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ORIGINAL ARTICLE

Evaluation of mercury contamination in dogs using hair analysis

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Abstract

OBJECTIVES: The present work is aimed at assessing the mercury contamination of dogs through the analysis of hair. For the determination of the total mercury in dogs, we chose skin derivatives – hair. The content of total mercury was also measured in the pelleted feed.
METHODS: Dogs were divided into two groups. The first group (group A) was fed granular feed containing fish and the second group (group B) granular feed free of fish. A total of 131 hair and granular feed samples were collected. The total mercury in hair and granular feed samples was measured using atomic absorption spectrophotometry on AMA 254.
RESULTS: The values of the total mercury content in the hair of dogs had a median value of 0.0375 $mg.kg^{-1}$ in group A and of 0.0336 $mg.kg^{-1}$ in group B. No statistically significant difference was found between the groups (p>0.05). The median values of the total mercury were 0.0048 $mg.kg^{-1}$ in group A and 0.0017 $mg.kg^{-1}$ in group B, respectively. A highly statistically significant difference between the groups was found (p<0.01).
CONCLUSIONS: No correlation was obtained between the total mercury content in the hair of dogs and granulated feed (r_s=0.2069, p>0.05). The reason may be a content of various mercury species in feed samples or a human failure (nonobservance of the prescribed diet).

Abbreviations
AMA - Advance Mercury Analyzer
THg - total mercury

INTRODUCTION

Mercury and most of its compounds are highly toxic and have an adverse effect on humans and animals (Scheuhammer *et al.* 2007). The most toxic form of mercury is methylmercury (WHO, 2007). Mercury originates from both natural (e.g. vulcanic activity, erosion) and anthropogenic sources (fossil fuel combustion, gold extraction, cement production, the chemical industry, and dentistry – amalgams). Chronic exposure to mercury from various sources, including dental amalgams, may cause a range of health problems (e.g. fatigue, anxiety, and depression) (Kern *et al.* 2014). Owing to its instability and ability of long-distance atmospheric transfer, mercury enters remote areas and can contaminate polar and subpolar areas (Fitzgerald *et al.* 1998; Ebinghaus *et al.* 2002; Sousa *et al.* 2013).

A highly topical issue is the occurrence of mercury in the aquatic environment in which, in sediment, a transformation of the inorganic form into the organic one and consequent accumulation in aquatic organisms, namely fish, can take place. (Fitzgerald *et al.* 1998; Ipolyi *et al.* 2004). In this way, mercury can subsequently enter the food chain of humans and animals. The organic form of mercury, accepted by living organisms, easily penetrates the blood-brain barrier and, afterwards, accumulates in the brain. Furthermore, mercury deposits in the structure of skin derivatives, which can be used in the monitoring of long-term mercury exposure (Sakai *et al.* 1995; WHO, 2007). Studies dealing with this issue in both humans and animals have been performed. Skin derivatives (e.g. hair) serve as a suitable non-invasive matrix for the monitoring and evaluation of mercury exposure via feed (Lieske *et al.* 2011; Sousa *et al.* 2013). Lieske *et al.* (2011) demonstrated that the hair of piscivore animals (e.g. otter, polar bear) could be successfully applied in the risk assessment of the contamination of the aquatic environment with mercury. Similar conclusions were also drawn in studies realized in Nordic regions where sled dogs are suitable model organisms because they feed primarily on fish (Dunlap *et al.* 2007). The monitoring of mercury content in hair has also been realized in cats, in which a correlation between the mercury content in hair and the mercury content in feed with fish (e.g. tuna, sardines) has been investigated. Higher mercury concentration was detected particularly in older individuals (Sakai *et al.* 1995). An influence of feed with fish on mercury accumulation in hair has been proven in monitoring studies in dogs as well (Sakai *et al.* 1995; Reynolds *et al.* 1999; Dunlap *et al.* 2007).

The maximum hygienic limits for mercury in feed have been established by the Directive of European Parliament and Council No. 2002/32/ES on undesirable substances in animal feed as amended by the Directive of Commission No. 2010/6/EU. The maximum limit of mercury in feed mixtures for dogs, cats and fur animals is 0.4 $mg.kg^{-1}$.

The aim of the present study is to investigate whether the addition of fish into granulated feed leads to an increased accumulation of mercury in hair and evaluate a correlation between the total mercury content in the feed served and the total mercury content in skin derivatives of dogs.

MATERIAL AND METHODS

To evaluate the contamination of dog organisms with mercury, a non-invasive method was used. Approximately 500 mg of hair, cut in the forearm region as close to the skin as possible, was collected in 131 different dog breeds. The dogs came from private owners. There were both pedigreed and crossbreed dogs (74 males, 57 females). Examined dogs were divided into two groups. In the first group (group A, n=42), there were dogs fed granulated feed containing fish (fish meal), in the second group (group B, n=89) dogs fed granulated feed free of fish. To determine the total mercury content, samples of granulated feed in the amount of about 10 g were also collected. Granulated feed was fed minimally one month prior to hair collection.

Before the actual measurement, impurities were removed from hair samples by washing in acetone, thereafter three times in distilled water and subsequently in acetone again. Samples were left in every agent for 10 minutes and stirred every 2 minutes, thereafter dried between two filtration papers. Samples of granulated feed were not adjusted, they were only crushed. The total mercury content in hair samples and granulated feed samples was measured on the single-purpose atomic absorption spectrophotometer AMA 254 (Advanced Mercury Analyzer), which enables the detection of total mercury. AMA 254 employs a thermic decomposition of samples. The total mercury content in hair and granular feed was determined by a direct method involving cold vapours using an AMA. The wavelength employed was 253.65 nm, the limit of detection was 0.01 ng of mercury, and reproducibility was below 1.5%. Statistical processing was performed by using UNISTAT 6.5 for EXCEL. The unpaired Mann-Whitney test was used to compare the differences between groups. For evaluating the relationship between mercury in the hair and granulation the Spearman coefficient was used. Statistical significance was assessed at the level p=0.05 and p=0.01.

RESULTS

Total mercury in granulated feed

The results of the total mercury content in granulated feed are presented in Figure 1. Data is presented as median values. The highest concentration was found in group A (0.0048 $mg.kg^{-1}$) ranging from 0.0006 to 0.0498 $mg.kg^{-1}$. The content of the total mercury in group B was 0.0017 $mg.kg^{-1}$ and ranged from 0.0002 to 0.0267 mg.kg^{-1}. A statistically significant difference was found between the groups ($p<0.01$).

Total mercury in hair

The results of the total mercury content in hair are presented in Figure 2. The data are presented as median values. The median concentration of the total mercury in the hair in group A was 0.0375 mg.kg^{-1} (ranged from 0.0104 to 0.7278 mg.kg^{-1}) and the content of the total mercury in the hair in group B was 0.0336 mg.kg^{-1} (ranging from 0.0003 to 0.4567 mg.kg^{-1}). Although a higher total mercury content was found in the hair in group A compared to group B, no statistically significant difference was found between the groups ($p>0.05$).

Correlation of total mercury content between hair samples and feed

No correlation was obtained between the total mercury content in the hair samples of dogs and the total mercury content in samples of granulated feed ($r_s=0.2069$, $p>0.05$).

DISCUSSION

In our study, we investigated the relationship between the total mercury content in the feed served and the total mercury content in the skin derivatives of dogs. As stated by Clarkson & Magos (2006), the mercury content in feed influences the mercury content in the skin derivatives and blood of mammals.

In the monitored samples, we investigated a statistical significance in the resulting values of total mercury in the granulated feed ($p<0.01$). A higher mercury concentration was detected in group A compared to group B. This fact is also documented by Gerlach *et al.* (2006), who state that mercury accumulates in fish and can transfer to the food chain. This finding is supported by the study of Kruzikova *et al.* (2008) stating that fish have a natural tendency to accumulate mercury in their bodies. The degree of mercury accumulation in fish is influenced by the fish species, breeding method and place of occurrence. The total mercury content differs in fish bred in aquaculture and wild fish. This was confirmed by Kim *et al.* (2012), who conducted their research on the Korean peninsula and compared the mercury content in wild fish and fish from aquaculture. Wild fish had higher mercury levels compared to fish bred in aquaculture. The fish were of the same species and had an identical body length. Mercury accumulation depends on the fish position within the food chain (Gerlach *et al.* 2006), predatory species have higher mercury levels compared to non-predatory ones. Mercury concentration in fish bodies rises with the growing trophic level. The amount of mercury in fish is influenced by the size and age of the fish (Cizdziel *et al.* 2002).

In the dog hair, we didn't found statistically higher values of total mercury in the group A and group B. On the other hand Sousa *et al.* (2013) detected difference and in his study described dogs eating fish and showing higher mercury concentration compared to dogs which were fed a commercial diet without fish. Sedlackova *et al.* (2013) stated that the mercury content in the hair of dogs depended on the consumption of fish, granulated feed containing fish and fish dainties. Some studies indicate that mercury levels in dogs may be affected by other factors, namely the geographical location and type of diet. Diet plays an important role in dogs coming from specific communities with higher fish consumption or consuming just one type of food (Sousa *et al.* 2013).

In the human population, we can observe the same correlation between fish consumption and mercury concentration in blood and skin derivatives. Nair *et al.* (2014) reported the correlation between mercury concentration and fish consumption in humans. Humans

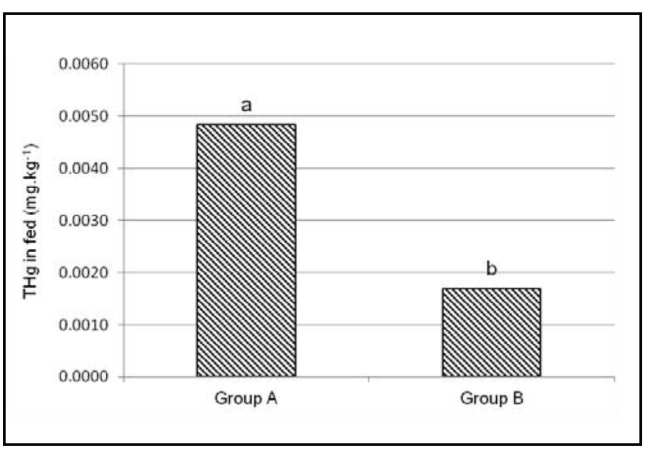

Fig. 1. Results of the median concentration of THg in feed. Significant differences ($p<0.01$) are indicated by different alphabetic superscripts (group A – fed granular feed containing fish, group B – granular feed free of fish).

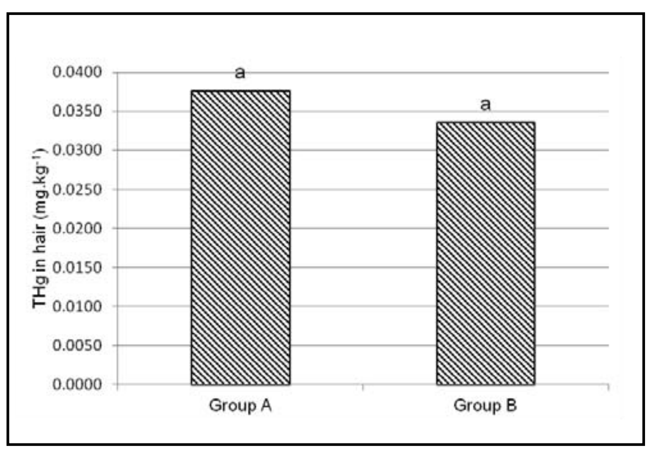

Fig. 2. The results of the median concentration of THg in hair. Significant differences ($p<0.05$) are indicated by different alphabetic superscripts (group A – fed granular feed containing fish, group B – granular feed free of fish).

having higher fish intake showed increased mercury levels in hair (Hruba *et al.* 2012; Liu *et al.* 2014). Hair is a suitable matrix for monitoring the effects of long-term exposure to mercury and it is a good indicator of fish consumption (Kruzikova *et al.* 2008; Kruzikova *et al.* 2009). Cejchanova *et al.* (2012) investigated that women who did not consume fish within the monitored period showed a lower mercury level in blood.

The difference in the total mercury content in the hair in group A and group B was not significant. This result could result from various proportions of fish, the use of different fish species, or the presence of various mercury species in the samples of granulated feed. Sedlackova *et al.* (2013) reported that individual forms of mercury had a different toxicity and half-life. For example, ethylmercury has a shorter half-life compared to more toxic methylmercury. The other factor could be human failure. The monitored dogs could have been served not only the monitored granulated feed, but also other food or dainties, or, if reared outside, they could also have had other sources of food. This is supported by the fact that dogs fed granulated feed free of fish had a maximum value of total mercury of 0.4567 $mg.kg^{-1}$, which proves that the dogs also received another feed containing fish.

Some studies also searched for a relationship between the total mercury concentration in hair and the age and gender of dogs, respectively. Statistically significant differences in the mercury concentration in hair depending on age have not been found (Hansen & Dancher, 1995; Dunlap *et al.* 2007). Similarly, Lopez-Alonzo *et al.* (2007) did not prove any significant correlation between the age of dogs and mercury concentration in hair. Malvandi *et al.* (2010) did not record any significant difference in the mercury content between dogs´ genders.

A low mercury concentration in the hair of dogs may be caused by the fact that dogs are not typical fish consumers compared to cats and humans (Sousa *et al.* 2013). The hair of cats has a significantly higher mercury concentration compared to dogs, due to a higher consumption of fish like tuna or sardines (Doi *et al.* 1986), as observed by Sakai *et al.* (1995) as well.

CONCLUSIONS

The relationship between the total mercury content in granulated feed containing fish (group A) and free of fish (group B) and in skin derivatives of dogs was monitored. A significant difference in the total mercury content between group A and those group B was investigated ($p<0.01$). However, a significant difference in the mercury content between hair samples of dogs group A and hair samples of dogs group B was not proved ($p>0.05$). No correlation was obtained between the total mercury content in the hair of dogs and the total mercury content in granulated feed ($r_s=0.2069$, $p>0.05$). No sample granular feed was crossing the limit 0.4 $mg.kg^{-1}$ (maximum limit of mercury in feed mixtures for dogs).

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