AEM Accepts, published online ahead of print on 5 October 2012
Appl. Environ. Microbiol. doi:10.1128/AEM.01798-12

Evidence Against Barium in the Mushroom *Trogia venenata* as a Cause of the Yunnan Sudden Unexpected Deaths

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Keywords: sudden unexpected death, Yunnan, barium, wild edible mushrooms

Running Title: Low barium in wild mushrooms in Yunnan

Abstract: This study examined barium concentration in the mushroom *Trogia venenata*, the leading culprit for sudden unexpected deaths in Yunnan, southwest China. We found that barium in *T. venenata* from Yunnan were low and comparable to other foods, inconsistent with barium in this mushroom as a significant contributor to these deaths.

Since 1978, over 400 sudden unexpected deaths (SUDs) have been reported in Yunnan in southwest China (11, 13). The vast majority of these deaths occurred to apparently healthy people in northwest Yunnan and over 90% clustered in the rainy season between June and August, generating significant concerns among health authorities, the general public, and all levels of government (13, 14). Recent intensive epidemiological and toxicological investigations identified the mushroom *Trogia venenata* as the lead culprit (11-15, 18, 20). Specifically, two unusual toxic amino acids, 2R-amino-4S-hydroxy-5-hexynoic acid and 2R-amino-5-hexynoic acid, in *T. venenata* have shown capable of causing hypoglycemia in mice (12, 20) that could lead to cardiac arrest and SUDs in humans. However, the two toxic amino acids in T. venenata could not explain all SUD cases (13-15) and questions remain about what other factor(s) in *T. venenata* or from other sources that could have contributed to these deaths.

When this mushroom was first suggested as a culprit for the SUDs, a leading hypothesis for its toxicity was that *T. venenata* contained high concentrations of the metal barium (14). This hypothesis was mainly based on the following two types of observations. First, previous studies have demonstrated that certain mushrooms could accumulate heavy metals, including barium (2, 3, 4, 7, 8, 10). Second, high levels of barium are known to cause high blood pressure, cardiac arrests and sudden deaths in humans (1, 3, 5, 17). Although there was no information about barium in *T. venenata* when the mushroom was first suggested as the leading culprit of SUDs (12, 13, 14), the speculation that barium in *T. venenata* might be the major cause of SUDs was picked up as a fact by almost all the major news media. These reports also generated significant concerns among the general public about potentially high levels of barium in wild edible mushrooms in southwest China. However, there has been little information on barium concentration in *T. venenata* or other mushrooms from southwest China to substantiate/refute the hypothesis.

In the summer of 2009 and 2010, we collected fruiting bodies of *T. venenata* from around five villages that had reported cases of SUDs and from around two communities that had no known SUDs. Relevant information about each of the seven villages/communities is presented in Table 1. *T. venenata* mushrooms from these communities all had identical or highly similar sequences (>99% nucleotide sequence identity to each other) at the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene cluster (data not shown), consistent with these *T. venenata* populations belonging to the same species. Barium concentrations in representative *T. venenata* mushrooms from around these sites were determined using Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) at Kunming Institute of Metallurgy following the procedure described by Li et al. (9). In the assays, we used the China National Standard Barium Solutions (GSB 04-1717-2004) as a reference for calibrating barium concentrations in wild mushrooms.

Our results showed that barium concentrations in *T. venenata* were low, ranging from 0.5 to 22 μg/g of dried mushrooms (Table 1). The mean barium concentrations in these mushrooms varied from 5.4 to 12.2 μg/g among the seven sites (Table 1). Previous studies have identified that barium compounds (e.g. barium acetate, barium carbonate, barium chloride, barium hydroxide, barium nitrate, and barium sulfide) dissolved in water could all cause adverse health effects in humans (1,5). Based on our data, to reach the lethal barium concentration by consuming *T. venenata*, and assuming that the consumed mushrooms all had the most toxic form of barium ($BaCl_2$, minimum lethal dose at 11.4mg/kg of body weight, 4), a person weighing 60kg would need to consume at least 35kg of dried *T. venenata* mushrooms (equivalent to about 350kg fresh mushrooms) with each containing the highest concentration of barium that we detected here (i.e. 22μg/g of dried mushroom, in Beishan village in Heqing county). This is an extremely unlikely event. In addition, there was no positive correlation between SUD mortality rate (Table 1) and barium concentration in *T. venenata* among the seven sites. Instead, though statistically not significant (p=0.526), a slight negative correlation was found (Pearson's correlation coefficient r= -0.292).

We further investigated barium concentrations in several wild edible mushrooms in southwest China to test if *T. venenata* preferentially accumulate barium. A total of 36 mushrooms belonging to 12 species obtained from seven mushroom markets were analyzed for their barium concentrations. These mushrooms were collected in northwest Yunnan and west Sichuan provinces. The species (and their mean barium concentrations in μg/g of dried mushroom; n, sample size) were *Albatrellus dispansus* (3.1, n=3), *Auricularia delicata* (29.5; n=3), *Boletus edulis* (5.5; n=5), *Cantharellus cibarius* (7.5; n=5), *Catathelasma ventricosum* (10.8; n=3), *Craterellus aureus* (6.9; n=3), *Lyophyllum shimeji* (4.0; n=1), *Ramaria spp.,* (3.9; n=1), *Russula virescens* (4.9; n=3), *Termitomyces radicatus* (16.4; n=4), *Thelephora ganbajun* (11.0; n=3), and *Tricholoma matsutake* (6.3; n=3). Though variations were found, both the mean and range (mean=9.1; range 0.5 – 51.0μg/g) of barium concentrations among the 36 tested wild edible mushrooms were similar to those in *T. venenata* (mean=7.4; range 0.5-22μg/g). These results are inconsistent with the hypothesis that *T. venenata* preferentially accumulate barium over other mushrooms in natural environments in southwest China.

The barium concentrations in mushrooms found here are similar to those reported in a recent study (19) that showed barium levels ranging from 0.82-22µg/g in wild mushrooms from four counties in northwest Yunnan. Overall, the barium levels in wild mushrooms in Yunnan from our study and from those in Yin et al. (19) are slightly higher than those found in wild mushrooms from other regions such as southwestern Moravia in the Czech Republic (mean of 1.43µg/g; 15) and the east Black Sea region in Turkey (a mean of 0.64 to 1.62µg/g among 18 species, 4). However, barium concentrations in wild mushrooms in Yunnan and other places are similar to those found in many foods in other parts of the world. For example, in a comprehensive survey in the United Kingdom in 2006 (6), among 20 food categories, barium concentrations ranged from 0.03µg/g (in fresh meat and poultry) to 131µg/g (in dried nuts).

While our results refute the hypothesis that there is a high barium concentration in *T. venenata* to cause SUDs in Yunnan, we cannot rule out barium as a significant contributor for the deaths. For example, high concentrations of barium were reportedly found in the blood, urine and hair samples from some victims of SUDs (14). At present, the source(s) of barium in these victims remains undetermined. In addition, we shall also like to stress that our study doesn't suggest that all wild mushrooms have low levels of barium or that all wild mushrooms are safe for human consumption. Mushroom poisoning is common and extreme care should be taken before eating unfamiliar wild mushrooms.

Acknowledgement

This study is jointly supported by grants from the Yunnan Provincial Department of Science and Technology (2010CI106), National Natural Science Foundation Program of PR China

(31100018 and 31060008), and Yunnan University (2010C04Z). The authors declare no conflict of interest.

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Table 1. Information about the 7 sampled sites for the mushroom *Trogia venenata* in northwest Yunnan, China.

County	Village	Geographic Coordinate (longitude, latitude)	No. SUDs / Total Population (Mortality)	Mean Barium Concentration in dried *T. venenata* (μg/g) (range; sample size)
Tengchong	Hengshan	98.65°E 25.42°N	13/36 (36.1%)	5.9 (3.9-9; n=4)
Bingchuan	Zhushengsi	100.38°E 25.95°N	12/43 (27.9%)	8.8 (6-11; n=5)
Dayao	Ajizu	101.03°E 25.7°N	29/120 (24.2%)	8.1 (0.6-13; n=5)
Heqing	Xipo	100.32°E 26.55°N	6/31 (19.3%)	5.4 (2.2-11; n=5)
Heqing	Beishan	100.28°E 26.48°N	7/134 (5.2%)	12.2 (6.2-22; n=3)
Tengchong	Qushixiang	98.6°E 25.22°N	0/~43500 (0%)	8.5 (3.7-13; n=5)
Xiangyun	Midian	100.83°E 25.68°N	0/~28000 (0%)	5.9 (3.7-8.9; n=5)