

EFFECT OF HIGH FLUORIDE AND LOW PROTEIN ON TOOTH MATRIX DEVELOPMENT IN GOATS

Jundong Wang,^a Jianhua Hong, Junping Li, Yuhong Guo,
Jianfeng Zhang, Junhu Hao
Shanxi, China

SUMMARY: An optical and scanning electron microscopy study was made of the effect of high fluoride and low protein intake on tooth matrix development in goats in an industrially polluted region of Baotou, China, during the dry grass season. The enamel and the dentine were severely damaged, and the boundary lines between them and between the cementum and the dentine were obscure and uneven. The fluorosed dentine had loose and crooked rows of collagen with non-uniform staining, in contrast to dense and neat rows of collagen in controls with uniform staining.

Keywords: Baotou, China, Fluoride pollution, Goat Teeth, Low protein, Tooth matrix.

INTRODUCTION

The Baotou region in Inner Mongolia of China has a rainy season (total annual rainfall 250-300 mm) from July to September. During the remainder of the year, with little rainfall, goats pastured in this region develop sawteeth that are brittle and show excessive wear, whereas teeth developed in the green grass season are comparatively hard and normal in appearance. When teeth are uneven, pasturing and mastication are impaired, and the life span of the animal is curtailed. In severely polluted areas livestock can survive only 2-3 years, thereby limiting reproduction and reducing the success of animal husbandry.^{1,2} Various efforts to solve these problems have been investigated, *e.g.*, removal of goats from high to low fluoride areas, transfer of adult nanny goats from low to high fluoride areas,³ use of stored green grass in the dry season as fodder,⁴ mechanical trimming of sawteeth,⁵ and dietary supplementation with minerals.⁶⁻⁸ Over the years we have experimented with methods to reduce fluoride exposure and have been able to show that supplying protein-rich feed is the best way to prevent wearing down of teeth in goats being pastured in high-fluoride areas.^{9,10}

How does fluoride affect the development of teeth in goats? Do various nutritional factors play a role, or do they indirectly modify the toxicity of fluoride? Focusing on these questions, we conducted the following investigation.

MATERIALS AND METHODS

Twenty native goats, aged 6 months, were sent to pasture in Baotou for 12 months in a severely fluoride-contaminated area. When the first pair of incisors (developed in the high fluoride period) had grown out and had worn for half a year, and the second pair of incisors (developed in the green grass pe-

^aFor Correspondence: Prof. Jundong Wang, College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801 People's Republic of China. Email: hjh7511@163.com

riod) had also breached and were used, the first and second pairs of incisors were collected as specimens. Incisors of control goats, which did not have any symptoms of fluorosis, were selected from a safe low-fluoride zone.

The teeth were fixed in neutral formalin for about 1 week. They were then transferred to methanoic acid-formalin solution (methanoic acid 5 mL, formalin 5 mL, distilled water 90 mL) for decalcification until calcium could no longer be detected chemically. For histo-pathological study the teeth were dehydrated, embedded in paraffin, sectioned, stained by the Van-Gieson method,¹¹ and photographed. For scanning electron microscopy (SEM: Japan EMASIC-40) the decalcified teeth were dehydrated, gilded, and photographed following Zhan Chongwan's method.¹²

RESULTS

Clinical appearance: The sampled F incisors were mottled and appeared worn. The first pair of incisors were even more affected than the second pair. The control teeth had no fluorosis symptoms.

Histo-pathology: Under an optical microscope, teeth of control goats presented clear and even boundary lines between enamel and dentine and between cementum and dentine. Moreover, the dentine and the cementum were thick and compact (Figure 1). In the F groups, the boundary lines between cementum and dentine were diffuse, the density of cementum and dentine was poorly-distributed, boundaries between enamel and dentine were uneven or crooked, and part of the enamel was damaged (Figure 2). The second incisors had distinct boundary lines between the cementum and dentine, and textures were comparatively compact. In contrast to the control

Figure 1. Tooth cementum of control goats: clear and even boundary lines between tooth enamel and dentine. Van-Gieson staining (×100).

Figure 2. Fluorosed tooth enamel: damaged tooth enamel and uneven boundary line between cementum and dentine. Van-Gieson staining (×100).

teeth (Figure 3), collagen of fluorosed dentine showed loose and crooked rows and non-uniform staining (Figure 4).

Under SEM, dentinal tubes of transverse section of the control tooth showed regular shapes and neat rows with uniform brightness of dentinal matrix (Figure 5), while the F dentinal tube section had irregular shape and unequal size, showing bright wall tubes but with darkness between them (Figure 6).



Figure 3. Collagen of control tooth dentine with neat rows and uniform. Van-Gieson staining ($\times 200$).

Figure 4. Collagen of fluorosed tooth dentine with loose and crooked rows and non-uniform. Van-Gieson staining ($\times 200$).

Figure 5. Dentine tubes of control tooth; regular shapes and neat rows with uniform brightness of dentinal matrix (SEM $\times 2000$).

Figure 6. Dentine tubes of fluorosed tooth; irregular shapes and unequal size with bright walls but darker area in between (SEM $\times 2000$).

DISCUSSION

In the industrially fluoride-polluted areas of Baotou, goat teeth developed in the dry grass seasons were worn very rapidly, while the teeth developed in the green grass seasons were worn relatively slowly. According to our earlier study, the fluoride content of herbage was high (soluble F 30-80 mg/kg) in the dry grass seasons (dust fluoride was dominant), while it was relatively low in green grass seasons (herbage grew rapidly, and therefore suffered less fluoride pollution). Moreover, the dust on herbage is frequently washed away by rain in the rainy seasons (soluble F 10-30 mg/kg).¹

In terms of nutrition, herbage in the dry grass seasons was notably deficient and lacked coarse protein (5.3%), compared with that in green grass (average protein 15%). In addition, it had more coarse fiber which resulted in poor digestion and evacuation in rumen, and less food intake by ruminal animals, causing them to be seriously undernourished in the dry grass seasons. Accordingly, we suggested that besides the effects of high fluoride on tooth quality in the dry grass seasons, perhaps malnutrition (especially protein deficiency) also had a negative influence on the development of teeth or enhanced the toxic effect of fluoride. To confirm this, we conducted a study and found that supplying protein-enriched bean feed during a dry green season relieved the excessive wear of teeth developed in this period.⁹

Other epidemiological surveys support these ideas. A 1995 New Zealand Health Ministry survey on the developmental quality of children's teeth in different cities and towns concluded that the health level of children's teeth was related to the income of their parents, i.e., the poorer the region, the lower the quality of the children's teeth.¹³ In India, Kodali also found that undernourishment and high-fluoride water affect tooth development.¹⁴ A comparative survey on tea-drinking fluorosis of teeth in pasture and non-pasture areas of the Xinjian Uygur Autonomous Region and the Inner Mongolia Autonomous Region in China revealed that herdsmen ingested a high level of fluoride, but their teeth were only slightly damaged. These phenomena suggest that other nutrient factors may be involved, and although the herdsmen lack diversity in their diet, their main food is protein-rich milk and meat, which help prevent the toxic effects of fluoride.¹⁵

All the above evidence indicates that, in the developmental phase, malnourishment has a negative influence on tooth development, and high fluoride combined with malnourishment affects the development of teeth matrix. The result is that larger quantities of imperfect collagen matrix are produced, and thus the quality of teeth is decreased, which results in extreme wear.

CONCLUSION

In dry and industrial fluoride-polluted areas, high fluoride (especially polluting dust fluoride) and malnourishment in the dry grass seasons cause

underdevelopment of the tooth matrix, subsequently affects its calcification and finally impairs the quality of the teeth.

ACKNOWLEDGEMENT

This research was sponsored by the China National Natural Science Foundation (Grant No. 39870571).

REFERENCES

- 1 Wang J D, Zhan C W, Chen Y F, Li J X, Wang W F, Cai J P. A study of damage to hard tissues of goats due to industrial fluoride pollution. *Fluoride*; 1992;25(3):123-30.
- 2 Wang J D, Hong J P, Li J X. Studies on alleviation of industrial fluorosis in Baotou goats. *Fluoride*;1995,28(3):131-4.
- 3 Wang H Z, Liu H W, Chen Y F. Prevention and treatment of goat fluorosis by shifting fluorosed goats to low fluoride areas. *Proceedings, Animal Fluorosis in China, Huhehaote* 1987;226-32.
- 4 Dong B S, Zhao G Y, Li Q X. Studies on the goat tooth of industrial fluorosis areas. *Inner Mongolian Journal of Animal Science and Production* 1992;47(2):1-4.
- 5 Wang H Z, Lin H W. Effect of trimming long teeth of industry-fluorosed goats by special scissors. *Proceedings, Animal Fluorosis in China* 1987;271-3.
- 6 Li G L, Zhao G Y, Dong B S. A study on alleviation of goat fluorosis with chemicals, *Proceedings, Animal Fluorosis in China, Huhehaote* 1987;244-8.
- 7 Chen Y F, Wang J D. Effect of Se pill on goat serum enzymes and immunological function in an industrial fluoride contaminated area. *Huanjing Kexue (Beijing) [Environmental Science]* 1991;10(2):64-7.
- 8 Chen Y F, Wang J D, Hong J P. Effect of Se spill on industrial fluorosis of goats. *Huanjing Kexue Xuebao (Beijing) [Acta Scientiae Circumstantiae]* 1991;11(4):490-5.
- 9 Wang J D, Hong J H, Li J X, Cai J P. The effect of nutrition supplementation during the annual dry grass season on tooth wear in industry-fluorosed goats. *Fluoride* 1994;27(3):136-40.
- 10 Wang J D. The control of industrial fluorosis of animals. *Chinese Journal of Veterinary Medicine*, 1999;9:18-9.
- 11 Gong Z J, Zhan R Y. *Histo-pathological section and staining technology*. Shanghai Science and Technology Press, Shanghai. 1994.
- 12 Zhan C W, He D J, Zhao T Z, Wang C D. Light microscopic and scanning electron microscopic observations on human fetal bones from an endemic fluorosis area, *Fluoride* 1986;19:18-22.
- 13 Huang J Q. Is fluoridated water healthy for teeth? *Foreign Medical Sciences: Medical Geography Section*. 1995;16(2):69-71.
- 14 Kodali V R R, Krishnamachari K A V R, Gowrinathsastry J. Eruption of deciduous teeth: Influence of under nutrition and environmental fluoride. *Ecology of Food and Nutrition* 1993;30(2):89-97.
- 15 Wang L F, Sun X Z, Xu X. Effect of climate and diet habit on tooth fluorosis prevalence. *Endemic Diseases Bulletin (China)* 1991;6(2):69-71.