## Canine hair as a model for tracing ethylmercury from Thimerosal-containing vaccines. Author reply

## Lenka SEDLÁČKOVÁ

Department of Veterinary Public Health and Animal Welfare, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Correspondence to:	Lenka Sedlackova, MSc.				
-	Department of Veterinary Public Health and Animal Welfare, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho str. 1/3, 612 42 Brno, Czech Republic. TEL: +420 541 562780; FAX: +420 541 562790; E-MAIL: H10023@vfu.cz				
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We thank Mr. José G. Dórea for his interest in our article *Total mercury content in canine hair before and after administration of vaccines containing thiomersal*, which deals with the determination of total mercury in hair of dogs after administration of thiomersal-containing vaccine (TCV) with ethylmercury as active substance. We thank him, in particular, for very inspiring and valuable comments that we will use in our future work.

We would like to state the following notes:

As far as the proportions of organic and inorganic mercury in canine hair are concerned: Methylmercury accumulates in hair. Hair is a suitable biomarker of human exposure to mercury, the mercury content in hair reflects the methylmercury concentration in brain (Cernichiari *et al.* 1995). As soon as mercury is incorporated into hair, it is stable and able to provide a history of exposure (Phelps *et al.* 1980). In our work, we presumed that ratio organic mercury:inorganic mercury in canine hair will be identical to the ratio methylmercury:total mercury in human hair (Cejchanová *et al.* 2008). We agree that other hair structures can also accumulate mercury as stated by Dórea and Pereira (1983).

We know that speed of hair growth in human is about 1 cm per month (Kružíková *et al.* 2009). Speed of hair growth in dogs is very significantly influenced by the breed. The average speed of growth per 1 month is about 1 cm, i.e. 0.3 mm per day (personal information prof. M. Svoboda, University of Veterinary and Pharmaceutical Sciences Brno).

In our work, dogs were vaccinated against rabies. We stated also other diseases (tetanus; leptospirosis; distemper; distemper and parvovirosis; infectious hepatitis; infectious laryngotracheitis; parvovirosis; and parainfluenza) against which the vaccines containing thiomersal preservative agent are applied in the Czech Republic (RVLP, 2013). We did not consider to be important how many times dogs had been vaccinated in previous period. In monitored dogs, we determined total mercury content in hair before vaccination and then in early intervals 5, 10, 15, 20 and 25 days after vaccination. The dose of vaccine is identical for both small breeds (Chihuahua) and big breeds.

The paired Wilcoxon test was adopted for the analysis of differences of individual time points against the initial value as the most straightforward method of analysis of time dependent data. Repeated measures ANOVA or other more sophisticated models can be applied too; nevertheless due to relatively low sample size, missing data in some time points and less straightforward interpretation we decided to show results as simple as possible. The results computed on data using their expression as percentage of initial value are given in the Table below; the interpretation of results is the same as for absolute values.

Time (day)	n	THg in % of initial value Median (min – max)	Median of differences from day 0 in % of initial value	p-value (Wilcoxon test)
10	30	100.5 (18.0; 1 002.2)	0.5	0.894
15	31	103.7 (14.0; 218.4)	3.7	0.382
20	29	89.9 (10.5; 1 734.8)	-10.1	0.627
25	28	102.7 (15.4; 303.5)	2.7	0.374

Sousa *et al.* (2013) report results obtained in draught dogs in their work. It is obvious that higher values of mercury content are connected with regular fish consumption in these dogs. In the Czech Republic, dogs are fed with fish rarely and only in exceptional cases (fish dry food and dainties), thus the obtained values of total mercury content in hair are low. In our work, we found the relation between total mercury content in hair and presence of fish products in dog food.

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