

Arsenic Residues in Hair Samples from Cattle in Some Arsenic Affected Areas of West Bengal, India

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Environmental pollution due to arsenic in particular has gained global attention owing to its several deleterious health effects on both human and animal population. Higher level of arsenic (0.20 - 3.7 mg/lit of water) in ground water has posed serious threats to millions of human populations in various parts of the world especially India, China, Thailand, Bangladesh and Taiwan (Chakraborty and Das 1997). Livestock reared in and around such localities are also the likely victims of such catastrophe arising from arsenic pollution. In 50 districts of Bangladesh and 9 districts of West Bengal, India higher level of arsenic in ground water above WHO's recommended limit of 0.01 mg/lit (WHO1993) and suffering of huge human population has been reported time and again. Chronic exposure to arsenic has numerous untoward effects which includes cancer of skin, urinary bladder and lung (Chen et al. 1992), hyperkeratosis, melanosis, leucomelanosis, raindrop depigmentation, gangrenous extremities, edema of dependent parts, abortion in pregnant women etc (Rahman et al. 2001). Animals exhibit signs of abdominal pain, diarrhea, salivation, lacrimation, constipation, anorexia, weight loss, groaning, increased pulse and respiratory rate, dark urination, discrete skin eruptions, vomition etc (Radostits et al. 2000).

Health effects of arsenic can be best assessed in animals as in human beings using certain indicators. Of which blood and urine may be of particular interest. But they mostly reflect higher body arsenic burden in case of acute and high level exposure and may not reflect chronic exposure, therefore, are not usually considered good indicators for chronic and continuous exposure as realized in practical field (Wilhelm et al. 1996). Reason is that arsenic remains only few hours in blood of most animal species and gets rapidly excreted through urine. Besides, non-toxic forms of arsenic such as arsenobetaine, arsenocholine are also excreted through urine which may falsely elevate urinary arsenic level (Francesconi et al. 2002). Therefore, some alternative indicators are considered. Hair analysis is a preferred method for monitoring toxic metal exposure in both human and animal studies. Hair is a keratin rich tissue and is exposed to the internal metabolic environment including blood, lymph and extracellular fluids. Wool or hair contents are highly influenced by the health status of individual (Raab et al. 2002). Besides, ingested non-toxic form of arsenic is not accumulated

in hair of animals in contrast to human being (Marafante et al. 1984). Hair is easy and safe to collect, ship and store as opposed to blood or urine, and the analysis is non-invasive and less expensive. This makes it an excellent choice in certain situations as a screening tool or biomarker of chronic toxicant exposure.

MATERIALS AND METHODS

Hair samples were collected randomly from tail region of cattle with the help of stainless steel scissors. A total of 72 samples were collected from affected localities and 29 samples from unaffected / control areas. Affected areas were chosen on the basis of prior reporting of groundwater arsenic contamination and suffering of human population. Control areas were chosen from those that are at least 300 km from affected area and with no report of groundwater arsenic contamination. Animals reared in those localities since birth were included in the present study.

Hair samples were washed properly as per standard method (IAEA 1978). It includes sequential washing with acetone, distilled water and acetone to remove adherent dirt and organic materials. After drying hair samples (approximately 1g each) were digested as per Harshey and Oostdyk (1988) using triple acid digestion mixture (HNO_3 : H_2SO_4 : HClO_3 = 4:1:1). Determination of arsenic in diluted samples was carried out in a hydride generation atomic absorption spectrophotometer (ECIL, 4141, India) at 193.7 nm and 10 mA current with air-acetylene as combustion gas. Vapor generation accessory was used to produce continuous flow of hydride vapor from 0.6% sodium borohydride and 10 mM HCl reaction mixture. The detection limit for arsenic was 0.02 $\mu\text{g/ml}$. Analytical accuracy was ensured by repeated analysis of test samples; 5 freshly prepared standards and reagent blanks, run with each analytical series. All the glass wares used for acid digestion were overnight dipped in 10% HNO_3 and rinsed in triple glass distilled water before use. Results were presented as mean \pm SE. Statistical analysis was performed using analysis of variance and paired t-test (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Clinical symptoms associated with slow accumulation of toxic metals are non-descript and overt clinical signs do not appear quickly (Quig 1998). However, an alarmingly high level of metal residues in animal system definitely predicts the possible health problems to human population due to consumption of products of animal origin (Radostits et al. 2000). Thus monitoring toxic residues of heavy metals in animal system may be used as a biological tool for assessing environmental deterioration. It is assumed that higher intake of arsenic through drinking water by the animals in affected areas might lead to higher arsenic burden in hair.

The hair arsenic residues in cattle from affected and non-affected areas are presented in table -1. The hair samples from affected areas (n=72) have significantly ($P<0.05$) higher mean arsenic level ($0.684\pm0.024 \mu\text{g/g}$) compared to controls (n=29) value ($0.301\pm0.025 \mu\text{g/g}$). There was no statistically significant ($P<0.05$) difference in hair arsenic burden in cattle with reference to age (table-2) and sex (table-3) within a particular group. Numerically, slightly higher level of arsenic was noted in male hair samples ($0.711\pm0.038 \mu\text{g/g}$) compared to their female counterpart ($0.671\pm0.031 \mu\text{g/g}$) in affected areas. Higher level of arsenic was detected in hair samples of younger age groups (<1 year & 1-3 year) than the older age groups (3-6 year & >6 year; n=33) in affected areas. However, arsenic residues in hair from affected areas under each of the age group were significantly higher than respective control value.

Table1. Hair arsenic residues ($\mu\text{g/g}$) in cattle from polluted and control areas

Area	N	Range	Mean \pm SE
Polluted	72	0.22-1.22	0.684 ± 0.024^B
Control	29	0.10-0.73	0.301 ± 0.025^A
Overall	101	0.10-1.22	0.574 ± 0.025

Mean values (\pm SE) bearing different superscripts vary significantly ($P<0.05$). Figures in parenthesis indicate range.

Significantly higher concentration of arsenic in hair samples from affected localities in the present investigation could be attributed to chronic ingestion of arsenic contaminated water that had arsenic level of $0.097\pm 0.008\mu\text{g/ml}$ (n=24) as compared to non-detectable arsenic level ($<0.02\mu\text{g/ml}$) in control areas. Mondal et al. (1997) observed arsenic level in human hair samples varying from 1 to 7.43 mg/kg as against normal range of 0.08 to 0.25 mg/kg when the drinking water arsenic level ranged between 0.05 and 2.22 mg/lit in some areas of West Bengal. A 10 times higher level of arsenic ($5.32\pm0.03 \mu\text{g/g}$) in sheep wool has been reported in an affected area compared to the unaffected area (Raab et al. 2002) and in some other study arsenic in hair samples from deer in a polluted area was 4.5 mg/kg compared to 0.2-0.6 mg/kg in unpolluted area (Marova et al. 1982). Little higher level of arsenic from hair of male animals may be due to their longer period of contact with contaminated water or due to the fact that females excrete a little quantities of arsenic through milk which may lessen their average body burden. Mitranescu et al (2003) reported higher values of arsenic in young and dark colored cattle in relation to soil arsenic contamination from industrial activities. However, Miranda et al. (2000) failed to record any age and sex wise discrepancies in blood arsenic level of calves. In the present study younger group of animals were seen to accumulate non-significantly higher concentrations of arsenic than the older ones. This could be attributable to higher methylating capacity of older individuals with subsequent rapid excretion of arsenic through urine (Hindwood et al. 2003). No such published data are available to correlate the findings of hair arsenic residues in animals with respect to age and sex.

Arsenic is rapidly excreted through urine and feces, but some of it accumulates in keratin rich tissues of the body like hair and nail etc. as a consequence of its higher reactivity and binding with tissue constituents, most specifically sulfhydryl groups (Vahter and Marfante 1983). This might be the reason for which hair arsenic level went high in cattle from affected areas. As the animals had considerable access to surface water, which contains nontoxic form of arsenic, in addition to groundwater, the exposure to this metalloid was not sufficient enough to be manifested as overt clinical signs of toxicity. Some authors even did not find any correlation between the amount of arsenic in hair and clinical signs of toxicity in cattle exposed to toxic level of arsenic (Buck, 1976).

Table 2. Hair arsenic residues ($\mu\text{g/g}$) from cattle of polluted and control areas in relation to age

Age	Polluted			Control		
	N	Range	Mean \pm SE	N	Range	Mean \pm SE
<1 year	18	0.46-0.90	0.745 \pm 0.035 ^B	21	0.21-0.73	0.400 \pm 0.052 ^A
1-3 year	21	0.33-1.02	0.683 \pm 0.036 ^B	5	0.23-0.41	0.300 \pm 0.033 ^A
3-6 year	16	0.22-0.98	0.649 \pm 0.065 ^B	8	0.12-0.40	0.222 \pm 0.034 ^A
> 6 year	17	0.32-1.22	0.654 \pm 0.059 ^B	7	0.10-0.50	0.266 \pm 0.052 ^A

Mean values (\pm SE) bearing different superscripts vary significantly ($P < 0.05$). Figures in parenthesis indicate no. of animal and range.

Analysis of hair arsenic content has been extensively used to confirm acute arsenic poisoning in human beings (Althausen and Gunther 1929) and is recently being widely accepted for assessing toxic element exposure to correlate with nutritional status, diseases and disorders related to environmental exposure (Hasan et al. 2004). Compared to other types of clinical specimen, hair has different uses and some advantages over blood or urine. While blood and urine tend to show the current or recent exposure, hair represents a higher time frame, potentially of years. Several trace elements occur in hair at higher levels, allowing for more analytically accurate results. So, hair is used as significant index of long-term exposure or as a marker for environmental contamination due to arsenic in both human and animal population (Hindmarsh 2002).

Table 3. Hair arsenic residues ($\mu\text{g/g}$) from cattle of polluted and control areas in relation to sex

Sex	Polluted			Control		
	N	Range	Mean \pm SE	N	Range	Mean \pm SE
Male	23	0.29-0.97	0.711 \pm 0.038 ^B	12	0.12-0.55	0.322 \pm 0.033 ^A
Female	49	0.22-1.22	0.671 \pm 0.031 ^B	17	0.10-0.73	0.286 \pm 0.038 ^A

Mean values (\pm SE) bearing different superscripts vary significantly ($P < 0.05$). Figures in parenthesis indicate no. of animal and range.

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