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Clinical investigation on chronic industrial fluoride poisoning in adult cattle of Kerala, India

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Abstract
The objective of the present study was to assess the extend of chronic industrial fluoride (F) poisoning and its effects on adult cattle inhabitating in and around Aluminium and Fertilizers Units at Eloor industrial belt of Ernakulam district of Kerala, India. A field investigation was carried in randomly selected cattle aged three years and above in the vicinity of Aluminium and Fertilizers industries. Cattle showed clinical signs of lameness, dental lesions, bony exostosis, reproductive problems and gastro-intestinal disorders suggesting fluorosis. Fluoride toxicity was confirmed by estimating the fluoride levels in the field and biological samples collected in this area. Samples were collected from the randomly selected ten healthy cattle aged three years and above from University Livestock Farm, Mannuthy as control. The F level in the biological and field samples were significantly higher (P<0.01) than the control samples. From the present study, it can be concluded that aluminium smelter and fertilizers units involved in the environmental pollution with fluorine in the Eloor industrial area and caused incidence of chronic industrial fluoride pollution in adult cattle.

Keywords: Industrial fluorosis, cattle, aluminium industry, phosphate fertilizer

1. Introduction
Pollution and degradation of ecosystem had assumed more importance owing to increased industrialization. Industrial process had been directly or indirectly responsible for release of toxic pollutants in the environment. Of all pollutants, which affect farm animals, fluoride causes more and widespread damage [1]. Prolonged exposure of fluoride results in its accumulation predominantly in hard tissues, teeth and bones causing diverse adverse effects that appear in the form of dental mottling (dental fluorosis) and bone deformities (skeletal fluorosis) in both humans and domestic animals[2]. Prolonged exposure of F also damages the soft tissues or organs (non-skeletal fluorosis) of humans, and domestic and laboratory animals [1]. The objective of the present study was to assess the effect of environmental pollution with fluorine by industrial effluents on health of adult cattle in Eloor, Kerala, India.

2. Materials and Methods
2.1 Study area: The study was conducted in the vicinity of Aluminium industry and Fertilizers unit situated in the Eloor industrial belt of Ernakulam district, Kerala. The Aluminium smelter is situated at Alupuram and Fertilizers unit at Eloor centre and Eloor north.

2.2 Clinical examination of cattle: Clinical examination were conducted in randomly selected 51, 36 and 32 cattle aged three years and above to investigate the health status of cattle in the area of Alupuram, Eloor centre and Eloor north, respectively. The prevalence and pattern of clinical lesions suggestive of fluorosis were noted in detail. The lameness was assessed based on the observation of cattle standing and walking, with special emphasis on their back posture. The mobility scoring system was followed depending upon the severity of the lameness (0- Normal, 1- Mild-stand with flat back, but arches when walk, 2-Moderate-stands and walks with an arched back and short strides, 3- Severe-pronounced arching of back, reluctant to move).

2.3 Collection of samples
Field samples: The field samples namely water, sludge and forage were collected from the study area as per the method described by Lorgue *et al*. [3]

Biological samples: The samples like blood, urine and milk were collected from thirty cattle aged three years and above as per the method described by Lorgue *et al*. [3] Samples of bone were collected from the slaughter house at Eloor Industrial belt.

2.4 Analysis of samples

2.4.1 Field samples: Surface sludge samples were dried, powdered and digested as per the method described by Horwitz [4]. Forage samples were cut and dried at 80^0C in hot air oven for 48h. The dried samples were ground to pass No.40 sieve and digested [5]. The fluorine content in the biological samples were estimated by microprocessor ionalyser/901.

2.4.2 Biological samples: The fluorine concentration was estimated in plasma, urine and milk using ion selective electrode. Dry, fat free bones were ashed at 550^0C and fluorine concentration was estimated using microprocessor ionalyser/901 as per the methods described by Singer and Armstrond [6].

2.5 Comparison of data: Ten healthy cattle aged three years and above were selected randomly from University Livestock Farm, Mannuthy as control. Blood and urine samples were collected from the control animals to estimate the fluorine level. Bone samples were collected from the slaughtered animals at Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy and the fluorine level was analysed. The data obtained from the control samples were compared with those obtained in Eloor industrial area.

2.6 Statistical analysis: The data obtained were statistically analysed by the completely randomized design (CRD) using SPSS computer software 17.00 [7].

3. Results and Discussion

3.1 Clinical examination of cattle

The major clinical signs observed in the cattle of Eloor industrial area are presented in Table 1. Signs of lameness, dental lesions, bony exostosis, reproductive problems and gastro-intestinal disorders were recorded on clinical examination of cattle of Alupuram, Eloor centre and Eloor north which suggested osteo-dental fluorosis. In the Alupuram area, 52.2%, 38.8% and 8.8% of the cattle were showed moderate, mild and severe lameness, respectively. In Eloor centre and north, most of the animals were affected with mild lameness. The cases of debility, lameness, dental abnormalities (Fig.1) and reproductive disorders were comparatively higher in the vicinity of aluminium smelter as compared to phosphate fertilizer unit.

Table 1: Distribution of major clinical signs observed in the cattle of Eloor industrial area (n=119)

Disorders	Number of affected animals	Percentage
Dental lesions	37	31.10
Lameness	20	16.81%
Bony exostosis	9	7.56%
Debility	11	9.24%
Reproductive disorders	32	26.89%
Gastro-intestinal problems	13	10.92%

The important signs found in the permanent teeth were mottling, discolouration, hypocalcification and excessive erosion (Fig. 1). The most recognizable, indexive and early signs of fluoride toxicity was dental fluorosis. In the present study, majority of animals showed characteristic lesions notably mottling, discoloration, hypoplasia, hypocalcification and excessive erosion, which are in agreement with the earlier observations of Choubisa *et al.* [2], Majority of the affected animals showed dental lesions mostly involving incisor teeth which could be used as diagnostic aid in fluorosis.

Fig 1: Permanent incisor teeth showing uneven erosion in the cattle affected with fluorosis

In the current study, affected animals exhibited lameness, reluctance to move and palpable bony exostosis particularly in metatarsals, metacarpals and mandibular bones. Excessive periosteal bone formation and hyperstosis were predominant in adult cattle. The joints became thickened and ankylosed, due to calcification of tendons at their points of attachment to the bone. Stiffness and lameness became apparent in the affected cattle. Hence, the affected animals were unable to feed and drink, which markedly affected the performance of animals. Shupe *et al.* [8] described that fluorides had high affinity and biological activity to the calcified tissues of body such as bone and teeth. Patra *et al.* [9] found almost similar

cases of bone abnormalities in cattle and buffalo due to industrial fluorosis.
Clinical examination of cattle in the study area revealed higher incidence of reproductive disorders. Change in Ca: P ratio was found in the present study and it might be responsible for delayed post-partum oestrus [10].

3.2 Fluoride content in the field samples
The F levels in water, sludge and forage samples are presented in Table 2. Significantly higher ($p \leq 0.01$) F levels were recorded in the field samples of Alupuram, Eloor Centre and Eloor North as compared to samples collected in the control area. The values for the area of Alupuram, Eloor centre and Eloor north were significantly differs from each other and the concentration was much more in the Alupuram area followed by Eloor centre and Eloor north. The fluoride level of water recorded in the Alupuram area (1.328±0.94) was higher than the permissible recommended by Indian Counci of Medical Research (1.00ppm). But the fluoride level of water in the Eloor centre (0.871±0.060) and Eloor north (0.834±0.053) was lower than the recommended permissible level.

Table 2: Flouride (ppm) content of field samples of Eloor industrial area (mean±SE)

Samples	Fluoride level in ppm			
	Control (n=10)	Alupuram (n=30)	Eloor centre (n=30)	Eloor north (n=30)
Water	0.094±0.003^{c}	1.328±0.294^{a}	0.871±0.060^{b}	0.834±0.053^{b}
Forage	3.832±0.158^{b}	302.507±38.968^{a}	251.700±23.113^{a}	228.249±29.017
Sludge	2.577±0.053^{c}	212.746±24.176^{a}	101.737±9.724^{b}	65.524±15.276^{bc}

*a, b, c- means with different superscriptions within the same row differs significantly ($P \leq 0.01$)

Fumes and dust from factories processing fluorine containing ores had been volatilized and then condensed in the surrounding area. It might cause toxic level of contamination as far as 14 km from the factory [11].

3.3 Fluoride content in the biological samples
The fluoride levels (ppm) in plasma, urine, milk and bone was significantly higher ($P \leq 0.01$) compared to control animals (Table 3). The most reliable monitoring method to find out recent F exposure was assessing F levels in plasma [12] (WHO Expert Committee Report, 1994). The plasma F content of cattle in the Eloor industrial area was in agreement with the findings of earlier report [11]. The amount of F level in the blood correlated with the amount of F in the diet [10].

Table 2: Flouride **(**ppm**)** content of biological samples of cattle of Eloor industrial area (mean±SE)

Samples	Fluoride level in ppm			
	Control (n=10)	Alupuram (n=30)	Eloor centre (n=30)	Eloor north (n=30)
Plasma	0.108±0.016^{c}	1.247±0.123^{a}	1.021±0.051^{b}	0.910±0.054^{b}
Milk	0.076±0.006^{b}	0.496±0.041^{a}	0.427±0.032^{a}	0.413±0.054^{a}
Urine	5.031±0.576^{c}	35.477±5.35^{a}	28.200±3.314^{b}	26.529±1.402^{b}
Bone	420.393±62.225^{c}	3523.057±591.885^{a}		

*a, b, c- means with different superscriptions within the same row differs significantly ($P \leq 0.01$)

Cattle ingesting even high level of dietary F showed little increase in F content of milk. In the present study, the level of fluoride in milk was lower than serum and this finding are in agreement with earlier reports of Hoogstratten *et al.* [13] Fluoride concentration in urine reflected the current fluoride intake and was highly related to length of exposure or total fluoride consumed. The level of fluoride in the urine was indicative of industrial fluorosis [14]. Older animals excreted more fluorides in urine than young animals raised under same conditions and ingesting the same amount of fluoride. This was due to bones of older animals had higher fluoride contents, so more fluoride was excreted [8]. Fluoride had high affinity to the calcified tissues of body such as bone and teeth [15]. Bone fluoride concentrations were better indicators of long-term F exposure [16]. Significantly higher ($P<0.01$) levels (ppm) of F were recorded in bone sample collected from local slaughter house compared to control samples.

4. Conclusion
The presence of F in field and biological samples and evidence of osteo-dental fluorosis in adult cattle indicate that study areas are highly polluted with F toxicant emitted from Aluminium and Fertilizers industries. The incidence of chronic industrial fluorosis was much more in the vicinity of aluminium smelter than the fertilizer unit.

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