ANTHROPOGENIC FLUORIDE CONTAMINATION AND OSTEO-FLUOROSIS IN BOVINES INHABITING UMARDA, JHAMARKOTRA AND LAKKADWAS VILLAGES OF UDAIPUR, RAJASTHAN, INDIA

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ABSTRACT
A study was conducted to assess osteo-fluorosis in bovines in Umarda, Lakkadwas and Jhamarkotra villages of Girwa tehsil, Udaipur, Rajasthan. For the evaluation of osteo-fluorosis 15-28 cattle (Bos taurus) and buffaloes (Bubalus bubalis) of different age groups of both sexes were examined. Various symptoms of osteo-fluorosis like stiff joints, poor body condition, muscle wasting, bony exostoses, diffidence to move, foot dragging, and snapping sound during walking were looked for in the animals. Chronic signs of skeletal fluorosis were shown by 52.92% of cattle & 49.47% of buffaloes while 39.40% of cattle & 36.64% of buffaloes showed mild to moderate signs of fluorosis. It was observed that cattle were more sensitive to fluoride as compared to buffaloes. Results suggested that fluoride contaminated hard ground water, fodder grown on F contaminated soil, gaseous emission from surrounding factories and rock phosphate mining were the main sources of fluoride in these villages. Apart from the natural fluoride sources these anthropogenic sources were mainly responsible for fluoride toxicosis in animals.

Key words: Bovines; Fertilizer Factories; Osteo-fluorosis; Rock Phosphate Mining.

INTRODUCTION
Fluoride toxicosis is a dreaded disease caused by excessive ingestion of fluoride. Prolonged exposure with high amount of fluoride exerts deleterious effects on human beings, animals and plants too (Conn et al., 2010; Sauerheber, 2013; Choubisa, 2018). Fluoride can enter into the body through contaminated water, food and by inhalation of contaminated gases. Another source of fluoride uptake in grazing animals can be contaminated soil ingestion. Fluoride is an inevitable industrial toxicant and pollutant of geological origin which is frequently found in water. Anthropogenic sources of fluoride like phosphate fertilizer factories, phosphoric acid forming factories, brick kilns, coal burning and mining of rock phosphate release fluoride into the environment and effluents & waste products of these factories contaminate the soil and groundwater when discharged untreated (Mishra et al., 2010). These fluoride emitting industries are serious issue of concern not only for man but also for animals (Choubisa and Choubisa, 2015; Choubisa and Choubisa, 2016). Fluoride enters in to water, from natural as well as anthropogenic sources like mining process and industries, where fluoride mineral is used as crude material for making fertilizers and other products (Mohammadi et al., 2017). Water and fodder are the main sources of fluoride intake in animals. According to WHO (2004) permissible limit of fluoride in drinking water is 1.5ppm. Main observable symptoms of fluoride ingestion are dental and skeletal fluorosis (Darchen et al., 2016). Fluoride generally affects hard tissues of the body like teeth, bones and cartilaginous tissues. Bones are important site for fluoride storage in animals; therefore bones give evidence of potential exposure to toxic level of fluoride (Ouko and Peter, 2016). Fluoride can alter a bone’s internal environment, structure and growth. It can induce many deformities like hyperostosis, exostoses, osteosclerosis and osteomalacia (Shupe et al., 1992). Fluoride contaminated groundwater and air cause skeletal fluorosis in animals. Skeletal fluorosis is a disorder related to prolonged accumulation of fluoride in bones resulting into fragile bones having low elasticity in tendons, fused bones and immoderate calcification (Kadu et al., 2012). Common symptoms of skeletal fluorosis are poor body condition, muscle wasting, locked up joints, diffidence to move, painful and rigid joints, bony outgrowths and osseous lesions in animals.
Therefore the present study was focused on assessment of osteo-fluorosis in bovines reared in the proximity of rock phosphate mining sites and processing units in three villages of Udaipur, Rajasthan.
MATERIALS AND METHODS

The study area included three villages- Jhamarkotra, Lakkadwas and Umarda. Umarda village is divided into 12 regions, Lakkadwas into 15 regions and Jhamarkotra is divided into 9 regions. These villages are a part of Girwa tehsil and surrounded by many phosphate fertilizer plants, brick kilns and rock phosphate mining sites. Umarda is surrounded by several processing units of phosphate fertilizers and brick kilns whereas Lakkadwas (Matoon mines) and Jhamarkotra (RSMML mines, largest reserve of phosphates in India) are mining areas of rock phosphate (Fig. 1 a, b, c).

For the evidence of skeletal fluorosis, a survey was done in all the three villages. A total of 1528 domestic animals including cattle (Bos taurus) and buffaloes (Bubalus bubalis) were observed for signs of osteo fluorosis. Both Indian, as well as exotic breeds as Kathiyawadi, Nagauri, Tharparkar, Holstein-friesian, and Jersey of cattle while Murrah and Surati breeds of buffaloes were reared in the study area. Symptoms of osteo fluorosis like stiff joints, poor body condition, hoof deformities, muscle wasting, bony outgrowths or exostoses, reluctance to move or restricted movement, osseous lesions, arched back bone etc. were looked for in the animals. On the basis of the severity of these symptoms of skeletal fluorosis the animals were divided into mild, moderate or severe categories. Other common complaints like lack of appetite, bloating, poor lactation, low birth rate etc. were also taken into consideration. At the same time animal’s water and food supply were also investigated.

RESULTS AND DISCUSSION

The survey for assessment of skeletal fluorosis in bovines revealed that animals in all the three villages were affected by natural as well as anthropogenic fluoride contamination. The study area has large deposits of rock phosphates and is regularly mined for related industries. The mining and processing units in this area release fluoride in the surroundings. The ground water and Udaisagar lake are main sources of drinking water; both of these are contaminated with fluoride. Of the three villages, Lakkadwas village was found to be the most afflicted. Lakkadwas is receiving highly contaminated water supply from Udaisagar lake and at the same Matoon mines of rock phosphate is also adding fluoride content to it. Ayad river which is laden with domestic, industrial effluents from Madri industrial area and sewage discharge of the city, supplies water to Udaisagar lake and in turn dumps all the polluted waste (Fig. 1 d). The fluoride concentration of Udaisagar lake (surface water) ranged from 2.1 to 3.6 ppm suggesting airborne F contamination of surface water as well. Rock phosphate leaching from Matoon mines is making the situation more worse (Das, 1999). Variability of fluoride in water depends on the soil and rocks of the area. Earlier studies have suggested that water-rock interaction is responsible for fluoride enrichment in ground water (Dehbandi et al., 2017).

**Table 1: Prevalence of skeletal fluorosis in cattle**

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of animals</th>
<th>Mild to moderate</th>
<th>Severe</th>
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<tbody>
<tr>
<td>&lt;1 year</td>
<td>208</td>
<td>41(19.71%)</td>
<td>65(31.25%)</td>
</tr>
<tr>
<td>1-4 years</td>
<td>267</td>
<td>118(44.19%)</td>
<td>149 (55.80%)</td>
</tr>
<tr>
<td>&gt;4 years</td>
<td>398</td>
<td>150(37.69%)</td>
<td>248 (62.31%)</td>
</tr>
<tr>
<td>Total</td>
<td>873</td>
<td>309 (39.40%)</td>
<td>462 (52.92%)</td>
</tr>
</tbody>
</table>

*Numbers in parenthesis indicate percentage

Table 1 depicts skeletal fluorosis in cattle of different age group. In the age group of less than one year, 208 calves were examined for the evidence of skeletal fluorosis and 19.71% of calves showed mild to moderate signs of osteo fluorosis, while 31.25% of calves showed severe signs of skeletal fluorosis. Poor body condition, muscle exhaustion and stunted growth were common symptoms in the fluorotic calves (Fig. 2a). Earlier studies also reported similar symptoms in calves (Choubisa et al., 2011). Sources of
fluoride in this young stage might be fluoridated water and milk. Calves generally depend on mother for their food supply and there are chances of fluoride transmission through milk (Hobbs et al., 1954). Calves are exposed to fluoride during development in uterus also because mothers are also exposed to high fluoride and reports suggest that fluoride could pass through placenta (Krook and Maylin, 1979). Maylin et al., (1987) also reported that calves born from fluoride intoxicated cows had severe symptoms of osteodental fluorosis such as mottling, discolouration of teeth, enamel hypoplasia, severe malfunctioning in cartilage cell differentiation, osteoblasts bone marrow atrophies and stunted growth. Immature cattle have high sensitivity and susceptibility and less tolerance to fluoride (Kumar et al., 2015), hence at very young stage of life calves reveal the signs of fluoride toxicity. Calves of the study areas were reared in the vicinity of rock phosphate fertilizer factories and brick kilns, and received fluoride through gaseous emission from these factories and fluoride contaminated fodder that resulted in dental and skeletal fluorosis.

In the age group of 1-4 years, 44.19% of the cattle showed mild to moderate signs of skeletal fluorosis and 55.80% of cattle showed severe signs of osteo fluorosis. Apart from extreme muscle atrophy especially in pelvic region, shoulder muscles and frail body, the cattle had stiff joints & legs, bony exostoses, long bones alterations, arched backbone, reluctance to move, painful gait, and lameness (Fig. 2 b). Vertebral column and ribs of the animals were the most affected especially thoracic and lumbar regions. Similar symptoms of industrial fluorosis had been observed in cattle population of Orissa by Mishra and Pradhan, 2007. Choubisa et al.,(2011) also observed neighbourhood fluorosis while Kumar et al.,(2015) reported similar symptoms of skeletal fluorosis in cattle of Bikaner and Nagaur district of Rajasthan. Chronic dental fluorosis in live stock in these areas has also been reported by Panchal and Sheikh in the year 2017. Water is the major medium of fluoride intake by animals and humans. Animals may consume naturally occurring fluoride as well as ingestion of contaminated forage increases chances of fluoride toxicity (Narwariya and Saksena, 2012). The topsoil, forage and grasses grown in industrial and rock phosphate mines areas are generally contaminated by industrial fluoride emission and the accumulation of fluoride on foliage depends on the atmospheric loading. Rock phosphate contains about 25% fluorne which is converted into vapor and around 25000 tons of fluorine is added in to the environment annually. The environmental chronic fluoride intoxication leads to dental and skeletal deformities (Begum et al., 2008). Excess sources of fluoride intake in animals of the study area were fluoridated drinking water, contaminated soil, fodder and gaseous emissions (Fig. 1 e & f). Pandey and Pandey (2011) reported that airborne fluoride was a major factor for fluorosis in the vicinity of phosphate fertilizer factories in the rural areas of Udaipur.

Most industrial fluoride emissions are airborne and may contaminate soil, water, and vegetation not only in the industrial vicinity, but also up to a substantial distance from the emission source (Ranjan and Ranjan, 2015). Airborne fluoride generally settles over plant leaves and is consumed by grazing animals. Thus herbivorous are expected to consume more fluoride than grainivorous (Swarup and Dwivedi, 2002). In the older age cattle belonging to more than 4 years age group, 62.31% of cattle exhibited chronic signs of fluoride toxicity. Animal of this age group were severely affected due to prolong and constant exposure to high fluoride. Restricted movement, painful and stiff joints, lameness, fragile bone, visibly enlarged bones, bony exostoses, osteosclerosis, and snapping sound during walking were observed as severe signs of skeletal fluorosis in cattle (Fig. 2 c). Few animals in this age group were totally immovable and posed a burden on the owner who had no other option than to abandon them or leave to die.

Highest prevalence of osteo-fluorosis in this age group is alarming as these animals when consumed by human or other carnivores would result in an increased intake of fluoride. Extreme muscle atrophy was observed in hip regions of these animals. Stiff painful joints & lameness restricted the movement of animals which resulted into low food intake and poor body condition. Industrial fluorosis and its effects on cattle have also been reported by Kumar and Arvindaksham (2015). Hyperostosis, exostoses and osteosclerosis were observed in cattle by Shupe (1992). Jena et al., (2016) also speculated these chronic signs of fluoride toxicosis in cattle reared in the vicinity of aluminium smelter in Odisha. Bony exostoses in metacarpal, metatarsal, ribs and frontal bones and lameness in cattle are also reported by Gupta et al.,

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(2013) as signs of skeletal fluorosis. Fluoride accumulated fodder is a threat to feeding cattle (Mondal et al., 2017). Vegetation used for fodder grown in the close proximity of the factories is polluted by gaseous emission as well as by F in water & soil.

Figure 2: (a) Exposed vertebral column and ribs with muscle deterioration in an eight months calf (b) Feeble body with pelvic muscle loss (c) Extreme muscle atrophy and stiffened bones leading to immobilization
Figure 3: (a) Feeble body with pelvic muscle regression in six months calf (b) Sinking pelvic muscles with bony lesions and lameness (c) Arched backbone, extreme muscle wasting and exposed pelvic bones
Table 2 depicts the status of skeletal fluorosis in buffaloes of different age group. In the age group of less than 1 year, 17.79% of buffalo calves showed mild to moderate symptoms of skeletal fluorosis and 26.38% of buffalo calves were found afflicted with severe skeletal fluorosis. Calves were feeble and showed muscle regression especially in the pelvic region (Fig. 3 a). Leg bones as metatarsus and tibia fibula appeared delicate and rib cage was also exposed. Choubisa (2014) stated that bovine calves are less tolerant and highly susceptible to fluoride.

Table 2: Prevalence of skeletal fluorosis in buffaloes

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of animals</th>
<th>Mild to Moderate</th>
<th>Severe</th>
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<tbody>
<tr>
<td>&lt;1 year</td>
<td>163</td>
<td>29(17.79%)</td>
<td>43(26.38%)</td>
</tr>
<tr>
<td>1-4 years</td>
<td>229</td>
<td>97(42.35%)</td>
<td>130(56.76%)</td>
</tr>
<tr>
<td>&gt;4 years</td>
<td>263</td>
<td>114(43.34%)</td>
<td>151(57.41%)</td>
</tr>
<tr>
<td>Total</td>
<td>655</td>
<td>240(36.64%)</td>
<td>324(49.47%)</td>
</tr>
</tbody>
</table>

*Numbers in parenthesis indicate percentage

In the age group of 1-4 years 56.76% of buffaloes showed severe signs of skeletal fluorosis while 42.35% of buffaloes were found to be mild to moderately afflicted with skeletal fluorosis. Frail body, bony exostoses, locked up joints, sinking and flattened hind muscles, exposed skeleton were common in buffaloes of this age group (Fig. 3 b). Lameness and immobilization were also noticed in buffaloes. Painful gaits due to periosteal exostoses at ligament & tendons, osteomalacia and osteoporosis have been observed in fluorotic animals by Samel et al., (2016).

In buffaloes belonging to more than 4 years age group, 57.41% showed chronic signs of osteo fluorosis while 43.34% showed mild to moderate symptoms of skeletal fluorosis. Extreme muscle atrophy, arched backbone, weak and delicate metatarsus and tibia fibula were observed in older age buffaloes. Hoof deformities, bony exostoses, feeble body and deteriorating muscles were quite noticeable in buffaloes of this age (Fig. 3 c). Yanhu (1991) reported industrial fluorosis in buffaloes in China and 30% of buffaloes were found affected with skeletal fluorosis. With increasing age the amount of fluoride intake, duration of exposure make these animals reluctant to move or graze.

Fluoride concentrations in plasma are not controlled homeostatically and thus chronic doses lead to accumulation in bone and plasma. Most of the fluoride in the body is contained in bones. The crystallites of developing bone are small sized, more in number and heavily hydrated, providing an enormous surface area for reactions involving fluoride as compared to mature bone. Fluoride is readily incorporated into calcified tissues, as bone and teeth, substituting for hydroxyls in hydroxyapatite crystals. Fluoride exchanges between bone and body fluids in a short term process at surface layer of bone and long-term process in remodeling bones. High concentrations of calcium in gastrointestinal tract are known to cause net excretion of fluoride (Whitford, 1996). So absorption of fluoride from water manifests the adverse effects on skeleton in long term. Owners should be made aware of the issue and supplements of calcium, vitamin C and Boron should be given to animals as a remedial measure (Sheikh, 2011; Bharti et al., 2007).

CONCLUSION
In the present study it was perceived that cattle were more susceptible than buffaloes in the age group of more than 4 years. Among the three villages surveyed, highest prevalence of chronic fluoride toxicity was shown by cattle of Lakkadwas village. It is probably due to the leaching of contaminated water from Udaisagar lake, located in Lakkadwas village which is filled by highly polluted Ayad river and rock
phosphate mines in Matoon near the village, added fluoride to the ground water as well. Gaseous emission from the factories made the situation worst. Results of our study stipulated that anthropogenic sources were mainly responsible for fluoride toxicosis in animals of the study area. Major anthropogenic sources of excess fluoride were industrial emission, mining process of rock phosphate, contaminated soil, water and vegetation. Animals are the pivot of the Indian village economy and it’s mandatory to provide a good health to animals. Also, the industries must develop proper waste treatment strategies to combat environmental pollution. If the drinking water sources contain fluoride more than the permissible limit, defluoridation of water using various methods is suggested. The villagers can be educated to store rainwater for domestic use and irrigation. Locally available plants and their products rich in calcium can be fed to the animals to lower the risk of fluoride toxicity.

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