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## Identification and morphological characteristics of dental neonatal line in sika deer (*Cervus nippon*)

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### Abstract

The dental neonatal line of the sika deer (*Cervus nippon*) was identified experimentally using chronological labeling methods. In the enamel, prominent dark lines were observed under transmitted light, and the number of increments between the dark line and labeling line was almost consistent with the day-age at the time of labeling injection. Therefore, we identified the dark line as the enamel neonatal line. In the dentin, the bright line was observed under polarized light. Since the bright line corresponded to the enamel neonatal line, we recognized the bright line as the dentin neonatal line. Neonatal lines intersected with the enamel-dentin junction at approximately one-third cervical in the first molar. Using these features, it would make possible to distinguish the neonatal line in wild sika deer.

Key words : neonatal line, sika deer, teeth

Growth incremental lines are found universally in the dental tissues of animals<sup>15,18)</sup>. These lines reflect individual physiological systems<sup>21)</sup>, and for practical purposes three varieties of incremental lines can be distinguished in enamel : cross-striations and striae

of Retzius (short-and long-period regular increments) ; Wilson bands (pathological accentuated striae) ; and the neonatal line which is occasioned by birth<sup>19)</sup>. Combining the counts of the normal periodic growth increments and accentuated increments provides a means of

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reconstructing the chronology of dental development<sup>1,5,12)</sup>, age at death<sup>2,3,7)</sup>, and the age at which stress occurs<sup>4,13,14)</sup>. The neonatal line appears in both enamel and dentin as corresponding lines, and it is recognized as a structural response to environmental disturbances at birth<sup>17,20)</sup>. Therefore, the presence of the neonatal line makes it possible to distinguish pre-natal from post-natal enamel and dentin<sup>7,16)</sup>, so the identification of the neonatal line is fundamental for chronological study, such as age estimations.

In the previous study, we detected the periodicity of incremental lines in the first incisor using age-unknown individuals, but could not identify the neonatal lines in sika deer<sup>8)</sup>. So, in the present work, we used age-known animals and tried to identify the neonatal lines in sika deer teeth. Morphological characteristics and the appearance location of neonatal lines were examined, because the features may help to identify the neonatal line in other age-unknown animals.

The sampling was carried out on Satomigaoka Deer Farm of the Ashoro District in eastern Hokkaido from 1999 to 2001. Fifteen fawns were found by observing does and searching the farm area (1.1 ha) during the fawning period. In five animals, accurate birth dates could be determined by direct observation of birth. Fawns were ear-tagged, and then re-captured to inject fluorochrome (calcein : 6 mg/kg) after an interval of three or four days. These deer were slaughtered for research by phlebotomy of KCl under anesthesia at variable ages in days.

The neonatal line is only seen in those teeth which are in progress of formation and calcification at birth<sup>17)</sup>. Just after birth, while incisors have completed crown formation, the first molar ( $M_1$ ) is developing in its crypt but crown formation is incomplete. So, we employed  $M_1$  as the materials for the present

study.

$M_1$  were extracted and fixed in 10% buffered formalin for one week, then embedded in resin (Epofix : Marumoto Struers K. K., Tokyo, Japan) and polymerized at room temperature for one day or longer. The polymerized tooth was cut longitudinally with a diamond-edged saw through the mesial and distal cusps in the bucco-lingual plane. After grinding to remove scratches, the cutting plane was dried and bonded to a glass slide with epoxy glue. The specimen was sectioned into 300-400  $\mu\text{m}$  thicknesses using a Crystal Cutter NOVA (Maruto Instrument Co., Ltd., Tokyo, Ja-

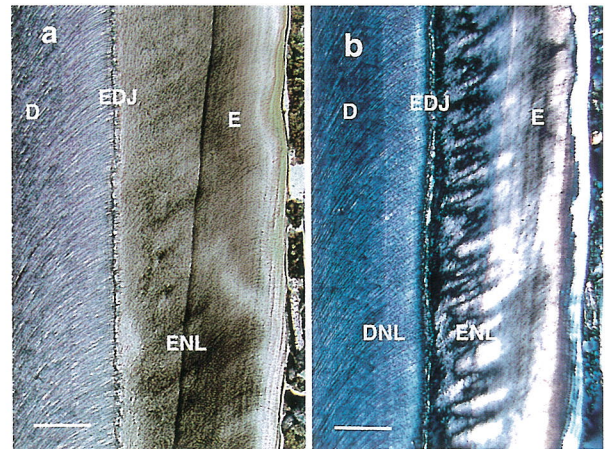


Figure 1 . The neonatal lines observed under transmitted and polarized light.

Micrographs of the thick section (approx. 100  $\mu\text{m}$  thickness) obtained from an animal of about 200 days in age.

- Transmitted light micrograph. The prominent dark enamel neonatal line (ENL) runs obliquely across the enamel. The pre-natal (inner) enamel is pale and free of imperfection, while the post-natal (outer) enamel has some distinct rough striae.
- Polarized light micrograph. The same section in a). In the dentin, the bright dentin neonatal line (DNL) is observed. The inner and outer sides of the neonatal line show a different hue in both enamel and dentin. Scale bar = 250  $\mu\text{m}$ . EDJ : enamel-dentin junction, E : enamel, D : dentin, ENL : enamel neonatal line, DNL : dentinal neonatal line.

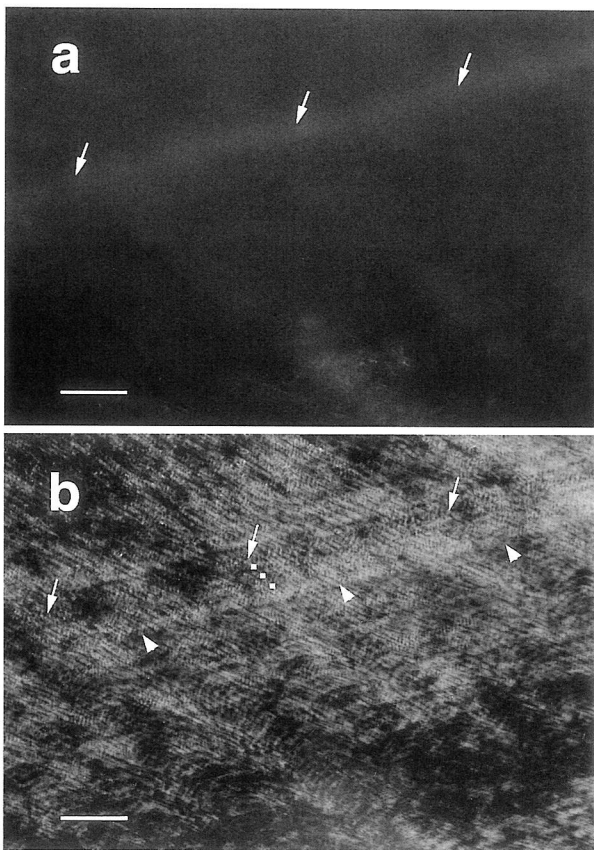


Figure 2. Comparison of fluorochromic labeling line and enamel increments.

Micrographs of the thin section (approx. 70  $\mu\text{m}$  thickness) obtained from the animal administered the labeling injection at 4 days of age.

- Fluorescence micrograph. Diffused calcein line is observed (arrows).
- Polarized light micrograph. Field of view is the same as in a). Three lines (dots), that is, four increments are observed between the "dark line" (arrow heads) and the fluorescent line (arrows). Regular incremental lines are more visible on the inner side of the enamel (bottom of figure). Scale bar = 20  $\mu\text{m}$ .

pan) and ground to a thickness of 100  $\mu\text{m}$  using descending grits of carborundum (600-4,000 grit) with paraffin oil. Specimens of five accurate age-known animals were ground to a thickness of about 70  $\mu\text{m}$  to observe the regular incremental lines. Then, the specimens were mounted with Entteran New (E. Merk

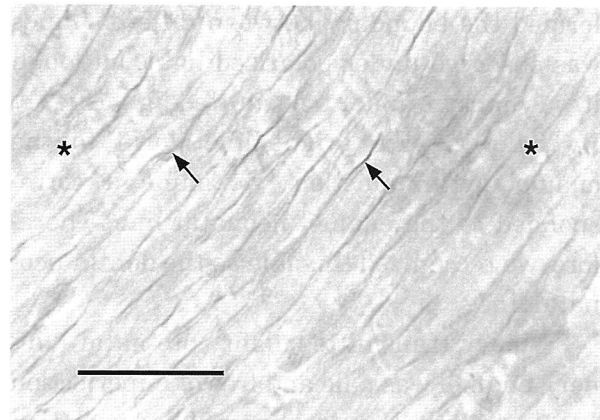


Figure 3. Higher magnification of enamel neonatal line.

Transmitted light micrograph of the thin section (approx. 70  $\mu\text{m}$ ). Enamel prisms run obliquely from bottom to top of the field of view. The enamel neonatal line crosses the figure (the line connecting asterisks). The line owes its visibility to the series of expansions of interprisms (arrows) at the expense of the prisms. The prisms showed a more or less irregular structure at the neonatal line. Scale bar = 20  $\mu\text{m}$ .

Darmstadt, Frankfurt, Germany), and examined under transmitted light, polarized light, and fluorescence microscopy (BX60: Olympus Co., Ltd., Tokyo, Japan). The excitation wavelength was set at 400-440 nm for calcein, and it showed green fluorescence.

In the enamel, we observed the characteristic dark line running across the enamel under transmitted light (Fig. 1a). In the five fawns with a known accurate birth date, the number of incremental lines between the dark line and fluorescence-labeled line was almost consistent with the day-age at the time of labeling injection (Fig. 2). In the previous study, we suggested the daily periodicity of enamel increments<sup>9</sup>, and it is well known that enamel short-period increments are formed daily in primates, including humans. Consequently, we identified the dark line as the neonatal line. In the dentin, the bright line which

formed the boundary between different hues was observed under polarized light, although it was unclear under transmitted light (Fig. 1). By following the bright line down to the enamel-dentin junction, we found it corresponded to the enamel neonatal line. Therefore, we recognized this line as the dentin neonatal line.

Under transmitted light, pre-natal (inner) enamel was pale and free of imperfection, while post-natal (outer) enamel had variation in color and some distinct rough striae (Fig. 1 a). In the same section, pre-natal and post-natal enamel showed a different hue under polarized light (Fig. 1 b). At higher magnification, the enamel prisms changed direction, and more evident or thicker interprism regions were observed at the neonatal line (Fig. 3). These characteristics were the same as those of the neonatal line in humans<sup>6,9,11,16</sup>. Note that in sika deer, the regular minute incremental lines appeared more distinctly in pre-natal enamel than in post-natal enamel under polarized light in the thin sections (Fig. 2b). On the other hand, the distinct increments were observed only in post-natal dentin under polarized light in the thin sec-

tions. The neonatal line was clear in the sika deer dentin with polarizing microscope, and the dentinal tubules were bent at the line. When there are other accentuated lines in enamel and identification of the enamel neonatal line is difficult, the neonatal line in dentin might be helpful for specifying the neonatal line in enamel, using the correspondence between them.

To investigate the location of the neonatal line, the distances from the cervix to the neonatal line's intersection with the enamel-dentin junction were measured on the buccal and lingual surfaces with the scale on the stage of the microscope. The Mann-Whitney test showed no statistically significant differences between the sexes, therefore, males and females were pooled. The mean values and proportions obtained from the measurement are shown in Table 1. The proportions indicate the ratio of crown formed post-natally to that pre-natally, calculated by dividing the distance from the cervix to the neonatal line by the total length (from the cervix to the cusp of the dentin horn). When the cusps were much worn, the mean total length (in mesial cusps, 16.3mm and 15.2mm, in distal cusps,

Table 1. The location of neonatal lines

	Mesial			Distal			Sign. <sup>c</sup>
	N	Distance <sup>a)</sup> (mm)	Proportion <sup>b)</sup> (%)	N	Distance <sup>a)</sup> (mm)	Proportion <sup>b)</sup> (%)	
Buccal surface	13	4.6±0.8	28±4	11	6.7±0.9	40±4	P=0.005**
Lingual surface	14	3.1±0.7	20±4	14	4.7±0.8	30±5	P=0.001**
Sign. <sup>d)</sup>		P=0.001**			P=0.005**		

Values are mean ± SD

a) Distance from the cervix to neonatal line's intersection with the enamel-dentin junction

b) Proportion of post-natal formation in crown extension, calculated by dividing the total length into distance of the neonatal line

c) Wilcoxon's test between sections obtained from mesial and distal cusps

d) Wilcoxon's test between buccal and lingual surfaces

\*\* Significance different p<0.01

16.6mm and 15.5mm, on the buccal and lingual surfaces, respectively) was used for calculation. The crown of M<sub>1</sub> had extended 60-80% at birth. Both the distances and proportions were significantly different within the teeth; the mesial cusps rather than the distal cusps, and the lingual surface rather than the buccal surface, had progressed the crown formation at birth. It was suggested that the lingual surface of the mesial cusps may be completed earliest, while the buccal surface of the distal cusps may continue in post-natal formation for a longer time.

In this study, we identified the neonatal line experimentally. It was recognized as the prominent dark line in the enamel under transmitted light, and as the border line between different hues in the enamel and dentin under polarized light. The neonatal line intersected with the enamel-dentin junction at approximately one-third cervical in the first molar. Identifications of the neonatal line using these features will be useful for age estimation and reconstruction of individual life histories, and may contribute to the management of wild animals.

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