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Clinico-epidemiological study of industrial fluorosis in calves reared near aluminium smelter plant, at Angul, Odisha

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Abstract
The aim of this investigation was to examine the effect of industrial fluorosis on calves reared in vicinity of aluminium smelter plants, Angul, Odisha. A total of 147 calves reared within 5 km radial distance from plant were screened and highest prevalence of both dental as well as skeletal fluorosis were observed in 8-12 month age group calves while the lowest prevalence was noticed in 0-4 month age of calves. The fluoride level in soil, feed (dry and green fodder and milk), water (surface and ground) in the vicinity of aluminium smelter were significantly higher than less exposed area i.e. Bhubaneswar (an area with low environmental fluoride level). A higher burden of plasma, urine and faecal fluoride were observed in calves from the surrounding of aluminium smelter plant than healthy calves reared in Bhubaneswar without any visible lesions referable to fluorosis. The fluorosis calves also exhibited a significant alteration in haemato-biochemical parameter along with serum micro and macro mineral content compared to the healthy control.

Keywords: Fluorosis, calf, dental fluorosis, haematological effect, biochemical alterations, soil fluorosis, water fluorosis

Introduction
Fluorosis in livestock is mainly attributable to industrial fluorosis (Singh and Swarup, 1994) [1]. Most of the emissions from aluminum smelter plants are fluoride-rich and can precipitate over a considerable distance over vegetation, soil and water bodies, thus causing industrial fluorosis (Patra *et al*., 2000) [2]. These industrial operations release F in both gaseous and particulate forms. Fluoride exerts residual toxicity due to its non-biodegradable nature and passes on to each phase of the trophic level through the food chain. Fluoride is readily distributed throughout the body, with approximately 99% of the body burden of fluoride retained in calcium rich areas such as bone and teeth (dentine and enamel) where it is incorporated into the crystal lattice. Calves exhibited the highest prevalence of fluorosis and this may be because, calves are more sensitive and susceptible and less tolerant to fluoride (Shupe, 1980) [3]. Besides apparent clinical manifestations, fluoride intoxication causes significant changes in biochemical constituents in serum and urine, some of which may be of diagnostic significance (Singh and Swarup, 1999) [4]. Being highly electronegative, the F anion can interact with many elements forming soluble or insoluble complexes. Therefore, changes in blood or tissue levels of macro, and trace minerals during chronic F toxicity are expected (Ranjan *et al*., 2011) [5]. In the present study, attempt has been made to evaluate the effect of industrial fluorosis, especially in calves in terms of prevalence, haemato- biochemical parameter and serum mineral content.

Materials and Methods
Talcher-Angul Industrial complex of Odisha, situated at latitude 20°95 'N to 21° 10 ' N and longitude 84° 55' E to 85° 28 'E, 139m above sea level (ASL), and 150 km away from Bhubaneswar, the state capital of Odisha, was selected as study site. For the present investigation, 6 villages namely Bonda, Tulasipal, Gardarkhai, Kulad, Jhajhariba hal and Languliabeda villages of Angul district were selected, that are located within 5 km radius of aluminum smelter plant of National Aluminum Company (NALCO).
To derive estimates of the prevalence of fluorosis in calves, A total of 147 calves, born and reared within a 5km radius from the smelter, were examined clinically for detection of dental

and skeletal lesions during door-to-door survey. Calves of either sex from 3 different age groups (0-4 month, 4-8 month and 8-12 month) were picked up randomly among which around 7 signs of dental lesions like deep yellow and brown discolorations, linear pigmented vertical streaks, loss of luster, attrition and mottling of teeth with or without pitting were reflected in most of the calves whereas skeletal lesion like bony exostosis (determined by manual palpation) was rarely encountered.

Water samples were collected from different sources such as ponds, tube well and dug well in the five selected villages and stored in pre-cleaned and sterilized polythene bottles of one-litter capacity following standard protocols. Feed and fodder samples (cultivated and native plants, straw, bran and concentrate mixtures) were collected and oven dried at 80 °C for 48 hr and ground with a mortar and pestle and stored in vials in order to determine the F content. Soil samples from a depth of 0-45 cm were collected from the polluted area, mixed, and stored in zippered polyethylene packets for further analysis. Milk samples (10 ml) were collected from dam of fluorosis calves and stored in sterilized bottles.

Blood samples, around 15 ml from each animal, were collected aseptically from the jugular vein with sterilized syringe and needle using heparin as anticoagulant. Half of the blood sample was centrifuged at 2000 rpm for 10 minutes to separate plasma for biochemical analysis and estimation of fluoride. The plasma samples were stored in a deep freezer at -20 ^{0}C temperature. Remaining whole blood was used for haematological analysis. About 20 ml of urine from each animal was collected directly in a 50 ml polyethylene bottle and were transported to the laboratory in an ice box and were stored in refrigerator for analysis of fluoride. Faecal sample was collected from the screened animals, directly from the rectum in pre-labeled plastic specimen bottle in morning time.

Fluoride concentrations of environmental samples and bio-samples were estimated by ion specific potentiometry using total ionic strength adjustment buffer and a portable fluoride ion specific electrode (Orion model 94 09 BN), and ISE meter (Orion Model-290A, Thermo Fisher Scientific Inc.) The detection range of the instrument is in between 0.019 and 1900 ppm.

Haemoglobin concentration (Hb) was determined by Sahli's hemometer method and packed cell volume (PCV) was determined by microhaematocrite method. Total leucocyte count (TLC) and differential leucocyte count (DLC) were determined by using the standard reference methods of Benzamin (1985) [6]. Activity of Alkaline phosphatase (ALP), Aspartate transaminase (AST), urea, creatinine, total protein, calcium and phosphorus were estimated in plasma samples by semi auto analyser (Model no- Microlab 300, Merck, India) using commercial reagent kits supplied by Crest Biosystems, Goa, India.

The level of Ca and P in plasma was estimated by semi-automated biochemistry analyser (Microlab 300, Merck, India) using Crest Biosystems Kit (Goa, India). The levels of plasma Mg, Zn, Fe, Mn and Co were determined using Double Beam Atomic Absorption Spectrophotometer (ELICO, Model No. SL243, India).

Data were analyzed statistically and compared using Student's't' test and expressed as mean ±SE, with $P<0.05$ considered statistically significant.

Results and Discussion

The level of F in soil, water and fodder in relation to distance from smelter plant showed a diminishing trend in table 1. This is due to centrifugal spread of fluoride in effluents around the plant. The surface water had higher fluoride than ground water due to contamination by sewage, sludge, fumes and suspended particulate matters released from the aluminium plant which were gradually deposited on water bodies. This is contrary to the picture of hydrofluorosis, where the fluoride concentration of ground water was much higher as a result of geothermal activity rather than industrial contamination (Maiti *et al*., 2003) [7]. The green fodder contained less fluoride than dry fodder irrespective of distance due to low dry matter content and high moisture content. The F levels of milk in dams of fluorosis calves were found to be prominently increasing with decreasing radial distance from plant than the cows inhabiting the unpolluted area.

Outbreaks of fluorosis among cattle population were reported worldwide near the aluminium smelter plants (Vandermissen *et al*., 1993; Raghib *et al*., 1994; Swarup *et al*., 1998)[8,9,10]. A total of 147 calves were screened from the study area and the prevalence of dental fluorosis and skeletal fluorosis are represented in table 2. Lesions consistent with skeletal fluorosis were not found in the youngest age group, and increased in prevalence with age and decreasing distance from the smelter. In the 4-8 month old group they occurred only in those calves residing within a 1.5 km radius of the smelter (14% prevalence), while prevalence varied from 6% (3-5km radius) to 27% (<1.5km radius) in the 8-12 month old group. On the other hand, dental fluorosis lesions occurred in all groups and at all distances within a 5km radius of the smelter, although the prevalence was highest at <1.5km. Based on age group, the prevalence of dental fluorosis lesions varied from 19-54% (0-4 months), 27-93% (4-8 months) and 38-100% (8-12 months), with lesions increasing in frequency with decreasing distance from the smelter. In this study, there was overall higher prevalence of dental fluorosis than skeletal fluorosis in all age group of calves as deposition of fluoride in teeth occurs during or before eruption, but the bone lesions are revealed after gradual deposition of fluorapatite crystals in bones for a longer period causing deformity (Radostits *et al*., 2009)[11]. The calves of 8-12 month age group showed higher prevalence of fluorosis than other age groups in form of brown discolouration of incisors and exostosis of long bones of foreleg whereas calves of 0-8 month age group revealed some extent of brown discolorations and rarely bony exostosis (only 2 animals).

Table 3 revealed that the plasma fluoride level of calves within 5 km radial distance from plant is above the normal level of 0.2 ppm (0.483±0.020 ppm) as stated by Radostits *et al*., (2009) [11]. and also much higher than the unpolluted area (0.086±0.005 ppm). The WHO (2006) [12] has set the guideline value at 1.5 mg/L of fluoride, but mild forms of dental fluorosis begin to occur at lower levels (Hussain *et al*., 2005)[13]. Although the fluoride level of surface water in the area within 3-5 km distance was found to be within permissible limit, but a lower prevalence of dental fluorosis and subsequently high plasma fluoride level were still observed as the fluoride level in both green and dry fodder were higher than the tolerance limit of 40 mg/kg (Krook and Maylin, 1979) [14]. Moreover, the higher fluoride level of soil also contributed towards the higher plasma fluoride level in calves as grazing cattle obtain over 50% of their dietary fluoride (and this may be >80% during winter) from soil ingestion and dietary fluoride absorptivity (bioavailability) of soil fluoride is from 20 to 38% (Cronin *et al*., 2000) [15]. The dietary intake of animals and the concentration of fluoride in

diet largely affect the degree of fluorosis. As the present study was conducted on farm-based animals (suffering from industrial fluorosis) which were mostly maintained on grazing with little supplementation of dry fodder, hence the minor variations in diet could not be studied.

In table 3, it was observed that there were elevated level of urine F and faecal F in fluorosis calves than calves from non-fluorosis area. F appears readily in urine after absorption and generally the urine F reflects the absorbed F on same day (Gopal and Ghosh, 1985) [16]. Fluoride concentrations in faeces reflect current F intake and correlate with length of exposure and level of F in the diet.

There is significant reduction in Hb, TLC and PCV in fluorotic calves than healthy calves. It is known that F intoxication depresses bone marrow activity in cattle (Radostis *et al*., 2009) [11]. Decrease in Hb may be also possibly due to toxic effect of F on the serum level of iron and poor retention of iron (Hoogstratten *et al*., 1965) [17]. Significant PCV changes in the study might be due to toxic effects of F on the RBC cell membrane and subsequently shrinkage of cell. Mandal *et al*., (2015) [18] also reported lower Hb, PCV level in fluoride toxicity on calve.

Alkaline phosphatase activity was significantly higher in fluorotic calves (185.35±0.940 IU/L) as compared to those from the non fluorotic area (147.12±1.051 IU/L). Since fluoride stimulates osteoblastic activity (Araya *et al*., 1990)[19], the increase in alkaline phosphatase can probably be related to abnormal bone formation and stimulated osteoblastic activity with increased fluoride concentration in the serum (Radostis *et al*., 2009) [11].

The significant enhancement of plasma AST and reduction of total protein and albumin, indicative of hepatic dysfunction, might be due to hepato-toxic effect of F compounds (Maiti *et al*., 2003) [7]. But the ALT level remained within normal range in fluorotic calves and this finding coincides with findings of Radostis *et al*., (2009) [11] and Swarup *et al*., (2001) [20].

There were a significant rise in the level of urea and creatinine in the fluorotic calves than the calves from non fluorotic zone. High levels of serum urea and creatinine in the affected cows and buffaloes are therefore indicative of degenerative changes in the kidney (Singh and Swarup, 1999) [4]. This increased level might also be due to catabolism of protein because of partial starvation in affected animals (Swarup *et al*., 2001) [20].

A significantly lower concentration of calcium was noted in fluorotic calves (7.037 ± 0.064 mg/dl) as compared to healthy animals (10.722 ± 0.182 mg/dl). This was probably because of the decrease in absorption as well as enhanced excretion of calcium via urine (Bharti *et al*., 2007) [21]. As fluoride is a highly electronegative element with a strong affinity towards electropositive elements, in the gastrointestinal tract, fluoride binds with calcium, thereby reduces their absorption. Moreover, decrease in calcium ATPase activity was responsible for increase in urinary calcium causing hypocalcaemia (Singh and Swarup 1999) [4].

The average serum phosphorus and cobalt content of fluorotic calves were found to be more than the normal value which was also reported by Ranjan *et al* (2008) [22]. in cattle. This is because circulatory parathormone level increases in fluoride intoxication (Singh and Swarup 1999) [4]. Which regulates metabolism of calcium and phosphorus which might cause hyperphosphatemia due to concurrent hypocalcemia.

In fluorotic calves there is decreased concentration of Fe, Mn, Mg and Zn than healthy calves. This might be due to the reaction of the highly electronegative F with these mineral, thereby reduction of their absorption. Increase in urinary and faecal excretion of various minerals may be another factor responsible for their decreased status in the body (Ranjan *et al* 2008) [22]. A decrease in Zn concentrations may be due to increased need for synthesis of Zn-dependent enzymes. Zn is poorly stored in body tissues, and therefore a decrease in its level occurs when increased demand is not accompanied with increased availability in the diet.

Table 1: The concentration of fluoride (ppm) in soil, water, fodder and plasma sample in less exposed area and in fluorotic area in relation to distance from smelter

Parameter	Distance from plant (km)			Less exposed area
	0-1.5	1.5-3.0	3.0-5	
Soil	255.68 ± 13.27^{d}	152.67 ± 7.71^{c}	67.1 ± 5.9^{b}	3.3 ± 0.21^{a}
Ground water	0.803 ± 0.08^{cA}	0.658 ± 0.088^{cA}	0.433 ± 0.091^{bA}	0.19 ± 0.04^{a}
Surface water	$2.983\pm.155^{dB}$	1.683 ± 0.113^{cB}	1 ± 0.074^{bB}	0.11 ± 0.02^{a}
Green fodder	175.97 ± 5.44^{dX}	103.53 ± 5.37^{cX}	45.5 ± 4.364^{bX}	$2.73\pm.02^{a}$
Dry fodder	242.33 ± 45.103^{dY}	131.33 ± 12.889^{cY}	91 ± 3.474^{bY}	7.15 ± 0.93^{aY}
Milk of dames	0.13 ± 0.007^{c}	0.107 ± 0.04^{b}	0.096 ± 0.02^{b}	0.059 ± 0.005^{a}

The values (Mean± S.E., n=6) with dissimilar superscript (small letter) within a row varies significantly at $p<0.05$.

The significant differences in the mean levels of F between ground water and surface water tested for different distance range and similar procedure has been done to test the differences in the means between green and dry fodder. Means bearing different superscripts (capital letters) differ significantly at 5% level ($p<0.05$)

Table 2: Prevalence of fluorosis in calves

Distance from smelter (km)	Age of calf (month)	Total number of calves screened	Prevalence of dental fluorosis	Prevalence of skeletal fluorosis
0-1.5	0-4	13	53.8% (7/13)^{a}	0 (0/13)
	4-8	14	92.8% (13/14)^{b}	14.28% (2/14)
	8-12	11	100% (11/11)^{b}	27.2% (3/11)
1.5-3	0-4	18	33.3% (6/18)^{a}	0 (0/18)
	4-8	20	65%(13/20)^{b}	0 (0/20)
	8-12	19	89.5% (17/19)^{c}	21.05% (4/19)
	0-4	21	19.04% (4/21)^{a}	0 (0/21)
3-5	4-8	15	26.7% (4/15)^{ab}	0 (0/15)
	8-12	16	37.5%(6/16)^{b}	6.25% (1/16)

A test of equality of proportion was conducted to know the significant differences in the proportions (Percentage) of dental fluorosis and proportions (Percentage) bearing different superscripts are significantly different at 5% level (p<0.05).

Table 3: The concentration of different parameters in calves in fluorotic area and in less exposed area

Parameters	Fluorotic area	Less exposed area
Plasma F (ppm)	0.483 ± 0.020^{B}	0.086 ± 0.005^{A}
Urine F (ppm)	11.717 ± 0.248^{B}	2.342 ± 0.220^{A}
Faecal F (ppm)	21.155 ± 0.676^{B}	6.622 ± 0.476^{A}
Haemoglobin (g%)	7.70 ± 0.22^{A}	11.13 ± 0.25^{B}
Total leucocyte count ($10^3/mm^3$)	6.86 ± 0.18^{A}	9.53 ± 0.31^{B}
Packed cell volume (%)	23.17 ± 0.54^{A}	33.5 ± 0.76^{B}
Calcium (mg/dl)	7.037 ± 0.064^{A}	10.722 ± 0.182^{B}
Phosphorus (mg/dl)	7.133 ± 0.092^{B}	4.834 ± 0.182^{A}
Alkaline phosphatase (IU/L)	185.35 ± 0.940^{B}	147.12 ± 1.051^{A}
Aspartate transaminase (IU/L)	86.375 ± 0.552^{B}	52.074 ± 1.401^{A}
Total Protein (gm/dl)	6.553 ± 0.172^{A}	7.083 ± 0.119^{B}
Albumin (gm/dl)	2.972 ± 0.078^{A}	3.576 ± 0.038^{B}
Urea (mg/dl)	36.437 ± 0.974^{B}	28.166 ± 0.742^{A}
Creatinine (mg/dl)	1.880 ± 0.072^{B}	1.160 ± 0.056^{A}
Magnesium (ppm)	19.77 ± 0.35^{A}	22.84 ± 0.23^{B}
Zinc (ppm)	1.3 ± 0.11^{A}	2.49 ± 0.16^{B}
Manganese (ppm)	0.092 ± 0.005^{A}	0.135 ± 0.003^{B}
Iron (ppm)	1.19 ± 0.07^{A}	1.57 ± 0.03^{B}
Cobalt (ppm)	0.199 ± 0.013^{B}	0.098 ± 0.006^{A}

The values (mean ± SE, n = 6) bearing dissimilar superscripts (capital letters in a row) differ significantly at P<0.05.

Conclusions

In conclusion, the present study revealed toxic levels of fluoride in environmental samples as well as higher prevalence of fluorosis in calves within 3km radial distance of aluminium smelter plant area, but less severe beyond that, for which grazing of livestock in this area should be avoided as far as possible and provision of ground water (such as deep well or bore well) as drinking water should be practised.

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Declaration of Interest

The authors declare that there is no conflict of interest.

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