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Source: Journal of Wildlife Diseases, 37(2) : 362-365

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-37.2.362>

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Theobromine Intoxication in a Red Fox and a European Badger in Sweden

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ABSTRACT: A red fox (*Vulpes vulpes*) and a European badger (*Meles meles*) were found dead on a golf-course in October 1997 near Stockholm (Sweden). At necropsy, both animals were obese and the main finding was acute circulatory collapse. Theobromine intoxication was suspected as chocolate waste was available at a nearby farm and no other cause of death could be detected. Gastric contents and samples of liver from both animals were analyzed by reversed-phase high pressure liquid chromatography for the presence of methylxanthines. Theobromine and caffeine were detected in gastric contents and theobromine was identified in the liver samples from both animals. This appears to be the first report of theobromine intoxication in the red fox and the European badger.

Key words: Chocolate, European badger, intoxication, *Meles meles*, methylxanthines, red fox, reversed-phase high pressure liquid chromatography, theobromine, *Vulpes vulpes*.

The red fox (*Vulpes vulpes*) is present throughout Sweden. Following an epizootic of sarcoptic mange (caused by *Sarcoptes scabiei*) spreading throughout the country since the early 1970's (Mörner, 1992) the population has decreased and is currently estimated to include 200,000 animals with an annual bag of approximately 45,000 (T. Mörner, pers. comm.). The European badger (*Meles meles*) is found in the south and central parts of Sweden as well as in cultivated areas along the northern coast. The badger is not an endangered species in Sweden. The estimated population is 150,000–200,000 animals, and 35,000 badgers are harvested annually (T. Mörner, pers. comm.).

The red fox and the badger are both opportunistic feeders. The red fox feeds on rodents supplemented by hares, roe deer, birds, frogs, beetles and berries. The diet of badgers include earthworms, snails, in-

sects, rodents, nestlings, eggs, frogs, berries, fruits and oats. European badgers are known among Swedish hunters to show a predilection for sweet food, such as cookies, honey and molasses, which can be used as bait in traps.

In October 1997, a red fox and a European badger were found dead on a golf-course north of Stockholm (Sweden; 59°45'N, 18°00'E). As several red foxes had recently been found dead nearby, both animals were sent for post-mortem examination to the Department of Wildlife (National Veterinary Institute, Uppsala, Sweden).

On arrival, there was minimal autolysis. The red fox was a young male weighing 6.1 kg and the badger was an adult female weighing 9.2 kg. Both animals were obese. Unilateral traumatic corneal lesions were seen in both animals as well as hemorrhages in the left third eyelid of the fox. Numerous flies and ticks (*Ixodes ricinus*) were detected on both animals. There were no signs of sarcoptic mange. Gross post-mortem examination of both animals revealed visceral congestion. The spleen of the badger was enlarged. The gastric content of the badger was abundant and homogenous brown and the stomach of the red fox contained the remains of a bird and a moderate amount of brown ingesta.

Tissue samples were collected and fixed in 10% buffered formalin for 24 hr and processed routinely. The histological examination showed signs of circulatory failure with congestion and acute non-reactive edema in liver, kidney, lung, lymph nodes, heart and meninges. Both animals had mild mononuclear corneal cell infiltration and corneal edema. Multifocal hem-

orrhages were observed in the cerebral cortex and in the cerebellum of the badger. Mild alveolar fibrosis, chronic granulomatous alveolitis and degenerated remains of pulmonary nematodes were detected in the badger. The spleen of this animal was mildly reactive. No larvae of *Trichinella* sp. could be detected in the diaphragm and the masseter muscle. Specimens of liver and spleen from both animals were submitted for routine bacteriological culture on Blood Agar Base No. 2 (Difco Manual, Difco Laboratories, Detroit, Michigan, USA) with 5% horse blood at 37 C for 48 hr. Samples were also pre-enriched in buffered peptone water at 37 C for 24 hr followed by enrichment in Rappaport-Vassiliadis broth and plating on Xylose-lysine-desoxylate agar and brilliant green-phenol red agar at 37 C for 24 hr. No pathogenic bacteria were isolated.

It was later revealed that chocolate waste was kept in plastic bags in an open barn at a farm near the golf-course. The chocolate waste, consisting of plain chocolate bars and small amount of filling, was used to feed cattle and pigs. The farmer had seen red foxes in the barn on several occasions. Due to suspected dietary exposure to chocolate waste, a chemical analysis of methylxanthines was performed on samples of gastric content and liver. The applied method was a modification of an analysis of so called doping substances in urine and blood samples from racing horses and dogs (Schubert, 1995). The samples were homogenized with four times the samples weight of water-methanol (1:4). Etophylline was added as an internal standard. Following centrifugation, the supernatant was collected and partly evaporated. The solution underwent solid-phase extraction by passing through a polymer column cartridge (Isolute® ENV+, 1 ml, International Sorbent Technology, Hengoed, UK) and excess water and impurities were removed by passing water, air and hexane through the cartridge. Methylxanthines were eluted with 10% methanol in dichloromethane. Reversed-phase high

pressure liquid chromatography (HPLC) was performed on a Hewlett-Packard 1090 UV-Diode-array instrument (Hewlett-Packard, Dover, Delaware, USA). Gradient elution was used and chromatograms and spectra were recorded. The concentrations of methylxanthines were calculated by internal-standard technique with a computer program included in the instrument's software package. Spectral examination together with chromatographic retention data showed the identities of the eluted methylxanthines. Theobromine (TB) was detected at high concentrations in samples from both animals. The gastric content of the red fox contained 420 µg/g and the sample from the badger contained 270 µg/g. Caffeine was detected in the gastric contents at lower concentrations; 10 µg/g sample in the red fox and 110 µg/g sample in the badger. TB was also detected in samples from liver. The liver sample of the red fox contained 64 µg/g sample and the sample from the badger contained 105 µg/g.

Methylxanthines are naturally occurring alkaloid substances which can be found in a variety of plants. Methylxanthines include caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), paraxanthine (1,7-dimethylxanthine) and theobromine (3,7-dimethylxanthine). Cacao, which is derived from seeds of the cacao tree (*Theobroma cacao*), contains TB and, to a lesser degree, caffeine (Tarka, 1982; Feldman, 1998). Chocolate is prepared by fermentation, drying, roasting and grinding of cocoa beans, and various amounts of sugar, and sometimes dried milk and fillings, are added to make the final product (Feldman, 1998).

TB toxicity from various cacao products has been described in dogs (Glauberg and Blumenthal, 1983; Hooser, 1985; Hooser and Beasley, 1986; Hornfeldt, 1987; Gustafsson, 1993; Hovda and Kingston, 1994; Strachan and Bennett, 1994). The LD₅₀-value of the dog is reported to be 250–500 mg TB/kg bodyweight (Hooser and Beasley, 1986). Deaths have been reported af-

ter ingestion of less than 100 mg TB/kg bodyweight (Glauberg and Blumenthal, 1983; Gustafsson, 1993; Strachan and Bennet, 1994). The main pharmacological effects of methylxanthines in the dog include central nervous system stimulation, diuresis, cardiovascular and metabolic effects, bronchial relaxation and increased secretion of gastric acids (Hornfeldt, 1987). Clinical signs of acute TB intoxication in the dog include vomiting, diarrhea, cardiac arrhythmias, bradycardia, diuresis, restlessness, excitement, ataxia, muscle tremors, seizures and coma (Hooser and Beasley, 1986). Death may follow in 6 to 24 hr post-ingestion and chronic exposure can result in sudden death after several days probably from cardiac failure (Hornfeldt, 1987). Toxic effects of TB have also been reported in pigs and poultry (Blakemore and Shearer, 1943; Black and Barron, 1943; Gunning, 1950), calves (Curtis and Griffiths, 1972), horses (Hooser, 1985) as well as in mice, rats, hamsters, guinea pigs, cats and rabbits (Tarka, 1982). The post-mortem findings in the red fox and the badger in this report were consistent with what is seen in dogs. The corneal lesions in both animals and the cerebral hemorrhages in the badger were thought to have been inflicted during seizures.

TB is metabolized by the liver and excreted via urine and feces (Miller et al., 1983). The half-life of TB may be as long as 23 hr in the dog (Grip-Jonsson et al., 1994). The metabolism of TB is mediated through N-demethylation and oxidation, and substantial quantitative and qualitative differences exist between species (Miller et al., 1983).

The ingested amount of chocolate could not be determined in these cases and the capacity of the red fox and the badger to metabolize TB is unknown. According to our knowledge no data on liver concentrations in cases of TB intoxication in animals have been published. However, as the red fox and the dog are both members of the Order Carnivora, family Canidae, it cannot be excluded that the fox is as sensitive to

TB as the dog. Using the LD₅₀-data of the dog, the red fox should have consumed 1.5–3.1 kg of chocolate (6.1 kg bodyweight multiplied by 0.250–0.500 g TB (=LD₅₀) = 1.5 to 3.1 g TB; 1 g TB corresponds to 1 kg of chocolate according to the manufacturer). This amount of chocolate could possibly have been ingested by a 6.1 kg red fox at one time but it seems more likely that the intoxication resulted from repeated ingestion. Using the same data, the calculated amount of ingested chocolate of the badger was 2.3 to 4.6 kg. However, as the badger belongs to the Order Carnivora, family Mustelidae, it may not be as sensitive to TB as the dog. This is also supported by our finding that the TB concentration of the liver at death in the badger was higher than in the fox. Taken together, the lack of any sign of vomiting or diarrhea (signs of acute TB intoxication), the obesity and the calculated amount of ingested chocolate strongly indicate that the intoxication was the result of chronic intoxication. However, the scarcity of toxicity data in these two animal species, particularly the badger, and the small sample size (one individual of each species) make all interpretations of results speculative.

Based on the two cases reported in this study, we conclude that the spectrum of species known to be susceptible to TB intoxication should be extended to include the red fox and the European badger and, therefore, it would seem wise to protect wildlife from exposure to chocolate waste.

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Received for publication 25 February 2000.