

Total mercury content in canine hair before and after administration of vaccines containing thiomersal

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Abstract

OBJECTIVES: Thiomersal is ethylmercury containing compound. It has been used as a preservative in vaccines since the 1930s because it is very effective in preventing bacterial contamination. Ethylmercury penetrates into growing hair in a similar manner as methylmercury.

DESIGN: A total of 48 hair samples were collected from vaccinated dogs. Each sample was accompanied with a questionnaire including data on age, gender, vaccinations. Total mercury content in hair, granules and vaccines was determined by the direct method of cold vapours using an AMA 254 (advance mercury analyser; Altec Ltd., Czech Republic).

RESULTS: At first we performed two pre-experiments. In first pre-experiment, the highest value of total mercury content was 0.732 mg.kg⁻¹. The content of total mercury ranged from 0.022 to 0.092 mg.kg⁻¹ in the second pre-experiment. The results were not statistically significant in the pre-experiments. In the main experiment the lowest concentration of total mercury in dog's hair was 0.002 mg.kg⁻¹ and the highest value was 0.560 mg.kg⁻¹. The median value of total mercury ranged from 0.023 to 0.033 mg.kg⁻¹. The results were not statistically significant in the main experiment. Total mercury content in vaccines corresponded with the declared quantity. Rather, results showed mercury content to be correlated with the consumption of feed containing fish.

CONCLUSIONS: Thiomersal preservative, contained in vaccine, does not increase content of total mercury in canine hair. Our results have shown that content of mercury in hair depends on fish consumption (fish granules, fish treats and fresh fish).

Abbreviations:

AMA - advance mercury analyser
EtHg - ethylmercury
MeHg - methylmercury
THg - total mercury

INTRODUCTION

Mercury is a metallic element that occurs in elemental mercury, inorganic mercury compounds, and organic mercury compounds. The toxicity of mercury depends on the form of mercury, route of entry, dosage, and time of exposure (Clarkson, 1992, 1997). Ethylmercurythiosalicylate (an organic compound of mercury) is contained in thiomersal (thimerosal, merthiolate) (Magos, 2001; Heron *et al.* 2004), and has been used as a preservative in vaccines since the 1930s owing to its effectiveness in preventing bacterial contamination (Ball *et al.* 2001; Pichichero *et al.* 2002; Geier *et al.* 2007). Thiomersal has been widely used in multidose vaccines (Folb *et al.* 2004; Pichichero *et al.* 2008; Barregard *et al.* 2011).

On July 7, 1999, the American Academy of Pediatrics and the US Public Health Service issued a joint statement calling for the removal of thiomersal, a mercury-containing preservative, from vaccines. This action was prompted in part by a risk assessment from the Food and Drug Administration (Ball *et al.* 2001).

The toxicity of low-dose exposure to ethylmercury (EtHg) is similar to that of methylmercury (MeHg). Vaccine with thiomersal may on rare occasions causes neurologic reactions like encephalopathy, Guillain-Barre syndrome, meningo-encephalitis, poly-neuropathy, or peripheral neuritis (Ball *et al.* 2001; Zareba *et al.* 2007; Dorea, 2011b). The neurotoxicity of ethylmercury is lower than that of methylmercury (Magos, 2003). Hypersensitivity reactions following thiomersal exposure are well-recognized. Known manifestations of acute toxicity following high-dose exposure to thiomersal include neurotoxicity and nephrotoxicity (Ball *et al.* 2001). Ethylmercury accumulates in the brain, where it converts to inorganic mercury much more rapidly than methylmercury dose (Clarkson, 2002). Exposure to thiomersal or thiomersal containing vaccines (TCVs) is associated with increased risk of autism, autism spectrum disorder (ASD), tics, attention deficit disorder, and emotional disturbances (Young *et al.* 2008; Price *et al.* 2010).

Ethylmercury is known to be less stable than methylmercury (Qvarnstrom *et al.* 2003). Due to its lower stability, it could be expected that ethylmercury, once incorporated into hair, would break down into Hg^{II} more rapidly than methylmercury (Dorea, 2009). Hair is commonly used as biomarker of exposure to methylmercury because concentrations of methylmercury in hair are proportional to concentrations in the blood and the brain (Cernichiari *et al.* 1995). Ethylmercury penetrates into growing hair in a similar manner as methylmercury (Zareba *et al.* 2007; Schoeman *et al.* 2010).

The aim of this study was to determine whether there is an increased content of total mercury in canine hair after treatment with the thiomersal preservative.

MATERIAL AND METHODS

Sample collection

A total of 48 hair samples were collected from vaccinated canines. Each sample was accompanied with completed questionnaire including data on age, gender, vaccinations. An important experimental control was consumption of fish or granules with the presence of fish meals because fish are the primary common source of methylmercury intake (Kannan *et al.* 1998, Kruzikova *et al.* 2009). Hair samples were collected from canines in a vicinity of the front leg.

Vaccine

Thiomersal contains 49.55% of ethylmercury (Geier *et al.* 2007). It is contained in the vaccine against rabies, tetanus and leptospirosis. It is also present in bivalent vaccine against distemper and parvovirus and it is contained in vaccine for the active immunization of canine animals against distemper, infectious hepatitis, infectious laryngotracheitis, parvovirus, and parainfluenza. In our experiment, a vaccine against rabies (with 0.01% of thiomersal) was used most commonly.

Experiment

Two pre-experiments were carried out prior to the main experiments. Samples were first analyzed from canine hair on day 0, which is the day prior to vaccination, and then samples taken after vaccination on days 2, 4, 6 and 8 ($n=9$). For the reason that there was no increase in the total mercury in the hair was found, a second pre-experiment was carried out. This time, samples were taken after vaccination on days 0, 3, 6, 9, 12 and 15 ($n=7$). The main experiment was carried out on days 0, 10, 15, 20 and 25 ($n=32$).

Determination of mercury content

Obtained samples were cut into small slivers (2–5 mm). Hairs were then washed in acetone, three times in water and once more in acetone (every ten minutes); samples were subsequently dried overnight (Cejchanova, *et al.* 2008). For the mercury analysis we used about 5–10 mg of the hair, 30 mg of granules (without pre-treatment) and 100 μ l of properly diluted vaccine.

Total mercury content in hair, granules and vaccines was determined by a direct method involving cold vapours using an AMA 254 (advance mercury analyser; Altec Ltd., Czech Republic). The AMA 254 uses a mercury vapour generation with subsequent capture and with concentrate on a gold amalgamator. The wavelength employed was 253.65 nm, the limit of detection was 0.001 mg.kg⁻¹ of mercury, and reproducibility was below 1.5%. Temperature program parameters for hair were set to values 10/100/30, meaning: 10 second for drying, 100 second for decomposition and 30 second waiting time. Temperature program parameters for granules were 10/150/45 and 60/150/45 for the vaccines. For each sample, two independent measurements

were performed. The AMA 254 computed the mean and the standard deviation. If standard deviation was greater than 10% measurement was repeated.

The accuracy of the results (total mercury content) was validated using the standard reference material CRM No. 13 HUMAN HAIR of the National Institution for Environmental Studies.

Statistical analysis

For statistical evaluation, we used the Wilcoxon test. This is used for the evaluation of paired experiments, when the monitored parameter does not correspond to the Gaussian normal distribution. Each given day was compared to day 0.

RESULTS

To begin, we performed two pre-experiments. In first pre-experiment, the lowest concentration of total mercury in hair was 0.003 mg.kg⁻¹, the highest value was 0.732 mg.kg⁻¹. The content of total mercury ranged from 0.022 to 0.092 mg.kg⁻¹ in the second pre-experiment. The median value is shown in the Table 1. The results of the pre-experiments were not statistically significant in.

All results of vaccinated canines from the main experiment are provided in Table 2. The lowest concentration of total mercury in canine hair was 0.002 mg.kg⁻¹ and the highest was 0.560 mg.kg⁻¹. The median value of total mercury is shown in Table 2, it ranged from 0.023 to 0.033 mg.kg⁻¹. The results were not statistically significant in the main experiment. There is no correlation between vaccine containing the thiomersal preservative and content of mercury in canine hair.

Values of total mercury concentration from canines that consumed fresh fish and granules with fish meals are given in Table 3. The highest content of total mercury ranged from 0.575 to 0.732 mg.kg⁻¹ in the first pre-experiment, these canines consumed treats of salmon or fresh salmon. Two canines consumed fresh fish in the main experiment (values ranged from 0.389 to 0.560 mg.kg⁻¹ and from 0.318 to 0.465 mg.kg⁻¹, respectively). Content of total mercury in the hair of canines that consumed granules with fresh meals was within the range of 0.074–0.132 mg.kg⁻¹ in the second pre-experiment, and 0.075–0.129 mg.kg⁻¹ in the main experiment. Total mercury content in vaccines corresponded with the declared quantity.

DISCUSSION

In US, use of the preservative thiomersal in human vaccines has been restricted since 1999. This restriction does not apply to animal vaccines. The main reason has been fear of ethylmercury exposure to the human organism. It has been hypothesized that ethylmercury might have a similar effect as methylmercury (Burbacher *et al.* 2005; Zareba *et al.* 2007). Mercury-

containing thiomersal vaccines were recognized safe at the UNEP (United Nations Environment Programme) meeting and excluded from the treaty (Kirby, 2013). This decision was endorsed by WHO. These concerns were premature. Ethylmercury has a much lower half-life. The blood half-life of ethylmercury in infants is 6 days (Pichichero *et al.* 2002). It might differ from the 40–50 days half-life of methylmercury (ranging from 20 to 70 days) in adults and breastfeeding infants (Clarkson, 1992). WHO (2012) published that the half-life of ethylmercury in blood is between 3 and 7 days in pre-term and low birth-weight babies. Burbacher *et al.* (2005) discovered that the half-life of ethylmercury in the blood of infant monkeys was between 2.1 and 8.6 days after exposure (i.m. injection) and thus significantly shorter than the 21.5-day elimination half-life following methylmercury exposure.

Tab. 1. Values of total mercury (THg) in dog's hair (mg.kg⁻¹) in the pre-experiments.

1 st pre-experiment				
Time (day)	n	Median of THg (min – max)	Median of differences from day 0	p-value (Wilcoxon test)
0	9	0.052 (0.005; 0.732)		
2	8	0.045 (0.003; 0.649)	-0.0011	0.861
4	9	0.049 (0.003; 0.613)	0.0002	0.309
6	9	0.049 (0.003; 0.587)	-0.0042	0.689
8	8	0.045 (0.003; 0.620)	-0.0043	0.425
2 nd pre-experiment				
0	6	0.044 (0.022; 0.092)		
3	6	0.030 (0.022; 0.074)	0.0029	0.345
6	7	0.039 (0.020; 0.102)	-0.0002	0.753
9	7	0.039 (0.021; 0.109)	-0.0011	0.917
12	7	0.041 (0.022; 0.132)	0.0034	0.028
15	6	0.036 (0.020; 0.081)	0.0028	0.345

Tab. 2. Values of total mercury (THg) in dog's hair (mg.kg⁻¹) in the main experiment.

Main experiment				
Time (day)	n	Median of THg (min – max)	Median of differences from day 0	p-value (Wilcoxon test)
0	32	0.032 (0.003; 0.389)		
10	30	0.033 (0.002; 0.522)	0.0001	0.861
15	31	0.029 (0.002; 0.506)	0.0007	0.309
20	29	0.032 (0.002; 0.560)	-0.0007	0.689
25	28	0.023 (0.001; 0.553)	0.0006	0.425

Tab. 3. Values of total mercury (THg) in hair ($\text{mg}\cdot\text{kg}^{-1}$) of individual dogs that consumed fish and fish products.

Dogs	Days (content of THg $\text{mg}\cdot\text{kg}^{-1}$)					Note
	0	2	4	6	8	
1	0.590	0.611	0.591	0.587	0.620	Fresh fish, treats of salmon
2	0.732	0.649	0.613	0.575	0.620	Fresh fish, treats of salmon
	0	3	6	9	12	15
3	0.092	0.074	0.102	0.109	0.132	0.081 Granules with fish meals
	0	10	15	20	25	
4	0.389	0.522	0.506	0.560	0.553	Fresh fish
5	0.318	0.415	0.465	0.448	0.423	Fresh fish
6	0.114	0.075	0.107	0.096	0.129	Granules with fish meals

In our study we used vaccines with 0.01–0.02% of thiomersal content. Total mercury content in vaccines corresponded with the quantity advertised. Usual dose of paediatric vaccine contains 12.5–25 μg of mercury (Ball *et al.* 2001). Pichichero *et al.* (2002) used vaccine which contained 0.01% of thiomersal (25 μg mercury per dose) and 0.005% of thiomersal (12.5 μg mercury per dose) in their study.

Total mercury content can be observed in blood, urine and hair. In our study on canines, we chose to determine total mercury in hair. This is a non-invasive method. So far only few studies have been published on this subject. Dorea *et al.* (2011a) demonstrated correlation between the number of vaccinations and concentration of ethylmercury in the hair of breastfed infants. However, the correlation was not statistically significant.

Zareba *et al.* (2007) presented results concerning ethylmercury content in the growing hair of mice. In this study, ethylmercury increased over the course of four days. The increased levels of ethylmercury in dog's hair were not found in our study (Table 2).

Many studies on the topic of mercury content in blood of infants, children, adults and animals following vaccination have been published (Ball *et al.* 2001; Pichichero *et al.* 2002; Schober *et al.* 2003; Burbacher *et al.* 2005; Zareba *et al.* 2007).

Pichichero *et al.* (2002) showed that quantity of mercury content in the blood of infants receiving vaccines formulated with thiomersal is well below concentrations potentially associated with toxic effects. There is no evidence of mercury toxicity in infants, children or adults who are exposed to thiomersal in vaccines (Andrews *et al.* 2004), but increased mercury levels were detected in stools after vaccination, suggesting that the gastrointestinal tract is involved in ethylmercury elimination (Pichichero *et al.* 2008). Harry *et al.* (2004) presented results concerning neonatal mice which were injected with a 10-fold higher dose of methylmercury chloride, ethyl-

mercury chloride and thiomersal. Measurements were performed over the course of 24 hours and seven days, respectively. The concentration of thiomersal in the brain did not decrease (from 1.7 ± 0.2 to 1.8 ± 0.3 $\mu\text{g}\cdot\text{g}^{-1}$). A decrease was observed in the blood (from 4.2 ± 0.3 to 1.5 ± 0.1 $\mu\text{g}\cdot\text{g}^{-1}$), the kidney (from 28.9 ± 4.2 to 17.8 ± 2.2 $\mu\text{g}\cdot\text{g}^{-1}$) and the muscle (from 5.4 ± 0.8 to 1.1 ± 0.1 $\mu\text{g}\cdot\text{g}^{-1}$). Zareba *et al.* (2007) reported reduction of ethylmercury content in blood of mice during 4 days.

Fish is very important and valuable food for human. It is the major source of n-3 polyunsaturated fatty acids which have positive effects in prevention of cardiovascular diseases but fish can be depository for several contaminants, such as mercury (Andreji *et al.* 2012; Mraz *et al.* 2012). In our study, it was the consumption of fresh fish, fish treats and granules with fish meals that was shown to influence the content of total mercury (Table 3). Kruzikova *et al.* (2009) presented results of positive correlation between the total mercury content in human hair and the consumption of marine and freshwater fish. Hair is considered indicator of fish consumption. Predatory fish are on the top of the food chain and therefore they have higher mercury content (US Environmental protection agency, 1997).

CONCLUSIONS

Thiomersal preservative, contained in vaccine, does not increase content of total mercury in canine hair. Our results have shown that content of mercury in hair depends on fish consumption (fish granules, fish treats and fresh fish).

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