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Level of Certain Micro and Macro Minerals in Blood of Cattle from Fluoride Polluted Localities of Udaipur, India

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Abstract Analysis of soil, fodder and water samples collected from some localities of Udaipur district, Rajasthan, India revealed high fluoride concentrations indicating the areas endemic for fluoride pollution. Concentration of micro and macro minerals was estimated in blood samples collected from cattle reared in these localities, and with clinical lesions suggestive of chronic fluoride toxicity. In comparison to healthy controls, zinc, copper and manganese levels were significantly ($p < 0.05$) lower, while cobalt and magnesium concentrations were significantly ($p < 0.05$) higher in fluoride-intoxicated cattle. Results of the present study suggested that interaction of fluoride with other minerals possibly played a role in pathogenesis of chronic fluoride intoxication.

Keywords Cattle · Fluoride · Minerals · Toxicity

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Chronic fluoride exposure is a serious health hazard for both man and animal. Cattle reared in fluoride endemic areas for a long time show overt clinical signs of osteo- and dental-fluorosis. Besides bone and teeth involvement, several other organs like kidneys, liver, adrenal glands, heart, bone marrow and brain are prone to toxic effects of fluoride and the disease in livestock is characterized by debility, anemia, poor production and reproduction efficacy, and increased mortality rates (Radostits et al. 2000).

Different mechanisms have been proposed to explain the toxic effects of excess fluoride in the body (WHO 2002). Interaction of this highly electronegative and reactive halogen element with other minerals in body tissues seems to be a possible mechanism of toxic effects. Many studies on induced-fluoride intoxication in laboratory animals have revealed alternation in micro and macro minerals status in blood and other organs or tissues. She et al. (2002) reported decrease in blood zinc and iron concentration in experimental fluorosis in rabbits. It was associated with decreased level of copper, selenium and zinc concentration in muscle tissues. Kanwar and Singh (1981) observed decrease in manganese, copper and zinc concentration in liver tissues from fluoride exposed rats. Decrease in calcium and increase in phosphorus level in blood of fluorotic men and animals have been reported by several workers (Khandare et al. 2005a; Xin et al. 2006). Contrary to the above findings, Wheeler et al. (1988) recorded increase in serum calcium, magnesium and phosphorus in fluorotic sheep. There seems to have paucity of reports on the status of micro and macro minerals in blood of cattle naturally affected with chronic fluoride toxicity. The present research work was aimed to assess the status of copper, zinc, cobalt, manganese, magnesium, calcium and phosphorus in blood of fluorotic cattle.

Materials and Methods

The study site is located about 15 km north of Udaipur city in southern Rajasthan and there are several operational superphosphate fertilizer plants in the area. Cattle maintained for more than two consecutive years within 4 km radius of the factory/ industrial area, either on or off wind environment were recruited for the present study. Cattle fulfilling above selection criteria were examined clinically for the presence of clinical signs and lesions suggestive of fluorosis. The animals were particularly looked for tooth lesions, bony exostosis in metacarpal, metatarsal, ribs and frontal bones, and/ or lameness. Dental lesions were scored on a 0 to 5 point scale as per the classification suggested by Shupe et al. (1979). Lameness was scored on a 4 point scale (0: no lameness; 1: mild lameness; 2: moderate lameness and 3: severe lameness). A total of 270 cattle were randomly examined out of total estimated cattle population of around 500. Blood samples were collected from non-pregnant lactating (milk yield 2 to 4 liters per day) cows with a score of $\geq$2 for either dental lesions or lameness, and with no other apparent clinical disease. Blood samples, around 10 mL from each animal, were collected from jugular vein using heparin as anticoagulant. Half of the blood sample was centrifuged at 2000 g for 10 minutes to separate plasma for estimation of fluoride, calcium and phosphorus. Remaining whole blood was used for estimation of other minerals. Composite ground water samples measuring 100 mL each were collected in polypropylene bottle. Soil samples, weighing around 100 gm each, were collected in clean polyethylene bags. Fodder samples were collected as per the method described by Wheeler and Fell (1983). Twenty samples of fodder (each equivalent to 100 gm dry matter) were collected and were pooled to make one composite sample.

Fluoride concentrations in water, soil and fodder collected from different areas of Bareilly City, Uttar Pradesh, India were estimated to rule out any possibility of fluoride pollution. Healthy non-pregnant, low yielding (milk yield 2 to 4 l per day) cattle were selected to serve as healthy controls, and blood samples were collected from these animals in similar manner. Fluoride concentration in plasma and water was measured by the method of Cernik et al. (1970). Digital Ion-analyzer equipped with a fluoride specific electrode (Orion Research Model 701A) was used in the present study. Samples were diluted to suitable concentrations and mixed with total ionic strength adjustment buffer II (TISAB II). Fluoride concentration in soil samples was estimated as per the method adopted by Madhavan and Subramanian (2002). Aqua regia was added to soil sample and the mixture was wet digested at temperature below 60°C. The pH of the digested sample was adjusted to 5.2 by mixing sodium acetate solution (45% W/V) and fluoride concentration was measured after mixing with TISAB II. Fluoride concentration in fodder was estimated by the method of AOAC (1995). The fodder was dried at 80°C and grounded to fine powder. The powered sample was dissolved in 10% hydrochloric acid and fluoride concentration in this solution was estimated using ion selective electrode after mixing with TISAB II. Calcium and phosphorus concentration in plasma were estimated as per the method suggested by Gitelman (1967) and Gomorri (1942) respectively.

Blood samples were wet digested as per the procedure described by Kolmer et al. (1951) for estimation of zinc (Zn), copper (Cu), magnesium (Mg), manganese (Mn) and cobalt (Co) concentration. Approximately 5 mL of the sample was mixed with 5 mL of double acid mixture consisting of 3 parts nitric acid and 1 part 70% perchloric acid and heated below 80°C till digestion. The digested samples were diluted with de-ionized triple glass distilled water and the concentration of different minerals were estimated by Atomic Absorption Spectrophotometer (AAS, Model-AAS 4141, ECIL, Hyderabad, India) at suitable wavelength and lamp current using air-acetylene mixture as fuel-oxidant mixture. The average reading of blanks was subtracted from test sample reading and standard reading to get the final concentrations (μg/g). The standards procured (Sigma Aldrich Chemicals Corporation, New Delhi and Sisco Research Laboratory, Mumbai, India) for each element were used to calibrate the equipment and to check the analytical quality with serial dilutions of test-specific standard solution, and to measure the absorbance to the test samples in reference to that to the two fixed concentrations of the standard. The detection limit of copper, cobalt, zinc, magnesium and manganese by AAS was 0.01, 0.02, 0.003, 0.01 and 0.01 μg/mL, respectively. The values obtained were expressed as mean $\pm$ S.E. and data were analyzed statistically to find out the significance of difference between control and polluted area mean values using student's t test (Snedecor and Cochran 1989).

Results and Discussion

Fluoride concentration in water, soil and fodder samples are given in Table 1. High fluoride concentration in fodder (534.42 $\pm$ 74.95 ppm) and water (1.186 $\pm$ 0.286 ppm) were reported earlier from our laboratory (Patra et al. 2000) and this was attributed to emission from superphosphate fertilizer factory located in the area. Recently, another superphosphate fertilizer factory has become operational in the nearby area and farmers and local authorities reported increased morbidity and mortality in animals to the Regional Disease Diagnostic Laboratory, Udaipur, Rajasthan. Therefore, it was decided to evaluate

Table 1 Fluoride concentration in soil, fodder (on dry matter basis) and water samples collected from fluoride polluted and unpolluted (control) localities

Sample	Mean ± S.E		Median		Range	
	Control	Polluted	Control	Polluted	Control	Polluted
Soil (μg/g)	0.71 ± 0.03 (n = 20)	25.81 ± 2.46* (n = 21)	0.73	22.4	0.39–0.98	14.4–54.1
Ground water (μg/mL)	0.17 ± 0.01 (n = 20)	1.37 ± 0.21* (n = 21)	0.17	1.06	0.11–0.27	0.41–2.9
Fodder (μg/g)	27.59 ± 3.18 (n = 7)	251.11 ± 26.07* (n = 7)	25.8	222.4	15.7–42.7	177–350

* Value (mean ± S.E.) differ significantly ($p \leq 0.05$) from control

n indicates sample size

the current status of fluoride pollution and its effects on livestock health. Fluoride concentrations in environmental samples observed in the present study were higher than the level observed in the past study, suggesting an increase in fluoride pollution magnitude. Fluoride concentrations in water, fodder and soil samples collected from Bareilly were found within the normal limits suggesting the area free from fluoride pollution.

Many parts of Rajasthan like Banswar, Ajmer and Dungarpur are reported to be endemic for fluoride pollution due to presence of fluoride rich rocks, soil and water (Madhavan and Subramanian 2002; Choubisa 1999). In present study fluoride concentration in ground water, soil and fodder samples were significantly ($p < 0.05$) higher than control. High fluoride concentration in fodder samples may be attributed to accumulation of dust and fumes emanating from superphosphate fertilizer factory (Radostits et al. 2000). Excess intake of fluoride through contaminated water and fodder must have contributed to the development of fluorosis in the livestock population in the area. High fluoride concentrations in both plasma (1.43 ± 0.15 μg/mL) and urine (30.16 ± 2.32 μg/mL) samples were observed in the present study. Soluble fraction of ingested fluoride is rapidly absorbed primarily in stomach and intestine, and reaches systemic circulation (Swarup and Dwivedi 2002). Thus, high fluoride concentration in plasma can be attributed to its rapid absorption following exposure to excess fluoride.

Concentration of Cu, Zn, Mn and Ca were significantly ($p < 0.05$) lower, while Mn and Co were significantly ($p < 0.05$) higher in fluorosis affected animals as compared to controls (Table 2). No significant difference was observed in phosphorus (P) level between the two groups. Singh and Swarup (1999) also observed significant decrease in serum Ca, Mg, Cu and Zn and increase in Mn and P levels in cattle supplemented with high fluoride containing mineral mixture for three years. Decrease in blood and tissue Zn level following fluoride exposure has also been reported in lambs (Vasisth et al. 1998), pigs (Xin et al. 2006) and rabbits (She et al. 2002). Kanwar and Singh (1981) reported significant decline in Mn, Cu and Zn in liver of rats following fluoride intoxication. Varying degree of hypocalcaemia has been reported in fluorotic animals (Jagadish et al. 1998; Maiti and Das 2004). Contrary to above, Wheeler et al. (1988) recorded increase in serum Ca, Mg and P in hydrofluorotic sheep. There seems to have no report available on status of cobalt in fluortic animals to compare the finding of the present study. Fluoride is highly electronegative halogen with strong affinity towards electropositive elements. In gastrointestinal tract, fluoride forms complexes with P, Mg and Ca and thereby reduces their absorption (WHO 2002). Spenser et al. (1985) reported 23% decrease in calcium absorption following fluoride exposure in man. Thus, lower level of Cu, Zn, Mn and Ca may be attributed to their reduced absorption from gastro-intestinal tract. Increase in urinary and fecal excretion of various minerals may be another factor responsible for their decreased status in the body. Krishnamachari (1986) reported increased fecal and urinary excretion of zinc after fluoride exposure in human. Spencer et al. (1977) observed that administration of 10 mg sodium fluoride per day in man did not alter the magnesium balance significantly, though there was a slight increase in fecal magnesium excretion. Increased utilization of zinc and copper for counteracting fluoride-induced oxidative stress may also be, at least partially, responsible for decrease in blood level of these elements. This is substantiated by the observation that supplementation of Zn, Cu and other antioxidant minerals like selenium have beneficial effects in fluoride intoxication (Liu and Kang 2003; Han-Bo et al. 2001; Khandare et al. 2005b). Bhargavi et al. (2004) reported that lower concentration of Ca, Cu and Mg in drinking water was associated with increased prevalence of dental fluorosis in man. They further observed that prevalence of dental fluorosis was negatively correlated with Ca and Cu content of drinking water. Thus, it can be concluded from the present study that alteration in level of different micro and macro minerals occur in fluorosis and such changes may also contribute to the toxic effects associated with exposure to excess fluoride (Fig. 1).

Table 2 Level of micro and macro minerals (µg/mL) in blood of cattle from fluoride polluted and unpolluted (control) localities

Minerals	Study locality	
	Control	Polluted
Copper (µg/mL)	0.63 ± 0.03	0.49 ± 0.02*
Zinc (µg/mL)	2.17 ± 0.13	1.82 ± 0.10*
Cobalt (µg/mL)	0.36 ± 0.01	0.54 ± 0.05*
Manganese (µg/mL)	0.21 ± 0.03	0.10 ± 0.01*
Magnesium (µg/mL)	16.16 ± 0.08	21.44 ± 0.09*

* Value (mean ± S.E.) differ significantly ($p \leq 0.05$) from control

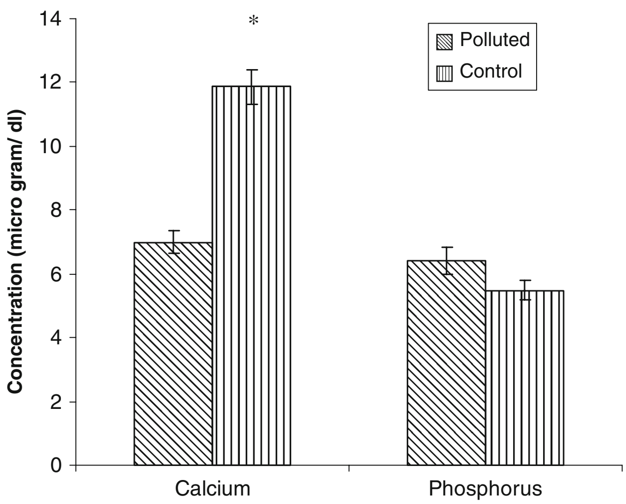

Fig. 1 Plasma calcium and phosporus concentration in cattle from fluoride polluted and unpolluted (control) localities. *Differ significantly ($p \leq 0.05$) from control

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