, exposure to As has been assessed by measuring its concentration in urine (Lindberg et al. 2008, Chang et al. 2019, Liao et al. 2022). Sex-related physiological differences, and thus the degree of arsenic accumulation or biotransformation, have been confirmed by other researchers (Lindberg et al. 2008, Chowdhury 2021, Liao et al. 2022), and therefore this factor is certainly of key importance. Lindberg et al. (2008) reported that women, but only of childbearing age, had higher arsenic methylation efficiency than men, supporting the influence of female sex hormones. Chowdhury (2021) also confirmed higher arsenic methylation efficiency in women compared to men. Differences in the concentration of these enzymes between male and female individuals may play a key role in the effective biotransformation of As. Relatively few studies have focused on sex-related differences in the metabolism and excretion of As, especially with respect to animals. This study revealed a higher concentration of As compounds in the urine of male dogs, which may indicate a more dynamic biotransformation and elimination of this xenobiotic from the body, taking into account the fact that all other factors, i.e. diet, drinking water, living environment of animals, body weight, etc. were identical or very similar. The groups of study animals only differed in terms of their sex. Thus, a statistically significant effect of sex on the level of arsenic compounds in the analytical matrix was confirmed (Table 1). Apart from different rates of arsenic biotransformation in male and female individuals, specific side effects are also associated with sex (Smith et al. 2000, Ahsan et al. 2006, Liao et al. 2022). For example, Smith et al. (2000) and Ahsan et al. (2006) reported a greater risk of skin diseases in men exposed to As.

In the present study As content was also measured in dog food, which is important for the assessment of the exposure of animals to this xenobiotic. Dogs received the same type and amount of food per body

weight, so despite the fact that food was a confirmed source of this xenobiotic, sex was the overriding factor determining the efficient biomethylation and level of elimination of As by urinary excretion. Waalkes et al. (2004) conducted an experimental study in mice that involved three administrations of increasing doses of As. The mean survival time in both males and females exposed to different doses of As did not change, but survival was reduced in males after exposure to the highest dose (85µl L^{-1}).

In this study the level of As(III) in most individuals in relation to the total level of As was in the range of 12-16%, regardless of the study group. The correlation between these two forms of As was calculated to identify the strength of their interaction (Table 2). Similar relationships have not been found in the literature. However, these data may be fundamental for understanding the interactions between the forms of As. The conducted analyses validated the hypothesis about the influence of sex on the urinary As level in dogs and on the rate of its elimination.

To summarize, due to the fact that the study groups differed only in terms of sex, while other parameters such as animal species, age, diet, body weight or location of the animal were identical, it is assumed that differences in the urinary As levels are closely associated with the sex-related rate efficiency of As excretion. This study confirmed a significantly higher concentration of both forms of As in the urine of male dogs, which indicates a higher metabolism and elimination of this element in males. To better understand this process, further studies should analyse the impact of various factors, including sex, on the As biomethylation process and differences in the proportions of As metabolites (monomethylarsonic acid and dimethylarsinic acid): MMA(III), DMA(III), MMA(V), DMA(V) in urine and the levels of key enzymes that play an important role in biomethylation and whose levels may vary between sexes. This study provides valuable information on the exposure of animals to this xenobiotic and valuable contribution to future research in this area.

Acknowledgements

We are grateful to the staff of the Animal Shelter for their consent to the collection of urine samples from dogs.

References

Ahsan H, Chen Y, Parvez F, Zablotska L, Argos M, Hussain I, Momotaj H, Levy D, Cheng Z, Slavkovich V, Van Geen A, Howe GR, Graziano JH (**2006**) Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: baseline results from the health effects of arsenic longitudinal study. Am J Epidemiol 163: 1138-48.

Chang JY, Ahn SC, Lee JS, Kim JY, Jung AR, Park J, Choi JW, Yu SD (**2019**) Exposure assessment for the abandoned metal mine area contaminated by arsenic. Environ Geochem Health 41: 2443-2458.

Chen H, Liu G, Qiao N, Kang Z, Hu L, Liao J, Yang F, Pang C, Liu B, Zeng Q, Li Y, Li Y (**2020**) Toxic effects of arsenic trioxide on spermatogonia are associated with oxidative stress, mitochondrial dysfunction, autophagy and metabolomic alterations. Ecotoxicol Environ Saf 190: 110063.

Chowdhury UK (**2021**) Biotransformation of inorganic arsenic: Influence of gender, arsenic dose level, and creatinine formation. Int J of Biochem Pept 1: 17-33.

Ghosh P, Banerjee M, Giri AK, Ray K (**2008**) Toxicogenomics of arsenic: classical ideas and recent advances. Mutat Res 659: 293-301.

Liao KW, Chien LC, Chen YC, Kao HC (**2022**) Sex-specific differences in early renal impairment associated with arsenic, lead, and cadmium exposure among young adults in Taiwan. Environ Sci Pollut Res Int 29: 52655-52664.

Lindberg AL, Ekström EC, Nermell B, Rahman M, Lönnerdal B, Perrson LA, Vahter M (**2008**) Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. Environ Res 106: 110-120.

Machado-Neves M (**2022**) Arsenic exposure and its implications in male fertility. Anim Reprod 19: e20220119.

Mukherjee AG, Gopalakrishnan AV (**2023**) The interplay of arsenic, silymarin, and NF-κB pathway in male reproductive toxicity: a review, Ecotoxicol Environ Saf 252: 114614.

POLISH NORM PN-EN-13805:2014. Foodstuffs. Determination of trace elements. Mineralization pressure [in Polish]. https://nobelcert.com/DataFiles/FreeUpload/EN%2013805-2014.pdf

Smith AH, Arroyo AP, Mazumder DN, Kosnett MJ, Hernandez AL, Beeris M, Smith MM, Moore LE (**2000**) Arsenic-induced skin lesions among Atacameno people in Northern Chile despite good nutrition and centuries of exposure. Environ Health Perspect 108: 617-620.

Timbrell J (**2008**) The poison paradox: chemicals as friends and foes. Oxford University Press, p 180.

Waalkes MP, Ward JM, Diwan BA (**2004**) Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. Carcinogenesis 25: 133-141.

Wei BG, Ye BX, Yu JP, Yang LS, Li HR, Xia YJ, Wu KG (**2017**) Blood Pressure Associated with Arsenic Methylation and Arsenic Metabolism Caused by Chronic Exposure to Arsenic in Tube Well Water. Biomed Environ Sci 30: 334-342.