



## Fluoride Toxicity In Domestic Animals Of Chandrapur District, Maharashtra, India

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Article History	Abstract
<p><b>Received-</b> 06-10-2022. <b>Revised -</b> 05-11-2022. <b>Acceptance-</b> 02-12-2022</p>	<p>A cross-sectional survey was carried out to evaluate the hazardous effects of fluoride on domestic animals in the fluoride-polluted areas of Chandrapur district in Maharashtra, India. Domestic animals, including buffalos, cattle, and goats from seven villages in the Warora tehsil, were the subjects of the current study. The animals showed signs of dental fluorosis to varying degrees, with subsurface water fluoride levels ranging from 0.53 to 5 mg/L. The elevated fluoride concentration functions as a potential pollutant. 1966 of the 5176 animals examined for dental fluorosis with varied degrees of the condition in the cattle (34.3%), buffalo (46.37%), and goats (42.69%), respectively. It was determined that the prevalence of dental fluorosis was extremely significant (<math>P &lt; 0.05</math>). Environmental toxins and contaminants that causes several metabolic problems and also influence blood hence to evaluate the effects of fluoride toxicity on haematological parameters the present study was conducted. High fluoride concentrations were found in serum samples from buffalo, cattle, and goats, in contrast to their respective controls. Haematological analyses of naturally fluoridated animals showed a significant decline in haemoglobin concentration, total erythrocyte count, packed cell volume and leukocyte count, all indicators of fluoride-induced anaemia. According to the differential leukocyte count, animals from fluoride-polluted areas had significantly greater lymphocyte percentages and lower neutrophil percentages than those from non-contaminated areas. There are significant differences between the mean values of haematological parameters in animals of fluoride polluted and nonpolluted localities. The current study's conclusions will undoubtedly aid in addressing potential operating procedures for health problems related to fluorosis in domestic animals in the study area.</p>
<p>CC License CC-BY-NC-SA 4.0</p>	<p><b>Keywords:</b> Fluorosis, Domestic animals, Buffalo, Cattle, Goats, Haematological and Dental parameters</p>

## INTRODUCTION

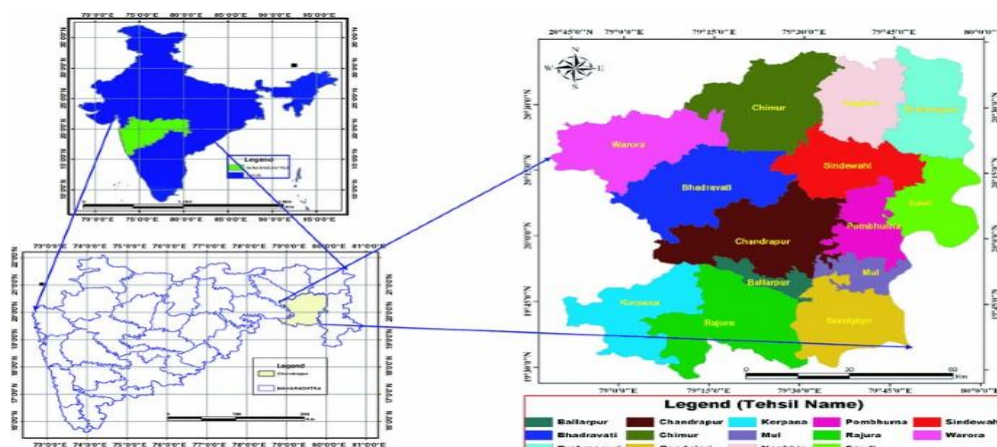
Domestic animal fluorosis is a global health concern that is prevalent in locations where fluoride is present in the environment, including in food, soil, water, and the air. Environmental pollution brought on by different substances and the harmful health repercussions that follow, whether overt or covert in human and animal forms, deserve major global attention. Throughout the world, fluorosis is a serious health issue that affects humans and animals.<sup>1-4</sup> Billions of people are impacted by fluoride exposure. In India, 20 million people have severe cases of the condition and 40 million are at risk of getting endemic fluorosis.<sup>5</sup> Fluoride-rich drinking water, feed supplements, and mineral mixes are additional sources of fluoride for animals in India.<sup>6</sup> Ingestion of fluoride-rich vegetation, or contact with high-fluoride industrial waste also increases fluoride level in animals. Numerous domestic animals, including livestock like cattle, sheep, goat, camel, donkeys and horses as well as pets like dogs and cats, are susceptible to fluorosis.<sup>7,8</sup> Choubisa *et al.*<sup>9-11</sup>, reported osteo-dental fluorosis in domestic ruminants like cattle (*Bos taurus*), buffaloes (*Bubalus bubalis*), camels (*Camelus dromedarius*), sheep (*Ovis aries*), and goats (*Capra hircus*) as a toxic effect of long-term fluoride (F) exposure in the Dungarpur district of Rajasthan, India. The obviously recognizable lesions in the tooth enamel dominated the clinical picture. According to studies, among domestic animals, cattle are the most sensitive and vulnerable, followed by sheep, rabbits, swine, rats, and poultry in decreasing order of sensitivity.<sup>12</sup> The prevalence of dental fluorosis in calves is comparatively higher than that in adult cows raised in the same F endemic locations.<sup>13, 14</sup>

In the Rajasthan district of Bikaner displayed signs of dental fluorosis calves (33.3%) and cows (40.0%).<sup>15</sup> Affected camels (*Camelus dromedarius*) with varying degrees of dental fluorosis have been documented. In addition, the enamel of the mandibular and maxillary teeth was stained from deep yellow to brown on both sides.<sup>12</sup> Because of industrial fluorosis, Rajasthan also had a high rate of recurrent miscarriages, stillbirths, and irregular estrous cycles in female goats.<sup>16</sup> Singh and Swarup<sup>17</sup> observed biochemical changes in serum and urine in fluorotic cow and buffaloes. The cows and buffaloes suffering with fluorosis had elevated serum urea, nitrogen, and creatinine levels. According to Susheela<sup>18</sup> fluoride overdose in humans results in anaemia or early erythrocyte death, meaning that the lifespan of RBCs diminishes due to membrane degradation, which causes them to transform into echinocytes.<sup>19,20</sup> Hematologic abnormalities include hypochromic anaemia, changes in erythrocyte size and shape, eosinophilic leukocytosis, lymphopenia etc.<sup>21</sup>. According to Hillman *et al.*<sup>22</sup> cattle with fluorosis developed hypothyroidism, anaemia, and leukocyte eosinophilia.

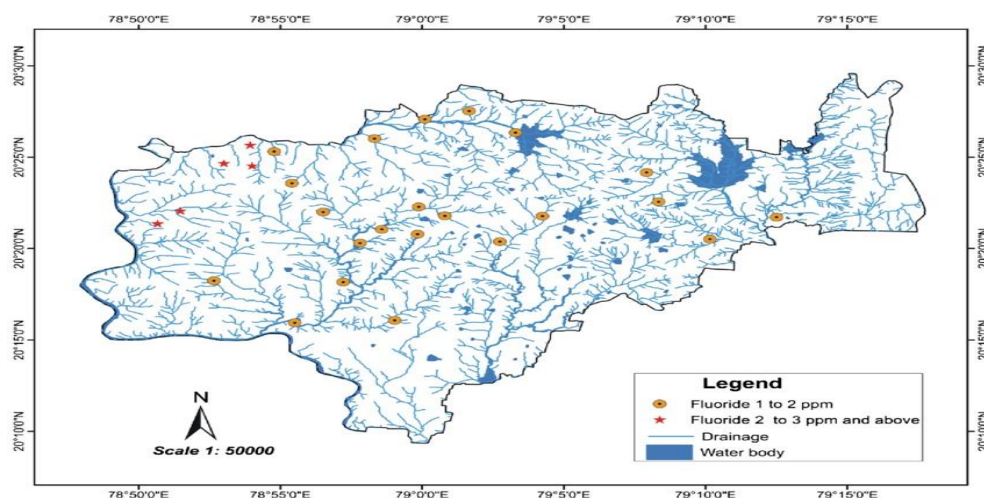
As per the data of state Maharashtra pollution control (2011), 875 and 1183 villages, and about 28 districts are affected by excess iron and fluoride respectively. Maharashtra is the state where about 30-50% districts are affected by fluorosis causes due to excess intake of fluoride through drinking water.<sup>23</sup> Ground water is the main source of drinking water for all the domestic animals. Most of the dugwell, borewells are contaminated with fluoride as the Dongargaon Fluoride Mine is also located in this study area. There are some reports in Chandrapur districts of eastern Maharashtra state, Central India, fluoride contamination occurs mainly in Warora, Korpana and Rajura areas. The finding pertaining to the F concentration and dental fluorosis,<sup>24</sup> skeletal fluorosis,<sup>25</sup> prevalence and severity of dental fluorosis,<sup>26</sup> skeletal fluorosis and nutritional status<sup>27</sup>, assessment of groundwater quality,<sup>28</sup> fluoride accumulation in food stuff,<sup>29</sup> red blood cell abnormalities<sup>30</sup> were reported earlier in people while the present work deals with the dental fluorosis in domestic animals and its effect on haematological parameters.

## MATERIALS AND METHODS

**Study area:** The region chosen for the current study is Warora tehsil, Chandrapur district, Maharashtra, India. Warora is in the north-western portion of Chandrapur district and is located between latitudes 19°55'5.7"N and 20°03'7.3"N and longitudes 79°06'28.4"E and 79°18'34.9"E.



**Fig. 1** Map of Chandrapur district of Maharashtra, India.(www.google.com)



**Fig. 2** Map showing drainage pattern in the Warora area along with sample location and distribution of fluoride.(https://doi.org/10.1007/s12517-021-08581-3)

**Survey:** The present study was carried out during pre monsoon 2022 in seven villages (Dongargaon, Pijdura, Tembhurda, Athmurdi, Madheli, Bandara and Chikani) of Warora tehsil of Chandrapur district (Figs. 1,2). In this district Dongargaon Fluoride Mine is also located. Chandrapur is one of the known endemic district for fluorosis where F contents in water (bore wells and hand pump) is ranging between 0.53 to 5 mg/L which is used as drinking water sources for human and domestic animals. In order to investigate the effects of F in various local species, a survey was carried out to determine the endemicity of fluorosis in domestic animals by going door to door in the early morning and the late evening.

**Animal examination and sample collection:** Animals in the fluoride-endemic area had clinical examinations to look for the classic fluorosis symptoms, such as tooth lesions, skeletal exostosis in the ribs, frontal bones, and metacarpals, as well as lameness.

**Collection of blood samples:** With prior permission of animal ethics Committee (CPCSEA), blood samples were taken from animals exhibiting fluorosis symptoms in fluorotic area, and in order to provide a comparison, an equivalent number of blood samples were taken from animals in non-fluorotic areas that appeared to be in good health. A jugular vein puncture was used to obtain about 10 ml of blood samples, out of which 5 ml were then placed in vials containing EDTA and 5 ml of blood were collected in clean sterile vials which allowed to coagulate in order to separate the serum.

**Diagnosis of Dental Fluorosis:** The research student and a trained veterinarian worked together to conduct clinical evaluations of dental fluorosis. The teeth exhibit obvious signs of fluorosis. Dental fluorosis was detected by closely examining and taking pictures of the teeth of both mature and young fluorotic animals. The Dean's Fluorosis Index was used to diagnose dental fluorosis. Based on the categorization by Shupe et al.<sup>31</sup> and Choubisa<sup>32</sup> dental lesions were examined and assessed, and it was scored on a 0–5 point scale.

**Biochemical parameters in blood serum:** Using a portable fluoride ion-specific electrode (Orion model) and an ISE metre (Orion Model), the F concentration in serum was determined using an ion-specific potentiometer, TISAB (total ionic strength adjustment buffer), and the modified Cernik *et al.*<sup>33</sup> method. Between 0.019 and 1900 ppm is the instrument's detection range. Five recently produced operating standards were used to calibrate the device.

Haematological counter (Abaxis HM5 Vet Scan automatic haematology analyzer) was used to assess the hematological parameters like, Haemoglobin (Hg), Total Leukocytes Count (TLC), Total Erythrocytes Count (TEC), Packed cell volume (PCV), lymphocyte and neutrophil percentages in affected animals of fluorotic areas and for comparison similar numbers of blood samples were also tested from apparently healthy animals from the non-fluorotic areas.

**Statistical Data analysis:** Statistical analysis of domestic animal of different species in different villages for dental analysis was performed using a Chi-squared test. The collected data of affected cases was compiled categorically for species and type of dental fluorosis for each village under study. Also Species wise independence of affected number of animals from all villages together is tested **using SPSS version 20.**

## RESULTS

**Evaluation of dental fluorosis:** High fluoride content and a variety of clinical symptoms of dental fluorosis were found in cattle, buffaloes, and goats in the surveys on clinical symptoms utilizing dental indices in 7 villages of the study region, from the 5176 animals screened, 1966 had dental fluorosis to varied degrees and it was extremely significant ( $P < 0.05$ ). The prevalence of dental fluorosis was found in the entire population under study areas. In all species, the prevalence of dental fluorosis in cattle (34.3%), buffalo (46.37%), and goats (42.69%), respectively and it is observed that there is significant infection among the various species of domestic animals at Dongargoan, Temburda and Chikani villages (Table 1). From the table 2 it is observed that  $P < 0.05$  is a statistically significant difference in the species wise dental fluorosis from all study villages of Warora tehsil.

**Assessment of dental fluorosis:** Evaluated the anterior teeth to rule out the different levels of abnormalities. One of the signs that all of these animals shared were mottled enamel, yellowish brown Incisors, discoloration, uneven enamel pits and patches, receding gums, exposed tooth roots, and abnormal tooth. On the enamel surface of the teeth, dental fluorosis initially manifests as a brownish-yellow stain, but as the animal ages, this stain eventually turns deep yellowish brown. It has been noted that symptoms get worse as animal age. Compared to juvenile animals, adults exhibited symptoms that were more severe. In addition to exposed incisor root cementum and projecting gingival, severely injured animals showed tooth loss, brittle and fractured teeth, loose teeth, and enamel degradation (Figure 3a,b,c,d). Animals were seen to have discoloration of teeth and trouble chewing and insufficient food which ultimately causes their early death.

**Evaluation of blood parameters:** This study was conducted to examine the blood parameter in fluorotic animals of Chandrapur district found to be decreased level of blood cell count and displays the average levels of serum fluoride in animals and the average values of their major hematological markers. There are significant differences between the mean values of haematological parameters in animals of fluoride polluted and nonpolluted localities. In comparison to their controls the F content in serum was considerably greater in the buffalo, cow, and goat ( $0.359 \pm 0.014^*$ ,  $0.292 \pm 0.017^*$ , and  $0.186 \pm 0.004^* \mu\text{g/ml}$ ), (Table 3). Indicators of fluoride-induced anaemia such as leukocyte count, total erythrocyte count, packed cell volume, and haemoglobin concentration were significantly decreased in fluoridated animals. Animals from fluoride-polluted areas exhibited considerably higher lymphocyte percentages and lower neutrophil percentages than those from unpolluted areas, according to the differential leukocyte count. By comparing the RBC counts of all the nonfluorotic animals, the current study explained the considerable reduction in RBC numbers. Haematological assays revealed that all of the animals' total leukocyte counts had dropped.



**Fig.3 Animals in the research area have different types of dental fluorosis:**

(a)Dental fluorosis in calf was only visible as light to dark yellowish, striated, horizontal lines, commencing with the teeth's roots (b) dental fluorosis takes the form of blackening of the enamel surface and brownish, irregular patches on cow's teeth (c) Pigmentation that is irregular or finely dot and strip like, excessive abrasions, irregular wearing, alveolar teeth that are receding, together with receding and bulging gingiva in buffalo (d) The tint of the incisors is yellowish brown spots of uneven enamel, pits, and discolouration that can be seen in goat.

**Table 1. Prevalence of dental fluorosis in different species of domestic animals amongst the seven villages of study area.**

Name of Villages	Total animal available in villages			Animal with Dental Fluorosis			Chi square test
	CATTLE	BUFFALO	GOAT	CATTLE	BUFFALO	GOAT	X <sup>2</sup>
Dongargaon	375	38	308	131/375 34.93%	24/38 63.15%	125/308 40.50%	<b>12.264412</b>
Pijdura	513	39	207	187/513 36.45%	22/39 56.41%	89/207 42.99%	7.7172469
Tembhurda	592	43	437	198/592 33.44%	18/43 41.86%	195/437 44.62%	<b>13.519378</b>
Athurmudi	230	73	128	78/230 33.91%	21/73 28.76%	60/128 46.87%	8.4258097
Madheli	688	58	292	230/688 33.43%	26/58 44.82%	117/292 40.06%	6.0343241
Bandra	35	13	24	11/35 31.42%	7/13 53.48%	14/24 58.33%	4.7418132
Chikani	625	81	377	214/625 34.24%	31/81 38.20%	157/377 41.64%	<b>12.447232</b>
Total	<b>3058</b>	<b>345</b>	<b>1773</b>	<b>1049/3058 34.30%</b>	<b>160/345 46.37%</b>	<b>757/1773 42.69%</b>	

X<sup>2</sup>- Chi Suare

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From the above table it is observed that there is significant infection among the various species of Dongargaon, Temborda and Chikani villages.

**Table 2: Species wise independence of dental fluorosis from all study villages of Warora tehsil.**

Name of Village	Cattle affected	Goat affected	Buffalo affected	Kruskal Wallis H test
Dongargaon	131	125	24	Significance = 0.016
Pijdura	187	89	22	
Tembhurda	198	195	18	
Athurmudi	78	60	21	
Madheli	230	117	26	
Bandra	11	14	7	
Chikani	214	157	42	
Total	1049	757	160	

$P < 0.05$ , this means that there is a statistically significant difference in the level of infection when observed species wise.

**Table 3. Serum fluoride concentration and mean values of haematological parameters in animals of fluoride polluted and unpolluted localities**

Parameters	Buffalo ( <i>Bubalus bubalis</i> )		Cattle ( <i>Bos taurus</i> )		Goat ( <i>Capra hircus</i> )	
	Healthy	Fluorotic	Healthy	Fluorotic	Healthy	Fluorotic
Serum fluoride ( $\mu\text{g/ml}$ )	0.092 $\pm 0.008$	0.359 $\pm 0.014^*$	0.088 $\pm 0.006$	0.292 $\pm 0.017^*$	0.0896 $\pm 0.009$	0.186 $\pm 0.004^*$
Hb (g/dl)	11.89 $\pm 0.14$	7.67 $\pm 0.21^*$	11.43 $\pm 0.15$	7.32 $\pm 0.18^*$	11.37 $\pm 0.17$	6.95 $\pm 0.33$
PCV (%)	32 $\pm 0.404$	23.52 $\pm 0.536^*$	33.75 $\pm 0.34$	23.26 $\pm 0.36^*$	24.28 $\pm 0.34$	16.70 $\pm 0.25^*$
TEC (millions/ $\mu\text{l}$ )	5.82 $\pm 0.0068$	4.67 $\pm 0.0652^*$	5.75 $\pm 0.007$	4.43 $\pm 0.049^*$	10.87 $\pm 0.0154$	9.82 $\pm 0.052^*$
TLC (thousands/ $\mu\text{l}$ )	6.40 $\pm 0.078$	5.517 $\pm 0.079^*$	6.50 $\pm 0.045$	5.63 $\pm 0.080^*$	11.24 $\pm 0.037$	10.42 $\pm 0.046^*$
<b>Agranulocyte</b>						
Lymphocytes (%)	61.41 $\pm 0.123$	69.67 $\pm 0.129^*$	60.66 $\pm 0.097$	70.14 $\pm 0.105^*$	61.44 $\pm 0.104$	71.36 $\pm 0.041^*$
Monocytes (%)	0.32 $\pm 0.0045$	0.50 $\pm 0.003^*$	0.17 $\pm 0.002$	0.50 $\pm 0.003^*$	0.17 $\pm 0.002$	0.43 $\pm 0.0040^*$
<b>Granulocyte</b>						
Neutrophils (%)	36.17 $\pm 0.181$	28.16 $\pm 0.11^*$	36.36 $\pm 0.068$	27.29 $\pm 0.060^*$	36.45 $\pm 0.057$	25.43 $\pm 0.079^*$
Eosinophils (%)	0.505 $\pm 0.0034$	0.608 $\pm 0.0182^*$	0.324 $\pm 0.0048$	0.535 $\pm 0.01^*$	0.331 $\pm 0.0023$	0.411 $\pm 0.0054^*$
Basophils (%)	0.17 $\pm 0.0018$	0.20 $\pm 0.0012^*$	0.17 $\pm 0.0018^*$	0.29 $\pm 0.0023^*$	0.17 $\pm 0.0029$	0.21 $\pm 0.0024^*$

Values within row with \* differ significantly at  $p < 0.05$  and  $p < 0.01$  between healthy and fluorotic animals.

## DISCUSSION

As reported earlier on dental<sup>24</sup> and skeletal fluorosis<sup>25</sup> in a population in Chandrapur district due to natural high fluoride concentration in water<sup>28</sup>, and food stuffs<sup>29</sup> as a main sources of fluoride toxicity. The present work has special emphasis on domestic animal's fluoride toxicity. Fluoride has a negative impact on dentin and enamel development. The symptoms were more severe in adult animals. Damaged ameloblasts and odontoblasts create a matrix that is unable to absorb enough minerals, leading to inadequate tooth formation as well as dental diseases. The current study found that buffaloes (46.3%) had the greatest prevalence rates of

dental fluorosis as compare to goats (42.7%), and cattle (34.3%), close resemblance exists between our findings and those of <sup>31, 34-37</sup> made quite comparable observations as well. Within the same F-infected communities, animals may show varying degrees of F-prevalence and severity, as well as varying frequencies of F-exposure and ingestion. Due to their habit of staying indoors and frequently consuming fluoridated water from hand pumps and wells, buffaloes have a higher prevalence and severity of dental fluorosis than cattle and goat, which are typically left in the pastures during the day and regularly drink water from various sources, primarily nullahs, ponds, and canals. Older animal's teeth suffered from excessive abrasion from years of exposure to fluoride.<sup>38</sup> When these dental lesions become serious enough to interfere with grazing and chewing, the animals succumb to starvation and cachexia at a young age.<sup>39</sup>

The current findings further show that fluoride toxicity can manifest below the maximum allowable amount of fluoride in drinking water and that it is influenced by a number of biological and non-biological factors. In Karnataka's endemic areas, elevated plasma F concentrations have also been seen in cattle.<sup>35</sup> Blood being most sensitive act as an indicator of countless metabolic disorders. The current study of haematological parameter elucidated the significant decrease in the number of RBCs as well as haemoglobin concentration in fluorotic areas of Chandrapur district and similar finding is consistent with the decrease in RBC count in sheep and cattle.<sup>40</sup> Anaemia and low haemoglobin production as a result of RBC lysis and lower RBC formation as a result of decreased bone marrow production could be the one explanation for this decline.<sup>41,42</sup> Toxic substances' interaction with red blood cells can impact the latter's ability to carry haemoglobin (Hb), hence reducing its concentration.<sup>43</sup>

The higher dosages of F to the rats observed leukopenia and lymphopenia; this may be because F directly harms leucopoiesis in lymphoid organs. Cows and buffalo have also been shown comparable outcomes.<sup>34,44</sup> The F compounds also had an impact on the animals' leukocyte differential counts, considerably boosting the proportion of neutrophil and monocytes, which is a first order immunological reaction to the chemicals.<sup>45</sup> Das *et al.*<sup>46</sup> showed a significant decrease in neutrophil and monocyte counts in rats that had been exposed to F for 28 days, and they came to the conclusion that this would result in reduced cellular immunity. Reduced WBC and neutrophil counts were most likely brought on by F's detrimental effects on the bone marrow and other haematological organs. Our current results are consistent with other earlier studies that found a little increase in WBC in dogs<sup>47</sup> and in human.<sup>48</sup> We noticed a similar trend for WBC in the Chandrapur district's Dongergaon village. The body's defences against infections are compromised by low WBC.

Gujrathi *et al.*<sup>49</sup> have noted decline in PCV during fluorosis in cattle and buffaloes. Our findings are also similar to them. PCV readings are indicative of the blood's ability to carry oxygen and are crucial in assessing the impact of stress on an animal's health.<sup>50</sup> Stress in the animals may be indicated by the decrease in WBC and PCV values.

## CONCLUSION

With mild to moderate as well as severe signs of dental fluorosis, the current investigation demonstrated the incidence of dental fluorosis in all species of domestic animals, with extremely significant ( $P < 0.05$ ) prevalence in Chandrapur district. Anaemia and stress are haematological abnormalities brought on by fluoride toxicity. Therefore it's vital to remember that fluoride's effects on blood might vary depending on exposure time, and personal susceptibility. Fluoride effects on blood may differ depending on the species. A multidisciplinary approach including professionals from the veterinary, medical, environmental, and social sciences is crucial in order to lower the prevalence of dental fluorosis and increase public awareness of domestic animal health.

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## CONFLICT OF INTERESTS

None

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Not applicable

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